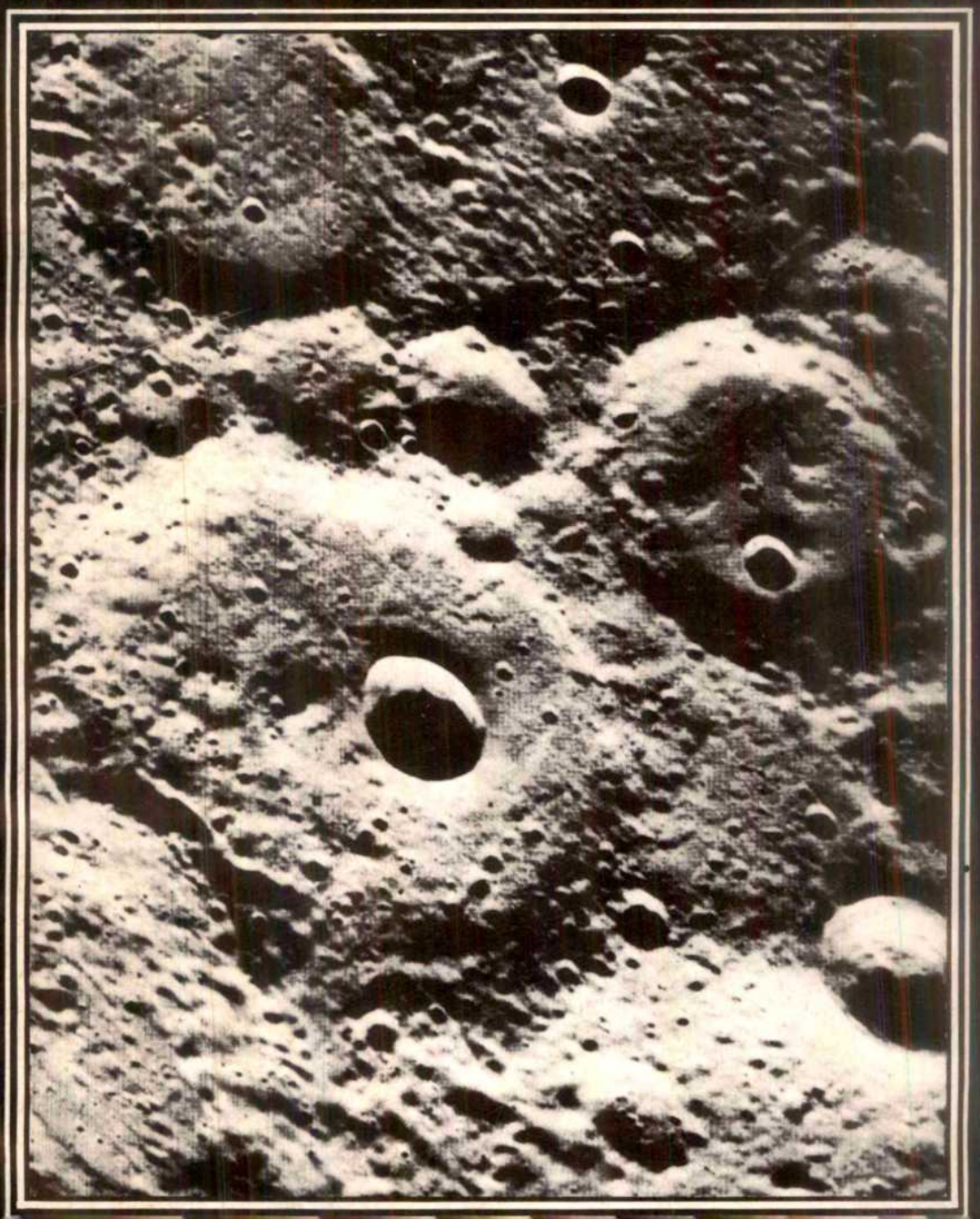
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Edited by Wolfgang Wieser

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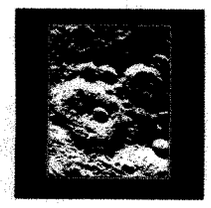
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Nature accepts three types of communications:

- Articles are up to 3,000 words in length with at most six figures and may either be reports of major research developments in a subject or broader reviews of progress.
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(i) If it is necessary to refer to several references by the same author at once, only one reference number need be given.

(ii) The last page as well as the first of any reference should be cited.

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A fuller guide appeared in *Nature* (**246**, 238; 1973).

nature

Volume 249 May 3, 1974

INSTITUTE OF ELECTRONICS

Chips with everything

THE science of human nutrition in Britain has fallen on hard times. Of that, even the practitioners themselves seem to agree. In the last war the nutritionist played a major and acknowledged role in keeping the public healthy (some would say healthier than ever before or since). These days scientists turn their noses up at the thought of becoming nutritionists and the public gets little or no advice on what constitutes a balanced diet—apart from keeping cigarettes out of it. The situation seems to be worse, if anyting, elsewhere in Europe.

Nutrition is not an easy subject to define, as it includes so many aspects of other disciplines. Nevertheless it may be helpful to make subdivisions such as

(i) clinical nutritionists dealing with individual cases and counselling at the individual level,

(ii) scientific nutritionists working, say, on vitamins, fortification or dietary assessment techniques,

(iii) epidemiological nutritionists handling the statistics of large populations and

(iv) public nutritionists working either with government or the food industry on monitoring and information.

Who becomes a nutritionist? It is here that the problems start. There is no prescribed way into the field, but a broad biological education, probably with a medical background, is desirable. However, the job lacks kudos and the attraction of the microscopic and submicroscopic in biology draws away many talented students from study of the whole man. In medical schools, nutrition is about on a par with chiropody in appeal to students, and, for lack of good teachers, has often been introduced incidentally in other courses. A clinical nutritionist will often find himself a different title such as endocrinologist. As a result, those in nutritional departments of universities find it extraordinarily difficult to recruit high-calibre postgraduates, who regard the subject as soft and indefinite.

There is also a problem in the student's perception of a career structure. There are several senior posts in nutrition which will fall vacant in the next few years and there will be difficulty, it is said, in filling these, as there are insufficient middle level positions. Students see the lack of obvious upward steps from junior posts and they leave for better guaranteed careers.

Nutrition is very obviously a subject where the interplay between universities, industry and the public health services should be strong, but the deficiencies in career prospects in the academic sector have not been made up for by an adequacy of challenging positions elsewhere. The food industry does not employ many nutritionists (in contrast with the animal feed industry with its obvious interests in fatter beasts—the whole man may not be a proper study of mankind, but the whole pig certainly is). Those that industry does use are frequently engaged simply in ensuring the accuracy of labels or approving advertisements. Those in public service too often find themselves engaged in routine activities, whilst there are large public health educational issues which go untouched.

Obesity is a case in point. Many believe that this is the major nutritional problem facing the Western world today, not only in itself, but for the many disorders which spring from it. Yet at present next to nothing is being done by public health authorities either in prevention or cure. Nutritional counselling even of the simplest kind barely exists. And for health clubs or dieting systems claiming to reduce weight safely there is no public supervision. This at a time when the choice of foods available to the consumer is large and there is a demand for information on good eating habits.

There are, fortunately, signs of change. The recent establishment of two Royal Society Sainsbury Fellowships should go someway towards producing better prospects for career advancement. Further, there is an indication that a new generation of students has a greater interest in multi-disciplinary people-oriented biology. And the new administrative areas of the National Health Service should each have a 'community dietician'.

Much remains to be done, however, in the field of health education. The responsibility is ultimately the government's and the duty is to make the public aware of the elementary principles of sound nutrition so that more sophisticated choices can be made when buying food. It has long been a British principle to assume that intuition is a good substitute for understanding, and therefore that detailed information about the nutritional properties of food is unnecessary. It is time that such an attitude was superseded.

The results of better nutritional counselling would be unspectacular, and there would be inevitable doubts that a long-term educational programme would have any more impact than have anti-smoking campaigns. This should not deter government from taking a few inexpensive steps in the right direction. They would at least stimulate a greater commitment to nutritional studies by the food industry. In turn, this might breathe more life into research in the universities.

100 years ago



A PETITION signed by twenty-six Professors in the Universities of Scotland has been presented to the Prime Minister, calling his attention to the treatment of the ladies admitted to matriculate as students of medicine in the University of Edinburgh, and afterwards refused the right to graduate, and urging the Government to take the whole subject of the University education of women into consideration, with the view of devising a remedy for the present anomalies.

From *Nature*, 10, 16, May 7, 1874.

Cold comfort from Friends

In a recent letter to the Secretary of State for Energy, Mr Eric Varley, Friends of the Earth (a charitable organisation committed to the conservation, restoration and rational use of the ecosphere) drew attention to the energy savings possible through improved insulation standards and urged that he encourage the introduction of measures which would put an end to unnecessary waste. Here Christine Thomas, a researcher with Friends of the Earth, describes the sort of savings which might be made with a policy for insulation.

Friends of the Earth recently spent a Saturday insulating the roofs of the homes of some 40 old age pensioners throughout the country. They did so in order to draw public attention to the waste caused by heat loss through poorly insulated buildings—an estimated 75% of the energy consumed for space heating in homes insulated to present standards.

Improved insulation can drastically cut this loss; domestic fuel consumption for heating could be reduced by as much as half while still maintaining the same degree of comfort, or indeed improving it. Such savings matter most for those on small or fixed incomes—in particular the elderly, whose health and incomes can least withstand excessive fuel bills. According to a recent national study about 6 million old people are living in rooms clearly too cold for comfort, between 500,000 and 700,000 of them having unacceptably low body temperatures: clearly candidates for hypothermia.

It was facts like these that prompted the Friends to join with Age Concern (the National Old People's Welfare Council) in a practical gesture to demonstrate the ease with which insulation standards could be improved. Laying glass fibre or mineral wool in an attic costs perhaps £20, and offers at present prices a fuel cost saving of some 16% a year in the average semidetached house. Cavity walls can be filled with a variety of foams and mineral substances; solid walls can be faced with insulating board; windows can be double glazed. An average investment of £300 would cover improvements to the roof, walls and windows, together with draught exclusion, cutting fuel consumption by almost a half. Such a sum can be recouped in less than 10 years by the concomitant savings. Double glazing, however, forms a disproportionately large part of this cost, by comparison with the heat loss it can prevent. Something like a reduction of 35% in fuel consumption can be achieved by increasing roof and wall insulation alone at a cost of around £100.

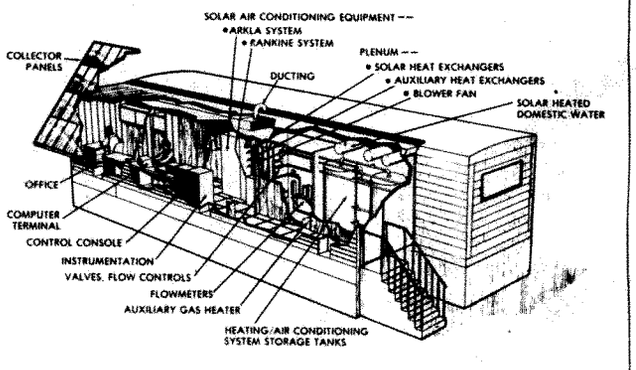
Since domestic heating accounts for 20% of the total energy consumed in Britain, the potential national saving would be about a tenth of the present amount of energy used, the equivalent of nearly 11 million tonnes of oil each year. Calculations show that the power demand that would be eliminated by such a reduction is some 38,000 MW, equivalent to the combined output of some 50 large nuclear reactors. It would, of course, be expensive to insulate existing housing to these standards; a sum of the order of £5,000 million would be required. This, however, compares favourably, with for instance, the capital cost of the power stations that would otherwise be needed: at least £10,000 million. Thus there could be a saving of more than £5,000 million in the long run, not to mention savings in environmental and social costs associated with the installation of large scale generating plant.

It would clearly be far cheaper to insulate all homes fully than to build unnecessary power stations purely to supply heat to waste. Furthermore, by thus relieving some of the pressure on present energy supplies, a very important breathing space would be gained: time to look closely at genuine energy requirements and how they can best be fulfilled, time to develop alternative sources of power, sustainable and safer sources.

The improvement of insulation standards is an area of energy conservation readily amenable to legislative encouragement. Minimum levels for new dwellings are set in the Building Regulations by stipulating the maximum values of U (a measure of the conductivity) permitted. The regulations are at present made under the Public Health Act, which stipulates that they can only be made with specific reference to considerations of health and safety. There is argument as to whether present standards are adequate even for this limited purpose, and proposals for amendments are at present being considered. This, however, seems as far as the present regulations can be pushed.

In order that higher standards, justifiable in terms of energy savings, can be set, the scope of the Building Regulations will have to be widened. This was discussed under the Heath administration when the Department of the Environment was promoting Part III of the Health and Safety at Work etc. Bill, which would have empowered the Secretary of State to make regulations specifically "furthering the conservation of fuel and power" (III, 61, 2, b).

At the discretion of a Local Council, Home Improvement Grants may at present be given for some upgrading of insulation. But the position is not clear; people are unaware of the possibility and many councils are reluctant to give such grants. Making insulation the subject of a Standard Grant would dramatically improve the availability of money; and, together with a positive campaign of public education to ensure that people took advantage of the scheme, would go a long way towards improving standards.



A TRANSPORTABLE laboratory to collect data on incoming solar energy and to carry out advanced research on solar energy heating and cooling systems under widely varying climatic conditions across the United States is completing tests in the Washington, D.C. area before beginning active experiments. The laboratory, housed in two large trailers, was taken to the Washington area for calibration and evaluation of its experimental systems and weather instrumentation at the National Bureau of Standards at Gaithersburg, Maryland. A major purpose of the research with the laboratory will be to obtain correlation of solar energy systems with local requirements which vary widely over the United States. In line with this purpose, local groups of designers, architects, building contractors, and zoning and building code officials in communities along its route will visit the laboratory to exchange information with researchers on specific needs in adapting such systems for use in their localities.

international news

Where freedom is an academic issue

AFTER seven months of junta rule in Chile academic life is stagnating. On the morning of the Allende government's overthrow in September the military occupied all of the country's nine universities, installed their own representatives in place of the deposed rectors, ordered mass suspensions of students and staff alike, and began a process of 'cleansing'. The result of this process is that those members of the academic community who avoided execution, torture or imprisonment, and who have not managed to join the thousands who fled the country, remain at the universities in puppet roles organising resarch according to the junta's decree, teaching what the junta thinks fit to be taught. If they were unfortunate enough to be working in the social sciences their chances of ever again doing academic work in their own country look remote. Even the Christian Democrats who balked at Allende's politics are now coming under fire, and as if the initial physical brutalities were not a sufficient token of the junta's intentions, Chile is being treated to a spectacle which makes clear its new rulers' attitudes to intellectual life: the burning of books.

Immediately after the coup the new regime closed most faculties and departments in the social sciences, history and philosophy. In the University of Concepcion 6,000 of the 16,000 students and hundreds of academics were suspended; the eastern campus of the University of Chile in Santiago was closed and over 8,000 students had their studies cut short, while 4,500 students and 100 academics in the university's school of fine art, music and architecture were suspended.

The Technical University in Santiago, where there was a massacre of students the day of the coup, has been changed into a "military polytechnic" and all of the students and teachers (about 20,000 students in all) have been expelled. All adult education programmes have been stopped. This puts an end to the educational schemes for workers that were carried out under the Popular Unity Government. It is estimated that 5,000 members of the Chilean academic community have fled to Mexico, and 1,000 to France, while smaller groups have escaped through foreign embassies to other European countries and to North and South America.

In Britain, where refugees had been welcomed with open arms from Czechoslovakia in 1968 and Hungary a decade earlier, there was official reluctance to mount a welcoming party for exiles who were themselves communist, or at least capable of living and working without complaint under a communist system. The removal of the Conservative government (one of the first to recognise the junta) in theory makes the way easier for Chilean academics looking for a base to re-establish their careers, but in fact only a handful have so far been accepted: 19 postgraduate students, two undergraduates and 14 visiting and research fellows.

An educational charity, the World University Service has joined with a pressure group called Academics for Chile in raising funds for displaced academics, and together they have produced more than £20,000 and pressed the British government for a contribution. The official response is that already £100,000 is being paid out in training and technical assistance to more than 70 Chileans, most of whom were in the country at the time of the coup, when aid was available on a government to government basis. The Department of Overseas Development is thought to be looking for new ways of continuing this aid now that a socialist government is in the driving seat.

Meanwhile, accounts of the average non-political academic's predicament at the time of the coup and since can be had at first hand in almost any university town in Europe. One such academic who left Chile after repeated harassment by the military and is now studying at a British university, recalled his experience in the following terms (his name is withheld because of his fears for relatives remaining in Chile):

"When the military government took over one of their first targets was the universities. On the day of the coup, the military occupied all nine universities in Chile and the Technical University in Santiago was bombed. The junta considered the universities the main focus of the social change that had swept the country since President Allende's government had come to power in 1970, and they seized power to put a stop to it.

"Their attention was especially concentrated on departments of economics, sociology, anthropology and history as these had been in the forefront of social change, with increasing emphasis being placed in the last few years on social research and educational projects in the shanty towns and amongst the workers and with increasing numbers of workers being given grants to study at the universities.

"After the coup the new government imprisoned many intellectuals even from the right-wing Catholic University in Santiago, and many are reported to have been tortured or executed. The rectors of all the universities were replaced by members of the armed services, and special 'prosecutors' were assigned to each department to hear information and charges placed against students and lecturers by informers.

"Many thousands of students and lecturers suspected of Marxist or socialist ideas who had escaped imprisonment were sacked, and in March all lecturers had to resign and reapply for their jobs. The appointments boards are appointed by the military rectors and the case of those lecturers sacked or refused reappointment on political grounds is grave. There is at present little hope of them being able to find other jobs in Chile, even as manual workers, as employment contracts are strictly scrutinised by the military authorities and no-one who has lost his job for political reasons can be re-employed.

"Whole university departments have been closed down, and I have heard of one department of social sciences where the whole department was imprisoned for publishing research on conditions in the shanty towns and in the country areas.

"As for myself, I was taken from my house by soldiers with no charges being made, and taken to the army barracks. There I was stood against the wall, guarded by four soldiers, for several hours before being interrogated. Already I found, the army had collected all my personal details and previous publications and activities, probably I thought from informers. I was accused of being a Marxist on the basis of my writings, but my interrogators obviously had no idea what Marxist philosophy really consisted of. I found it impossible to put up a legal and rational defence as the whole legal and moral basis of action had been overturned

and actions and writings which had been previously considered perfectly legal were now retrospectively considered illegal, such as not taking part in the anti-Allende demonstrations and strikes before the coup.

"I was interrogated ten times in all, and eventually I left the country rather than risk the alternatives of imprisonment, suspension or of being allowed to continue teaching only courses approved by the government.

"These restrictions in education have reached down as far as the primary schools, and in my opinion the military government is trying to turn the clock back in their educational policies by stopping grants for workers to study at the universities and by strictly controlling the matter and methods of teaching. The restrictions now being placed on freedom of speech are becoming intolerable to most intellectuals, even to those Christian Democrats who opposed Allende and had welcomed the coup."

The reference in this account to the overturning of the legal and moral basis of action is echoed in a further report from Dr H. M. Gerschenfeld, a signatory to a letter about the plight of Chilean academics published in *Nature* in March. According to the military government's Law no 111, suppressing the legal governing bodies of universities, 34 professors of the University of Valdivia were expelled without right of defence, says Dr Gerschenfeld. In some cases the professors were offered the alternative of resigning, and if they refused to do so, they were expelled.

Among more serious casualties of the new order were Professors Merio Ramirez (Education) and Jorge Pena (Music), shot at La Serena; Dr Enrique Paris, secretary of the University of Santiago, died while undergoing torture; Professor Kirkberg, rector of the Technological University of Santiago, imprisoned on Dawson Island.

As for institutions which have suffered under the junta, Dr Gerschenfeld writes as follows:

"The destruction of the departments of psychology and sociology occurred in essentially all of the universities in the country. Even internationally supported schools such as the well known Facultad Latino-americana de Ciencias Sociales (FLACSO) was closed. The Institute of National Studies (CEREN) of the Universidad Catolica at Santiago was also dismantled. Seventy to 100% of the faculty members in the departments of geography, literature, fine arts, history and geology were removed from their positions in Santiago.

"The authorities of the universities who were nominated by the military government also expelled a large number of students on the grounds of sympathising with the government of Dr Allende. The data are fragmentary, but the examples are indicative: for instance, of the 254 first year students of the Science School at Santiago, 174 were expelled, at Concepcion, of 70 students in the last year of the medical school, only 10 were readmitted after a selection on political grounds.

"The number of scientists, teachers, and professional people who are leaving Chile is so great that the military junta has become alarmed. There have been rumours in the last month that the government will impose a tax of 3,000 to 5,000 American dollars to each person having a university degree who wants to leave the country, even if they have been expelled from their jobs at the university or elsewhere. We could not confirm this directly, but the appeals for help from our Chilean colleagues mention this menace and urge the European scientific community to do something for them."

An idea of the lengths to which the military government will go in order to interfere with the career of an academic whom they consider hostile is illustrated by the experience of Juan Rada, who at the time of the coup was a third year student of the sociology of education at the Catholic University of Santiago. Rada had played an active part in the running of the university as a student representative on the higher council. He was a known supporter of Allende's social change programme, and within days of the junta's takeover he stood up and spoke against the military rulers during a meeting of the higher council. He received a phone call during the night (from one of the regime's supporters as it happened) warning him that the junta had decided he was dangerous.

Rada sought refuge in a foreign embassy, and since he was unable to obtain a safe conduct he left the country by a route which he prefers not to disclose—although he is prepared to describe a fast car ride to the airport with motor-cycle outriders. He arrived in Britain earlier this year, and since he had not actually sat his final exams at the time of the coup he asked permission to qualify at London University. When the university wrote to Chile in order to learn about Rada's academic record there they were told that nobody of that name had ever been at the Catholic University of Santiago. As far as the university where Rada had served on the higher council was concerned, he had never existed. Rada was able to establish his bona fides, and to take his exam, and he is now a postgraduate member of London University's Department of the Sociology of Education. He is in touch, regularly, with former colleagues who remained in the country. Their reports, he says, add up to a single message: "Intellectual life is dead."

Business report

John Gribbin and Roger Woodham

Gordon and Breach Science Publishers Inc., of New York, have filed a petition under Chapter XI of the United States Bankruptcy Act. The company's 1973-74 list contains 85 journal titles, most of them scientific; but, according to the management of the British subsidiary of the company, production has not been affected by the filing.

The G & B petition itself follows a similar petition filed under Chapter XI of the Bankruptcy Act by a subsidiary, Media Directions Inc., on November 2, 1973. According to a representative of the British company, the petition now filed by the American parent on March 29, 1974, was necessary "to protect the parent from repercussions from that action".

The Managing Director of the British-based operation — a wholly owned subsidiary of the American parent says that "production, promotion, distribution and other publishing operations are not affected by the filing, and subsidiaries of the American company are not involved. I should stress that we intend to continue our normal publication of journals and new books."

The first meeting of the G & B creditors was held in New York on April 24, before Bankruptcy Judge Edward J. Ryan. The proceedings were adjourned until May 13. Under American law it is possible for a company to bring the court and creditors into the running of that company without ceasing to trade.

According to figures circulated to major creditors by attorneys for the $4,161,297 and 21 cents, while assets total $2,302,940 and 84 cents including an item of $2 million for "stock in trade".

● "THE most significant new product range in the history of the company" was how Mr Ralph Price, chairman of Honeywell in the United Kingdom, described the new Series 60 computers when they were launched last week. Only time will tell whether Honeywell has the upper hand when companies like ICL and IBM unveil their models for the late 1970s and early 1980s in the next year or two.

Honeywell's total turnover in 1973 was $1,170 million, and the profit before tax was $93 million. Although not actually disappointed with the growth of sales in the past year or two, senior management at Honeywell hope that sales will grow faster with the Series 60.

The advent of Series 60 is a positive move on Honeywell's part towards the marketing of fewer, but more flexible, pieces of hardware and software. The new range builds on the best of

Russians seek joint facility in Australia

Peter Pockley, Sydney

Six weeks after the new Australian-Soviet science agreement was initialled in Canberra, it has been revealed that the Russian delegation which finalised these arrangements proposed at the same time that 'a joint scientific facility' be established in Australia. Although this was essentially a renewal of a previous proposal in 1971, the recent press stories caused a flurry of comment in the Parliament and diplomatic circles. There was reportedly a reaction from the United States Ambassador who was said to be concerned that the proposed 'facility' could be a threat to the various American defence bases in Australia.

Of these, two are in the big league —the naval communications base at North-West Cape (Western Australia) and the military satellite station at Pine Gap in the centre of the continent. A security screen surrounds the operations of these stations and there was immediate speculation that the Russians were 'trying out' Australia's diplomatic commitment to the American alliance by indirectly setting up a facility which could conceivably also act as an electronic monitor of American activities.

Both the Prime Minister, Mr Gough Whitlam, and the Foreign Minister, Senator Don Willesee, were, however, at pains to emphasise that no decision had yet been made and that the proposed facility—to be managed jointly by both nations—would be 'to photograph space objects and to contribute to atmospheric studies'. It is not, as reported by some news agencies, intended to be a tracking station The proposal is now being studied by various departments before Mr Whitlam's planned visit to Moscow in the middle of the year.

For readers in the Northern Hemisphere, who have become accustomed over decades to continual contract with the Soviet Union of a military and diplomatic kind, the sharp Australian reaction to such an apparently mild proposal may seem excessive. But, rare though it is in the Southern Hemisphere, even remote possibilities of direct influence by the Soviet Union on events here have always been greeted by over-reaction.

The most celebrated example in recent times was the case of Vladimir Petrov, a Russian diplomat-cum-spy operating out of Canberra, whose defection in 1954 was turned to long lasting political advantage over his Labour opponents by the then Prime Minister, Mr Robert Menzies. The first Russian scare occurred, rather extraordinarily, in the Crimean War when the new settlers actually built harbour forts in a show of self-reliant bravado against the imaginary invaders from the steppes.

The present concern has been a two-day political wonder but it has its roots in the fears of a possible buildup of Soviet naval power in the Indian Ocean on Australia's undefended western seaboard of about 2,000 miles. As with the Australian-Soviet science agreement itself, this latest matter was treated by politicians and press alike as primarily a diplomatic affair, with science as the passenger in the vehicle.

Honeywell's present lines and those of the erstwhile computer arm of General Electric (USA) which Honeywell took over three-and-a-half years ago. At that time Honeywell and General Electric actively marketed 10 computer series, 20 processors and 12 software systems —now there are just four, eight and six. The largest of the new series, Level 66 is based on the Honeywell Series 6000 which has brought in about $1,000 million since it was launched in 1971.

The development costs for the new series have hardly been trivial; most of the $50 million earmarked for development by Honeywell in each of the past three years has gone on the Series 60, and much of the development work was done in Europe. (Level 66 computers are, indeed, to be manufactured at the Honeywell factory in Newhouse, Scotland.)

Honeywell says that the Series 60 will be suited to existing computer users and newcomers alike. Prices for Level 66 systems start at £450,000—of £10,000 a month on a rental basis—but Honeywell do have relatively small companies and organisations in mind for the less sophisticated Level 61 which costs £22,000+£400+a month). Honeywell is using the phrase 'attack machines' to describe the Level 61, for it sees many potential customers buying this as their first computer, possibly to do such mundane jobs as stock control.

Cash problems for public interest law

Colin Norman, Washington

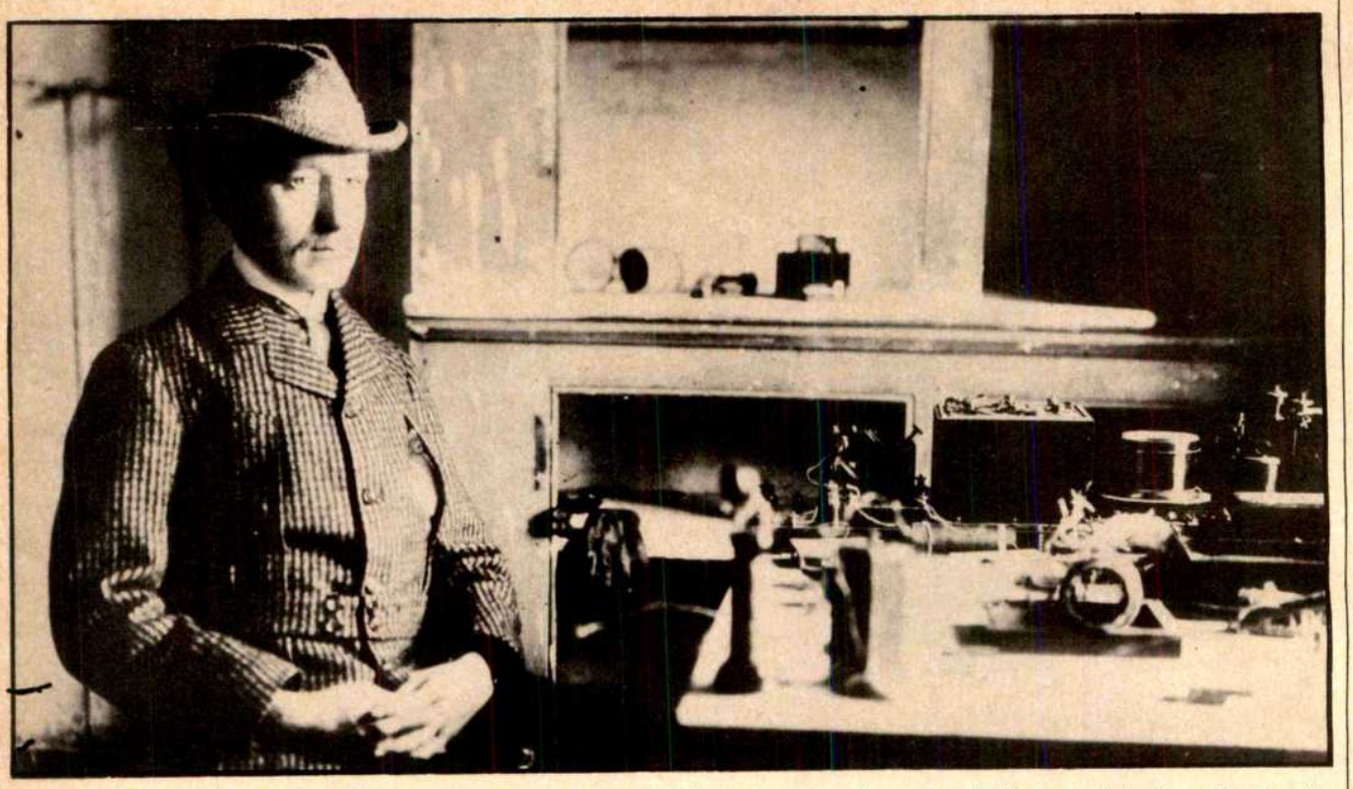
SINCE they first rose to prominence in the late 1960s, public interest law firms have made an impressive contribution to the development of government policies in the United States, ranging from the regulation of food additives to the protection of the environment. Staffed by a handful of lawyers and supported chiefly by philanthropic organisations, they have provided the cutting edge of the environmentalist movement and in many cases have formed an effective counterforce to the big business lobby which traditionally dominates the Washington political scene.

But they are now facing the prospect that one of their largest single sources of funding, the Ford Foundation, may withdraw its support in the next couple of years and put its money elsewhere. Ford has been pumping millions of dollars into public interest law since the 1960s, and since it traditionally provides seed money for the establishment of projects, rather than financial support over long periods of time its withdrawal from the field has long been anticipated.

Some idea of the influence of the foundation on the field of public interest law can be gauged from the fact that it provides between 85 and 90% of the budget of the Center for Law and Social Policy, the law firm which was in the forefront of the legal battle against the trans-Alaska pipeline and which have been effective in setting standards for treatment of the mentally retarded. The foundation also contributes some 40% of the operating funds of the Natural Resources Defense Council, which has won several landmark court victories against the Environmental Protection Agency and which argued the law suit which eventually forced the Atomic Energy Commission to publish an environmental impact statement on the liquid-metal fast-breeder reactor programme.

Faced with such the prospect that their funds may dry up, many of the law firms are exploring possible new financial arrangements which will put their activities on a sounder footing and provide them with a more permanent source of funds.

Two recent events have brought the matter to a head. The first is that last week the Ford Foundation announced that it will provide four of the largest firms with $2.3 million to finance their activities at least until the end of 1975 but it accompanied the announcement with the statement that Ford's role after the grants expire "cannot be determined now". And the second is that the Ford Foundation itself convened a conference in San Diego in



Marconi at Signal Hill, Newfoundland, in 1901 with instruments used to receive the first trans-Atlantic wireless signal.

THE Science Museum, London, is commemorating the centenary of Marconi's birth with an exhibition which opened on April 25. This is more likely to appeal to someone with a keen interest in wireless or in the history of science than to the casual visitor; the most interesting exhibits are letters and other literary material which are clearly presented and easy to read, but require time to digest.

Other exhibits emphasise the social rather than the scientific significance of wireless, with pieces of original equipment (including the kite used to fly the aerial from which the historic transatlantic broadcast of December 12, 1901, was made) and personalia including Marconi's swordstick.

One of the curiosities on show is a copper earthing tube dug up in the 1960s from the garden of the bungalow where Marconi lodged in 1896–97; the tube was in the ground outside Marconi's old room and was probably used by him in his earliest experiments in Britain.

But the written and printed matter is by far the most interesting. A cutting from the *New York Times* of February 13, 1927, reports Marconi's views about the prospects for wireless transmission of power, "radio movies" and television. He was rather optimistic about the latter, and the reporter looks forward to the day when "Secretary Kellogg (sits) in his office in the State Department and converses face to face with Premier Baldwin in Downing Street". In other ways, Marconi was equally visionary, foreseeing the replacement of oil as a fuel source by solar energy, but dismissing the idea of tapping geothermal power because heat from the Sun is both a simpler and cheaper source.

By 1932 (according to the *Daily Mail* of December 6) Marconi's vision had expanded still further. Musing that messages could be sent on further than around the Earth, and prompted by the interviewer, he said: "Interplanetary communication—who can tell? There is a great deal more to be found out and made perfect."

John Gribbin

mid-April to discuss possible new arrangements for financing the firms.

Although the foundation has made it clear that the firms should be thinking seriously about how they should live without Ford money, it is equally clear that if it came to the crunch, Ford would be unlikely to pull the rug from underneath public interest law firms and allow them to expire or cut back their operations drastically. Thus the emphasis will be on finding new sources and methods of finance.

Two possible arrangements were suggested in the San Diego meeting and, although no concrete decisions were taken, at least two possibilities were given careful consideration and hold great promise. One idea is to set up a permanent fund, financed by private donations, philanthropic organisations and perhaps even by professional legal organisations. Such a fund would probably operate much like a foundation, but the way in which the money would be distributed has not yet been worked out.

Another possibility is that courts will award legal fees to public interest law groups which successfully sue the government and industry. But the problem there is that the acceptance of such fees could well endanger the firms' legal status as tax-exempt organisations, and until the Internal Revenue Service issues some guidelines on the matter the firms are precluded from taking such fees. Ironically, some major pieces of environmental legislation, such as the Clean Air Act, and the Federal Water Pollution Control Act contain specific provisions which direct courts to award attorneys' fees to organisations which successfully bring court cases against the government, but until the IRS gets round to issuing its guidelines, those fees cannot be awarded to public interest groups.

Thus the next couple of years could be critical for the future of an extremely important element in governmental decision making in areas which have a profound effect on scientific matters.

Quake prediction no bonanza

David Davies

In the late 1960s travellers' tales suggested that Russians were having some success in detecting premonitory symptoms to earthquakes. Physical properties of rocks in Central Asia were said to change prior to quakes, although Western geophysicists, deeply immersed in plate tectonics and lacking any sight of the Russian data, were not moved to do much. Then in 1972 American interest suddenly grew explosively. By early 1973 precursors to earthquakes had been seen in many parts of the world, a dilatancy theory had been proposed to explain the observations and a simple relationship between the duration of the precursor and the size of the earthquake had been suggested. The annual American Geophysical Union meeting in April 1973 was a heady experience. This year's meeting was sober by comparison, and there was much concern about funding.

The situation is a classical one of what one could call the four black spots of present day science policy:

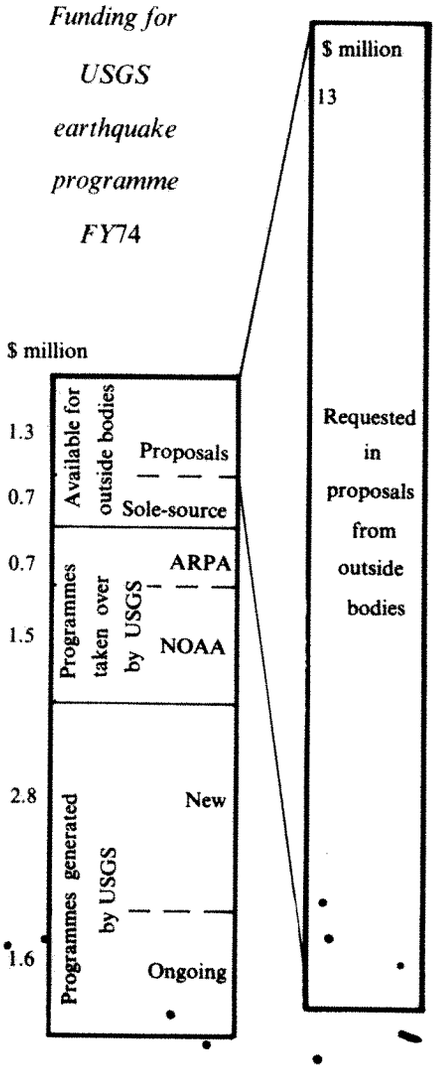
- Too many people chasing too little money.
- Government, industry and universities working in the same field.
- The political need for a mission-oriented approach in conflict with scientists' desire for in-depth understanding.
- The problem that the contractural system poses for the university scientist.

On the scientific side, it had been a relatively quiet year. There had been two instances, one in New York State, the other in Riverside, California, where events had been foretold by means of variations in seismic wave travel times before the earthquake. The same technique was also showing some promise in other places, although there seemed a growing feeling that the quality of data was often inadequate and that the near-focal conditions are vastly more complex than a first modelling would suggest. On the other hand there is promise both in gravimetric and tilt observations and some of the laboratory and field experiments (including earthquake control by fluid injection) have gone well.

Responsibility for the American 'earthquake hazard reduction programme' is vested in the US Geological Survey (USGS). Much more than prediction is covered—risk analysis, mapping and rock mechanics are other fields of activity. In the fiscal year July 1971-June 1972 (FY 1972) the programme amounted to $1.6 million, but the impact of the San Fernando earthquake put up the allocation to $4 million by FY73. In moving into FY74, the first year in which prediction could be discussed seriously, the USGS found itself picking up responsibility for earthquake work previously done by the National Oceanographic and Atmospheric Administration (NOAA) and fluid injection studies of the Advanced Research Projects Agency (ARPA). Thus although the budget for FY74 was $8.6 million, by no means all the increase was in the form of new money for research. The figure shows the distribution. Apart from sole-source contracts—for instance to support seismic networks operating at present, universities, state governments and industry had $1.3 million to fight over. No increase is seen in this sum for FY75. "We need a big earthquake to shake more money out of the government" as one official put it.

The figure of $1.3 million took many by surprise, as it was generally expected that in the mid-1970s the USGS would dispense the largesse that in the past had come from ONR, NASA, NSF and ARPA and which had made for so many thriving geophysical laboratories. External funding applications for FY74 amounted to $13 million from two hundred proposals—the USGS is able to support 32 of them. On one official's reckoning it could have intelligently used $8 million this fiscal year. Expertise is going unused, he thinks. As it is, some seismic stations in California have already closed for lack of funding. Other agencies no longer have a charter to do research in this field, so failure with the USGS really does mean failure to fund. The prediction programme is a very lean operation in which immediate relevance is necessary of every project. "A hard-nosed mission-oriented approach" as one project officer described it—himself with a modest fame as having already engendered such an approach in three other government agencies.

Funding for USGS earthquake programme FY74



It is inevitable that there is conflict in such an underfunded situation. More generalised and global research suffers at the hands of the more particular and American. There is a suspicion that the USGS will swallow up all the good ideas that come from outside (university scientists pointedly remark that the first good papers in the subject came from MIT, Stanford, Columbia and Caltech, not the big government laboratories which now have most of the available money). And, most immediate, people in universities suddenly find themselves up against government contracts officers, zealous to ensure scrupulous accounting. It is this more than anything else which seems to have persuaded rational men that they are being persecuted. The staff of the USGS are aware of these undercurrents of bitterness and are trying to establish better headquarters procedures.

There are some very obvious defects at the moment and action needs to be taken at governmental level (university scientists are also going to have to learn to live with the system a bit more). The lack of funds is quite ludicrous. The United States loses on average $630 million annually through earthquake damage—a figure which will doubtless go up when California next suffers a serious quake. To keep talent out through lack of one-hundredth of that sum is a false economy. Worse, it is worrying that the programme is required to be so nationally oriented (the USGS is, of course, part of the Department of the Interior). Most countries look to their own scientists to solve their own problems, but the Western world has increasingly looked to the United States to grapple with global problems, at risk of being branded imperialists. If ever there were a problem knowing no frontiers this is it, and success would be that much more likely if more American geophysicists could be financially encouraged to think and collect data in a more global framework.

correspondence

Russians at conferences

SIR,—"Have the Russians arrived?" International conference-goers will know how the question passes from one to another as they assemble at the start of the meeting. The uncertainty of whether the Soviet delegation will appear or not (and if they appear, on which day?) has long provided a touch of drama for the other participants and a headache for the organisers. The first clue for the organisers is often a message from the travel agency acting for Intourist that they are asked to book hotel accommodation for so many Soviet delegates. The names may not be known, only the total number, and the scientific contact with them will commonly not be direct but through some official Soviet committee or authority.

I have recently returned from an international conference abroad where this scenario was duly re-enacted, but this time I experienced it directly as I had to preside over the meeting. Writing now in my private capacity, not as president, I have to report sadly that nothing has changed; it is as bad as ever.

We had asked a prominent Soviet scientist, let us call him Professor Y, to give one of the specially invited review papers and our invitation was accepted. As the time drew near the secretary of the meeting wrote to him several times to ask about his paper, but with no reply. Telegrams were tried, and then many telephone calls to Moscow. Professor Y was unknown to them, they said; or, Professor Y had gone away. Faced with this concerted silence, we began to fear for our colleague. Finally, four days before the opening of the conference we received a telegram from Professor Y that his paper would be presented on arrival. (All the other papers for the conference had already been reproduced for distribution.) Our hopes rose, but we remembered the old Russian proverb "Do not sell the bearskin until you have shot the bear." The Soviet delegation, expected to be twelve, turned out to number seventeen, including the usual chaperone from Intourist, and Professor Y was not among them. They bore with them copies of his paper for distribution and a verbal message from him asking me to present it. I did so, believing that my colleague was in no way to blame for these bizarre events and wishing to help him in his difficulties as much as I could (the rules of the conference just allowed it). It is worth adding that some or all of the five extras in the Soviet party did not have enough foreign currency to pay the fee and so were unable to be registered for the conference.

The Soviet authorities who are responsible for these things should realise that this sort of behaviour on their part is harmful to Soviet science. Organisers of conferences already think twice before inviting papers from Soviet authors, knowing the uncertainties that will result. We should like to include them, on equal terms with the others, but how can we go on doing so in face of this continued official discourtesy to the scientific community?

Yours faithfully,

J. D. NYE

H. H. Wills Physics Laboratory, University of Bristol, UK

Great crested grebes



The Zoological Society of London

Darwin's omission

SIR,—I wish to draw the attention of historians of science to the omission by Darwin (in his *Origin of Species* and even in his *Selection in Relation to Sex*) of any mention of cases where the two sexes are similar in plumage. Sexual selection for Darwin involved the choice by females of brighter, and often larger, males. Darwin presumably did not have the opportunity to study sexually similar species: once he returned to Down House he was not able to observe freshwater or marine birds.

The same omission occurs in the books on evolution by Ernst Mayr, in Barnard Rensch's *Evolution above the Species Level* and, strangely enough, in the early edition of my own *Evolution, the Modern Synthesis*. I say strangely, for in the years 1914 to 1917, I myself had described the elaborate mutual display of grebes, herons and egrets; and later returned to the display of swans, albatrosses, and penguins, which had been studied in detail by others. The first account of mutual display was given by E. Selous in 1901–02, and by myself in 1914; in both cases, of great crested grebes (*Podiceps cristatus, Proc. zool. Soc. Lond.*, 1914). My failure to mention mutual display in my book seems to have been due to my not realising its frequency or its biological value in strengthening the emotional bond between members of a pair. This point emerges clearly from the work of Lorenz and Tinbergen.

I wish to stress the strikingly different results of unilateral and mutual display, in regard both to display characters and to behaviour. A regular feature of mutual display is that the returning bird presents the sitting mate with nest material (in grebes a similar presentation occurs on open water). Another feature of mutual courtship is the extent of ritualisation; but this also applies to many male unilateral displays where the sexes have different plumage, as in bustards, pheasants, some grouse, and so on. Today the importance of ritualisation has been confirmed: see the Royal Society's symposium on Animals and Man (*Phil. Trans. R. Soc.*, 1966).

The red breast in both sexes of the robin (*Erithacus rubecula*) is not connected with courtship: Lack has shown that the colour is a mark of territorial ownership, for both sexes have winter territories. In dimorphic species the songs of males, and of 'duetting' birds such as some African Shrike, are also proclamations of territory.

Yours faithfully,

JULIAN HUXLEY

31 Pond Street, London NW3, UK

Rat race

SIR,—a recent advertisement emphasises the present crisis in biology laboratories, where research is severly hampered by the shortage of experimental animals. "A position of Wissenschaftlicher Rat is vacant" in Freiburg (*Nature*, March 1, xx; 1974); applicants should have experience of cell-mediated immune reactions.

Yours faithfully,

B. JOVE

Hove, UK

news and views

Transferring foreign genes to plants

It is well established that purified DNA can be taken up by bacteria and that the recipient cells are capable of incorporating DNA into their chromosomes and expressing the genes thus acquired. Attempts to perform similar experiments with eukaryotes, however, are often viewed with scepticism although an increasing number of workers are investigating this problem.

In their simplest form these experiments involve the use of two closely related organisms which differ with respect to an easily identifiable phenotypic characteristic. Attempts are then made to transfer this character to individuals that lack it by applying purified DNA from the appropriate donor. The experiments of Hess involving white and red flowered plants of *Petunia* fall into this category (Hess, *Z. Pflanzenphysiol.*, **60,** 348-358; 1969; **61,** 286-298; 1969; **63,** 461-467; 1970; **68,** 432-440; 1973). In some instances the test organism has a deficiency in an essential metabolic pathway and corrected individuals are selected by their ability to grow without the appropriate nutritional supplement. In many experiments the donor DNA is derived from organisms totally unrelated to the recipient and evidence for uptake and possible incorporation into host chromosomes has been obtained. Occasionally bacteriophages have been used as an enriched source of specific genes and there is evidence for the synthesis of enzymes coded for by the phage DNA (Carlson, *Proc. natn. Acad. Sci. U.S.A.*, **70,** 598-602; 1973).

To establish that true transformation is possible in eukaryotes, it is necessary to study the fate of applied DNA by biochemical techniques, to show that changes in the recipient organism are directed by the donor DNA and to demonstrate that these changes are inherited. Ledoux and his colleagues have been carrying out some of these experiments for more than a decade and have obtained some remarkable results (Ledoux and Huart, *Eur. J. Biochem.*, **23,** 96-108; 1971). In this issue they present data which suggest that thiamine deficiency in *Arabidopsis thaliana* may be corrected by bacterial DNA and furthermore that this correction can be inherited.

Arabidopsis is particularly suitable for such experiments. It is comparatively small and easy to manipulate and grow aseptically and it flowers within a few weeks of germination. Ledoux and his team used a number of lines with lesions in the branched pathway for thiamine biosynthesis. These plants normally require either exogenous thiamine, pyrimidine or thiazole for continued growth and reproduction but they found that a small number of the plants grew and produced seed in the absence of supplements after they had been treated with the appropriate bacterial DNA.

Ledoux states that DNA with a molecular weight in excess of 10^7 is necessary and the DNA solution should be applied to the ungerminated seeds during imbibition. DNA-corrected plants grow more slowly than supplemented mutants and only about one third of those that grow actually produce seed. The observed frequency of correction can be as high as 10^{-2} compared with a spontaneous reversion rate of the *Py* locus of less than $\cdot 5\times10^{-6}$, and $4\times^{-5}$ for reversion induced by ethyl methane sulphonate. DNA from phage T7 and 2C is ineffective and DNA from *Escherichia coli* unable to produce the thiazole moeity of thiamine does not correct the corresponding *Arabidopsis* mutant although it is effective with plants deficient in the pyrimidine pathway.

Seeds from corrected plants were germinated and selfed to produce F_1 to F_3 progeny. Although these plants showed some variegation in chlorophyll content no typical thiamine-deficient segregants were observed. This suggests that the parental lines are homozygous which is a rather surprising result although it should be noted that Hess observed a similar situation with *Petunia*. Ledoux suggests that in the case of *Arabidopsis*, during meiosis and afterwards, the gametes containing the information for thiamine production are favoured over mutant gametes. In support of this proposal he cites the finding that if during DNA correction plants showing signs of abortive flowering are supplemented with thiamine then lethal mutants appear in the F_2. Although this result would also be expected in the absence of applied DNA, in this case no corrected plants would be anticipated. In crosses between progeny of DNA-corrected plants and either wild type or mutant individuals, lethal mutants and a variety of leaky mutants are obtained. There is no evidence for cytoplasmic inheritance of the corrected character.

Obviously the mechanism involved in these DNA-mediated corrections is of major interest, but it would be premature to attempt a deailed interpretation. From the figures quoted by Ledoux the frequency with which the corrections are obtained is too high to be explained by spontaneous reversion of the mutation. On the other hand direct evidence for the synthesis of a bacterial enzyme in these plants is not available at present. Other experiments by the Mol group suggest that foreign DNA can become integrated in some way into plant DNA although the significance of these findings has been questioned (Hotta and Stern, in *Informative Molecules in Biological Systems* (edit. by Ledoux) 176-178, North Holland; 1972). One thing is certain; experiments of this nature hold out intriguing possibilities and they will continue to generate interest and occasionally heated discussion.

From our Plant Cell Physiology Correspondent

Number of cells at the time of X activation

ALTHOUGH female mammals possess two X chromosomes, in any particular adult cell only one of these homologues is active, providing a dosage compensation which ensures that females and males both display the same number of active-X-linked genes. The fertilised egg and very early embryo of eutherian mammals, including man and mouse, possess two active X chromosomes; that one of these two chromosomes is randomly inactivated in each cell at an early stage of genetic development was first suggested by Lyon (*Nature*, **190,** 372; 1961).

The random nature of the process is suggested by the expression of sex linked genes in adult females. In heterozygotes in which one X chromosome carries a wild type and the other carries a mutant allele, those cells in which the wild type X chromosome is active must show wild phenotype whereas cells instead possessing the active mutant X chromosome display the mutant phenotype. One visible

consequence of this random inactivation is the variegation seen in the coats of female mice heterozygous for X-linked coat colour genes. The equal representation of wild and mutant phenotypes, and the presence of patches of each coat colour, suggest that at the time of decision in the early embryo, in each cell one X chromosome (chosen at random) remains active whereas any others are inactivated (in animals possessing several X chromosomes only one stays active). In the subsequent divisions of genetic development, the state of each homologue is faithfully reproduced; the adult cells descended from any particular embryonic cell after the time of decision therefore constitute a clone characterised by the presence of the same active X chromosome (with the exception of the germ cells in which both X chromosomes remain active).

Two critical questions to ask about genetic development in eutherians are therefore: when does X activation take place; and what is the basis for its random nature? The closest estimate of the time of decision derives from the work of Gardner and Lyon (*Nature*, **231**, 283; 1971) with chimaeric mice. Instead of the usual complete fusion between two embryos, they injected a cell taken from a blastocyst into another blastocyst. Because the donor and recipient carried different gene markers for coat colour, any cells in the adult coat descended from the injected embryonic cell can be distinguished. Since donor sex-linked markers showed variegation in the heterozygous condition, X activation must take place after the blastocyst stage (otherwise the donor cell would have suffered previous X activation and its descendents would all be of one phenotype in the adult).

By taking advantage of a mutant which influences the random nature of X activation, Ohno and his colleagues (*Cell*, **1**, 175; 1974) have recently developed an ingenious approach to estimate the number of cells in the embryonic mouse at the time of decision. Their experiments rely on the concept that the clonal composition of embryos immediately after X activation can be expressed as the binomial $(p+q)^n$, where n is the number of cells and p and q are the probabilities of activation of each chromosome, both equal to 0.5 in the wild type animal. In other words, given a population of animals it is possible to predict the frequencies with which any particular clonal composition will be generated. Unfortunately, this formula cannot be directly extrapolated to the adult animal, because distortions in the initial clonal composition must inevitably take place as the embryo grows; for only some, and not necessarily a random selection, of the embryonic cells will be utilised during formation of each adult clone. The formula which determines the clonal composition of an adult phenotype must therefore be written as $(p'+q')^{n'}$, where n' is the number of progenitor cells from which the adult clone is descended, and p' and q' have values corresponding to the relative frequencies of each active X chromosome in the set of progenitor cells—and this, of course, in turn depends upon the clonal composition of the embryo from which the progenitor cells are descended.

Because these changes in cell composition make it impossible to determine n directly from the adult phenotypes, Ohno *et al.* have used a special situation to allow this analysis. In one particular condition only, changes in cell composition cannot occur during development; this is the case when all the copies of one X chromosome (either the maternal or paternal) were by chance activated. This generates a monoclonal embryo, which can in turn give rise only to monoclonal adult. But these two extreme cases each occur with a very low frequency (0.5^n); as Ohno *et al.* point out, this creates the paradox that, even given a value for n as low as 20, such fractions would occur with frequencies too low determine by experiment in any researcher's lifetime (of the order of one in a million mice). To achieve a more manageable situation, they therefore utilised a mutation of the X chromosome (0^{hv}), which they have previously characterised, and which causes preferential activation of the X chromosome carrying it in heterozygotes where the other chromosome is wild type (*Nature new Biol.*, **245**, 92; 1973; *Cell*, **1**, 3; 1974). The 0^{hv} mutation seems to be an extreme version of alleles at the Cattanach's "controlling element" locus which maps in the middle of the mouse X-linkage group; and earlier analyses of chromosomes carrying this allele have suggested that the mechanism of decision is for one X chromosome to be activated whereas any others in the cell are inactivated (reviewed by Brown and Chandra, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 195; 1973).

When the 0^{hv} X chromosome carries the mutant gene *Blo* and the other X chromosome carries the corresponding wild type allele, regions of the coat of the mice are light coloured if derived from cells in which the 0^{hv} *Blo* X chromosome was activated, and are dark if derived from cells in which the wild type X chromosome was activated. Because of the preferential activation of the 0^{hv} X chromosome, the frequency of occurrence of mice with coats largely of *Blo* phenotype is appreciable. To obtain values for the occurence of two, rather than just one, of the possible clonal compositions, in addition to assessing the proportion of mice with coats derived entirely from *Blo* cells, Ohno *et al.* also determine the frequency with which mice displayed coats almost entirely of *Blo* phenotype, but with just one dark spot (corresponding to one coat progenitor cell in which the wild

BL Lac identified with giant galaxy

THE peculiar object BL Lacertae seems to lie at the centre of a normal giant elliptical galaxy. This identification provides an estimate of the distance to BL Lac, and this in turn suggests that the central source is similar in spectral index and luminosity to QSOs such as 3C48, 3C279 and 3C345, provided those objects are at the distances indicated by a cosmological interpretation of their redshifts.

Those are the principal conclusions reached by Oke and Gunn as a result of a four year study of BL Lac (*Astrophys. J. Lett.*, **189**, L5; 1974). The observations were made with the multichannel spectrometer attached to the 200-inch telescope; some of the observations were made using a disk with diameter 5″ and a surrounding ring with inner diameter 10″ to study the extended regions of the source while obscuring light from the bright central feature, and these showed Hβ in absorption at a redshift of $z = 0.07$.

Other data support this redshift estimate, and show that the galaxy is one of the most luminous known, with a magnitude of about −23. Together with the growing evidence of similarities between BL Lac and QSOs this identification is one of the key advances of recent years. Far from being a blueshifted QSO, as had seemed possible, BL Lac can now be fitted into the cosmological picture of receding QSOs quite satisfactorily. The distance to the source is some 350 Mpc, and this raises the same questions about its energy generation mechanisms that apply for very remote QSOs. The energy requirements are extreme, but as Oke and Gunn say "It is interesting, however, that the same phenomena at approximately the same level of outrageousness exist for BL Lac as for higher-redshift QSOs if they are placed at the distances suggested by their redshifts". J. G.

type X chromosome was activated).

These frequencies should have been determined by the clonal compositions of the cells which provide the immediate progenitors for the coat, that is they depend on the formula $(p'+q')^{n'}$, and so provide experimental values for the frequencies of the fractions $(p')^{n'}$ and $(p')^{n'-1}.q'$. To relate these values to the initial clonal compositions at the time of X activation (that is to p, q and n) requires knowledge of n'. Given a value for n', it is then possible to test various combinations of p, q and n to see which values would generate cell populations from which animals monoclonal for the coat would be produced at the observed frequency. This involves summing several possible situations, since, for example, a monoclonal coat may arise not only whenever the original embryo was monoclonal but also whenever the progenitor cells of the coat by chance were descended only from cells with an active 0^{hv} X chromosome; when the number of immediate progenitor cells is sufficiently large, the entirely mutant adult phenotype should occur only in individuals which started their development with very one sided clonal compositions.

The *Blo* mutation is therefore particularly well suited to such analysis, for the development of the cells in which it is expressed has previously been studied in some detail. *Blo* acts in mesodermal cells of somite origin, thought to result from the reproduction of 35 pairs of somite clones distributed along both sides of the vertebral column (Lyon, *Phil. Trans. Roy. Soc.*, **B259,** 41; 1970), suggesting a value for n' of 70. Animals with coats entirely of the *Blo* phenotype are too rare to measure in appreciable frequency, so Ohno *et al.* measured the frequency of occurrence of animals with a coat one half of which (corresponding to one side of the vertebral column) shows the *Blo* phenotype. This frequency, 41 in 2400 or 1.7×10^{-2}, provides an estimate of the frequency of the class p'^{35}; the square of this frequency, 2.9×10^{-4}, can thus be taken as an estimate for p'^{70}.

This total frequency might be produced by several combinations of p, q, n. To decide upon the most likely, Ohno *et al.* utilised their estimate of the frequency of occurrence of the class $p'^{69}q'$, that is the proportion of animals with only one wild type clone apparent in the coat; this was 3 in 2,400 or 1.25×10^{-3}. Although the accuracy of this estimate is not great, because of the small number of animals involved, this value can usefully be compared with the estimate for p'^{70}. Taking both values into account, Ohno *et al.* were able to show that a close fit to the data would be produced by $p=0.8$, $n=60$. In other words, the 0^{hv} allele confers an 80% probability that its chromosome will be activated; and the number of cells in the embryo at the time of decision may be close to 60.

How accurate is this estimate and what further tests may be made of it? One assumption upon which these experiments rely is, of course, that all 35 pairs of somite cells arise independently from some undifferentiated pool and not from a smaller pool of cells which then multiply upto 70. It would be useful if this point could be confirmed in future. Given the comparatively small numbers of animals which can be examined under the experimental conditions imposed by this system, the measured frequencies must clearly be taken as approximations and not precise values; but the value for n of 60 cells is consistent with the previous suggestion that the time of decision is soon after the blastocyst stage. It is therefore likely that 0^{hv} influences only the decision on activation and does not change the time at which it is made. Increased accuracy for these estimates would obviously be lent by determining clonal compositions and thus values for p, q and n from some other adult phenotype. But these qualifications do not detract from the importance of this seminal study, whose sophisticated manipulation of the mouse system has provided a useful estimate for the cell number in a mammal when the decision is taken on X activation.

B.L.

More about Mercury

by our Cosmology Correspondent

MARINER 10 has done more than just send back some impressive pictures of Mercury, looking so very much like the Moon that one radio astronomer remarked recently that for all he knew they were in fact previously unreleased Lunar Orbiter photographs. The six non-imaging experiments on board the spacecraft have provided a new insight into the nature of the closest planet to the Sun, and this shows clearly how Mercury fits into the overall picture of the Solar System.

The photographs have, of course, contributed to this. The front cover illustration this week shows a region very similar to the terrain of the Moon, with a large, flat bottomed crater about 100 km in diameter (about the same size as the lunar crater Copernicus), linear grooves probably produced by crater ejecta, and so on. Together with pictures of Mars obtained by the Mariner series of spacecraft and by Soviet spacecraft, the latest NASA achievement emphasises the broad similarities between the inner planets of the Solar System. It now seems almost beyond doubt that Venus too will be found to be cratered and scarred by impacts and volcanic activity, although the evidence there may have been largely eroded away.

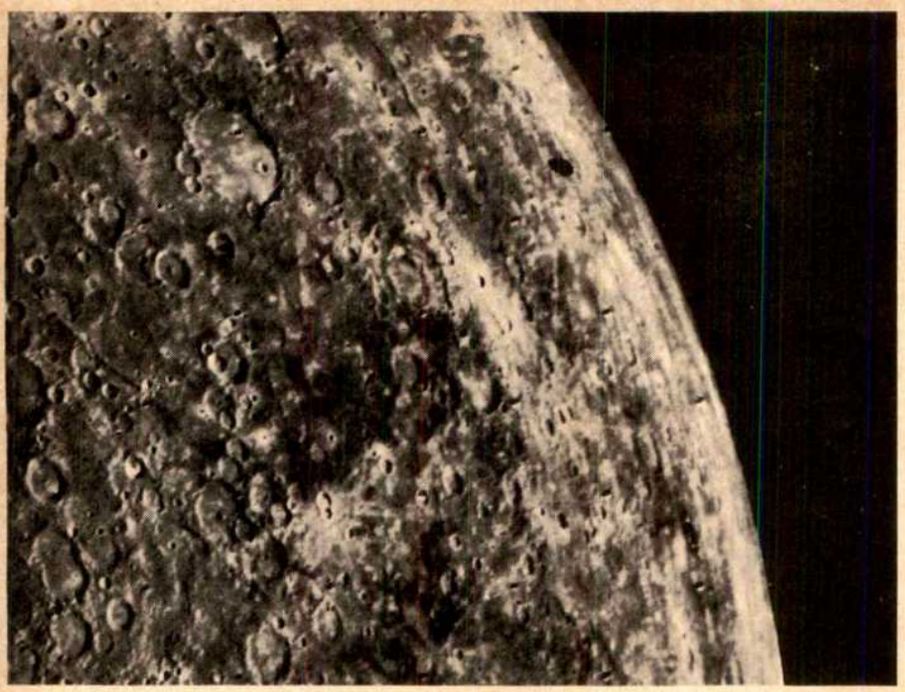
If the overwhelming impression of the visual evidence is that the inner Solar System forms a family of related planets, the non-visual data confirm this and indicate the way in which the distance of each planet from the Sun determines its individual characteristics within the family. Mercury turns out to be rather more massive than was expected, with a density of 8 g cm^{-3} implying that as much as two-thirds of it is composed of iron or iron group elements. The presence of a clearcut gradient in the densities of the planets in sequence outwards from the Sun obviously ties in neatly with the idea that more volatile elements condensed further out in the collapsing proto Solar System. And a large amount of iron could also explain the unexpectedly large magnetic field of Mercury. At some 200 to 300 gammas for the extrapolated strength of the field at the planet's surface, this is about 1% of the Earth's field; the favoured view at present is that this is a permanent field, and is not induced by a dynamo effect.

Among the other surprises already arousing interest is the discovery of a tenuous atmosphere around Mercury. This discovery will delight amateur astronomers, since reports that such an

Littorinid group

LITTORINIDS are present on the rock shores of all continents and present some intriguing taxonomic problems. The first symposium restricted to this family of the prosobranch Gastropoda was held at the Royal Scottish Museum, Edinburgh on March 19 under the chairmanship of Sir Maurice Yonge. Of the talks on taxonomic trends in the Littorinidae, perhaps the most noteworthy contribution was the evidence of J. Heller (University of Liverpool) that the polymorphic species *Littorina saxatilis* (Olivi) should be regarded as containing at least four separate species.

The meeting unanimously endorsed the suggestion that an informal littorinid group should be formed, with an international membership, as far as possible comprising all workers actively interested in these gastropods. Anybody wishing to learn more about the group should write to the appointed coordinator C. Pettit, Manchester Museum, The University, Manchester M13 9PL, England.



Southwestern quadrant of Mercury, viewed from Mariner 10 4 h before closest approach. Distance of spacecraft from planet was 198,000 km; the largest craters visible are some 100 km across. (NASA photograph.)

atmosphere could be seen have been made by members of that fraternity from time to time, but met with a cool reception from the professionals. Wise after the event, those professionals now say that such a tenuous atmosphere can be maintained, in spite of the low escape velocity from the planet, because of particles released through radioactive decay. Uranium releases helium (alpha particles), and potassium yields argon; another effect, capture from the solar wind, probably accounts for the presence of neon.

Clearly it will take a while for the new information to be digested and incorporated into theoretical models, and in September there is the prospect of more information as Mariner 10 passes by Mercury again. In the meantime, it is interesting to reflect on a comment attributed to Bruce Murray, leader of the Mariner 10 TV experiment team (*Aviat. Week Space Technol.*, **100** (14), 16; 1974), which indicates the rapid development of spacecraft systems and also answers the 'doubts' of that radio astronomer. The Mariner 10 pictures cannot be directly compared with Lunar Orbiter pictures, it seems, "because the Mariner 10 photos show more detail".

Models of growth and competition

from our Animal Ecology Correspondent

THE dynamics of competitive interaction between two species have long been the target of approximations. The formula

$$\frac{dN_i}{dt} = r_i N_i\,(1 - N_i/K_i - \alpha_{ij} N_j\,K_i)$$

(where N_i is the density of species i; r_i is the exponential growth rate of this species when its population density and that of its competitor j are low; K_i is the carrying capacity of the environment for species i when its competitor j is absent; α_{ij} is the linear reduction (of K_i) of species i's growth rate caused by its competitor j) has stood for more than 50 years since being proposed by Lotka (*Elements of Physical Biology*, Williams and Wilkins, Baltimore, 1924) and Volterra (*Leçons sur la Theorie Mathematique de la Lutte pour la Vie*, Gauthier-Villars, Paris, 1931). The model was validated in 1934 by Gause (*The Struggle for Existence*, Williams and Wilkins, Baltimore) who studied the dynamics of yeast and protozoan competition. The linearity of the model suited this kind of interaction. For arthropod interactions the model is less perfect. Gilpin and Ayala (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 3590; 1973) have recently put the model to the test using arthropod species and have come up with some important modifications.

Their competing species were *Drosophila willistoni* and *D. pseudoobscura*. Adult flies, in known densities, were put in culture vessels containing known amounts of food. After 1 week the surviving adults were counted, as were their offspring which emerged from the culture medium during the following 4 weeks. Zero isoclines, which depict the separation point between positive and negative population growth, were constructed from continuous populations, maintained by serial transfer. The single species equilibrium, or environmental carrying capacity, is the maximum population the culture medium can stand. As a competitor increases in abundance the zero isocline falls away from the maximum point. Where it intersects the zero isocline for the competitor species is the equilibrium point for the interaction of the two species.

The data from the *Drosophila* cultures were fitted to a large number of competition models chosen according to the criteria of simplicity, reality, generality and accuracy. The model that stood up best was

$$\frac{dN_i}{dt} = r_i\,N_i\,(1 - N_i/K_i)^{\theta i} - \alpha_{ij}\,N_j\,K_i)$$

The interpretation of the parameters is straightforward. r_i is the exponential rate of growth of species i when its density and that of species j is low; K_i is the carrying capacity of the environment for species i in the absence of its competitor j; α_{ij} is the reduction of growth rate of i as a result of the activities of j, and $\theta\,i$ denotes the asymmetry of the single species growth rate of species i. In the absence of competition, that is when $\alpha=0$, the growth rate conforms to the logistic. In other words the relationship between growth rate and density is parabolic. The point of maximum growth rate is $K/2$. The experimental data pointed out this restriction. The addition of θ removes it and allows the point of maximum growth rate to be less than, or more than, $K/2$.

This is an important modification to the Lotka-Volterra equation. Vertebrate populations in which growth rate tends to be controlled abruptly by the imposition of territoriality and other social factors cannot adequately conform to the continuously linear fashion demanded by the old model (when $\theta=1$). For them θ will tend to be larger than 1. Invertebrate populations, in which eggs, larvae and pupae are ignored, will tend to have a θ value less than 1.

Gilpin and Ayala propose this new model to encompass intraspecific and interspecific competition. Population ecologists shall have to wait before assessing its general applicability.

Biogenesis of chloroplasts and mitochondria

from a Correspondent

RECENT work on the biogenesis of chloroplasts and mitochondria was discussed at a symposium of the British Society for Cell Biology at the University of Warwick on March 28-29. Both

types of organelle are potentially autonomous, since they each contain DNA, DNA polymerase, RNA polymerase, and a protein-synthesising system, but all the evidence suggests that most organelle proteins are encoded in the nucleus and are synthesised by cytoplasmic ribosomes. The major problems discussed were the identification of the genes in the organelle DNA and of the proteins synthesised by organelle ribosomes.

The interactions between nuclear and organelle genomes in the synthesis of organelle proteins are complex, but are yielding to modern techniques. Two general principles are emerging: (1) one of the functions of the organelle DNA is to encode some subunits of multisubunit organelle proteins, the remaining subunits being encoded in nuclear DNA; (2) those protein subunits encoded in organelle DNA are synthesised by organelle ribosomes, whereas protein subunits encoded in nuclear DNA are synthesised on cytoplasmic ribosomes. For example, D. E. Griffiths (University of Warwick) reviewed data on yeast mitochondrial ATP-synthetase, which is composed of ten subunits; six of these are nuclear coded and made on cytoplasmic ribosomes, whereas the other four are made by mitochondrial ribosomes and are presumed to be encoded in mitochondrial DNA. The major chloroplast protein (fraction I protein or ribulose diphosphate carboxylase) is composed of large and small subunits. R. J. Ellis (University of Warwick) described the synthesis of the large, but not the small subunit, by isolated intact chloroplasts and by free chloroplast ribosomes. Tryptic peptide analysis of mutant tobacco plants has shown that the large subunit is encoded in chloroplast DNA whereas the small subunit is encoded in nuclear DNA. The emphasis of work in this area is now shifting away from the identification of products of the different genomes towards a study of the regulatory mechanisms existing between them.

Elegant studies of mitochondrial DNA isolated from cytoplasmic *petite* mutants of yeast were reported by P. Slonimski (CNRS, Gif-sur-Yvette); this DNA is derived from wild type DNA by large deletions which are replaced by reiterations of the non-deleted segments. The resulting gene redundancy can be detected by electron microscopic studies of half-denatured molecules. Stable *petite* mutants in which different parts of the mitochondrial DNA are repeated and amplified up to 100 times can be isolated, thus opening the way for the selective purification of different mitochondrial genes. Such studies on chloroplast DNA are much less advanced because of the difficulty of inducing mutations in the DNA of this type of organelle; R. Hagemann (Martin Luther University, Halle) reported that some naturally-occurring chloroplast mutations in higher plants are correlated with the absence of specific thylakoid proteins associated with photosystems I and II, and other mutations result in a loss of chloroplast ribosomes.

All of the products of protein synthesis by mitochondrial ribosomes, and most of those by chloroplast ribosomes, are membrane-bound. This raises severe problems of purification and isolation, but these problems are being steadily overcome. In a *tour de force*, R. O. Poyton (University of Connecticut) reported the isolation in milligram quantities of each of the seven subunits of yeast cytochrome oxidase. Four of these are made by cytoplasmic ribosomes and are water soluble when isolated, but the other three are made by mitochondrial ribosomes and remain insoluble. The three insoluble subunits are synthesised by isolated mitochondria, provided that oxygen is present; in anaerobic conditions, only one subunit is made. The mechanism of this interesting effect is unknown, but could reflect a specific effect of oxygen on mitochondrial transcription or translation.

On the second day, further work in the area of organelle biogenesis was discussed. G. S. P. Groot (University of Amsterdam) suggested from an analysis of base sequence homology in nuclear and mitochondrial DNAs of different yeasts that mitochondrial DNA evolves faster than nuclear DNA, and L. A. Grivell (University of Amsterdam) reported that yeast mitochondrial ribosomal RNA is extraordinary in its complete lack of ribose methyl groups and a very low level of base methylation compared with other ribosomal RNAs. A contribution on chloroplast RNA by M. R. Hartley (University of Warwick) was notable, in that it reported, for the first time, the detection of a specific messenger RNA from a plant source (other than a viral messenger). This messenger is for the large subunit of fraction I protein which can be correctly translated by a cell-free protein synthesising system from *Escherichia coli*. Studies of specific messenger RNAs from plants have lagged far behind those from animals, so this advance should open up a new area of plant molecular research.

Outstanding problems that were raised, but not resolved, were the function of the genes in chloroplast DNA, 90% of which are still unidentified, and the mechanism of the uptake of specific proteins into and across the outer membranes of mitochondria and chloroplasts.

Neural attenuation in echolocating bats

from our Animal Behaviour Correspondent

THE sonar system possessed by many bats enables them to perform such feats as flying unhindered through an array of fine wires and selecting one insect from a swarm of many. The echoes from surrounding objects must be perceived a few milliseconds after the bats have emitted an extremely intense outgoing sound pulse (often several thousand dynes per cm^2). Somehow the bats' ears must be protected from their own cries and yet still be sensitive enough to pick up the much fainter returning echoes. There are at least two mechanisms for accomplishing this. The muscles of the middle ear contract synchronously with vocalisation (they can contract and relax again up to 60 times s^{-1} on some species) and this has the effect of attenuating self-stimulation.

In addition to this peripheral mechanism, there are neural mechanisms of attenuation within the auditory pathways. Suga and Schlegel (*Science*, **177**, 82; 1972) made tape recordings of the calls of vespertilionid bats and recorded the response of a bat's auditory nerve both to this tape recording and to the same call when made by the bat itself. Although the response of the primary auditory nerves was virtually the same to both sorts of sound, the inferior colliculus (part of the mid-brain concerned with hearing) showed much less response to the live than to the tape-recorded sound. Attenuation, by some 25 dB, to the bat's own voice was occurring somewhere between the auditory nerve and this part of the brain.

Now Suga and Shimozawa (*Science*, **183**, 1211; 1974) have pinpointed the exact site of this neural attenuation. By recording from various nuclei along the auditory pathway when bats of the genus *Myotis* were either vocalising themselves or listening to tape recordings, they found that neural attenuation occurs in the nucleus of the lateral lemniscus. They point out that although such mechanisms are of obvious importance for bats with their special problems in echolocation, similar muscular and neural attenuation mechanisms may also operate in human speech, since our vocalisations rarely appear disturbingly loud.

Nuclear physics at Glasgow meeting

from a Correspondent

At the combined Institute of Physics conferences on nuclear structure and high energy physics, which took place at the University of Glasgow on March 27-29, the nuclear structure talks reflected in the main the specific interests of Glasgow workers. Experimental work in Glasgow concentrates on electron scattering, photoreactions and the use of photoreaction neutrons. The principal local activity in nuclear theory is the application of the shell model to fairly light nuclei.

E. Spamer (Technische Hochschule, Darmstadt) discussed the techniques by which the momentum resolution of the Darmstadt linear accelerator is being improved by a factor of five. He emphasised that this makes electron inelastic scattering applicable to the study of a great many more states than hitherto, at higher energies and in heavier nuclei. One would, for example, hope to learn more about the newly detected quadrupole resonance found above the familiar giant dipole resonance in medium nuclei.

R. Owens (University of Glasgow) dealt with photonuclear reactions in the higher energy region in which one hopes to obtain information about high momentum components in nuclear wave functions. This hope has been much deferred, and calculations with short range correlations seem at present to call for a further extension of the energy range in these experiments.

Short range correlations were also a feature of the contribution by J. M. Irvine (University of Manchester) who dealt with low lying levels of the p-shell nuclei. He has used a two-body interaction fitted to scattering deuteron data and a variational method applied to wave functions with both central and tensor correlation corrections, to obtain an effective interaction for use in a large basis shell model calculation with the Glasgow code. With reasonable restrictions on the short range central correlation, and by adjusting the strength of the tensor correlation to fit the absolute binding energy of each nucleus in turn, many other levels can be fitted with no further adjustment of parameters.

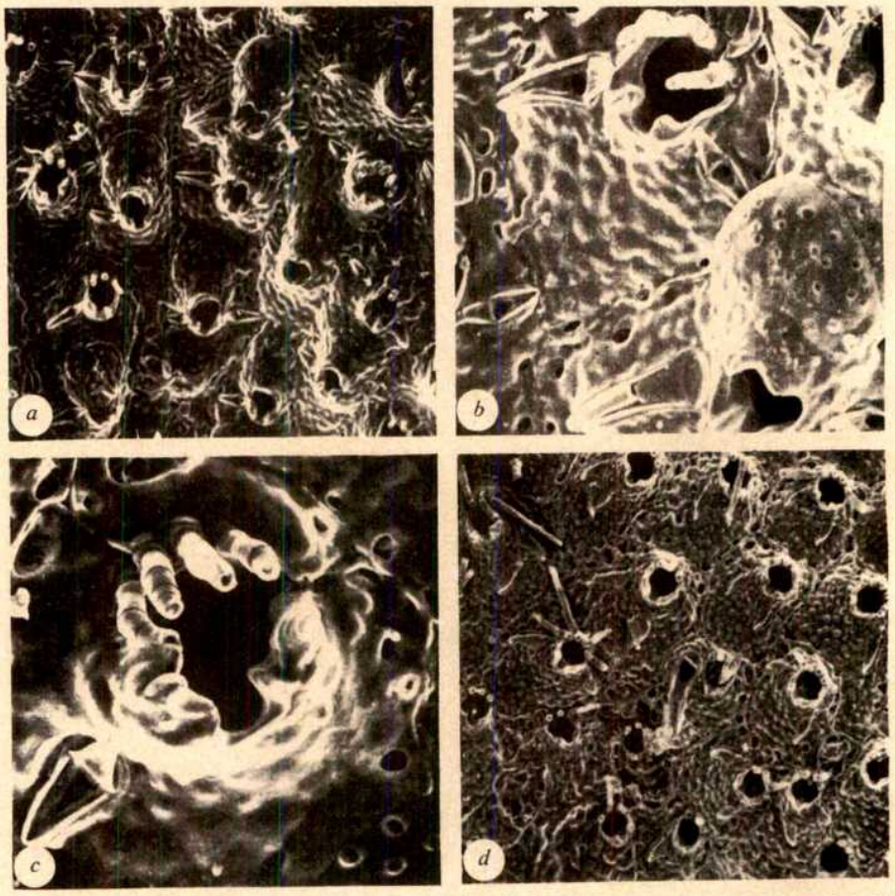
The talk by R. Arvieu (University of Paris, Orsay) also touched on the subject of large scale shell model calculations. He discussed the statistical approach to the shell model, in which one calculates the mean energy and moments of the level density distribution for various configurations. This can be used, for example to learn, in advance of investing in a large shell model calculation, which configurations are most essential and must be retained. H. A. Weidenmuller (University of Heidelberg) also discussed statistical aspects of nuclear physics, but in the more familiar context of the statistical model of neutron resonances. He emphasised that although statistical shell model calculations give the density of levels in a configuration in Gaussian form, as opposed to the semi-circle law of Wigner's random matrix model, the two models agree on the fine structure of the statistics of level spacings. The predictions of these models are well confirmed by data with up to 180 neutron resonances in a particular nucleus. Weidenmuller also discussed recent work at Heidelberg which extends the range of validity of the Hauser Feshbach prescription for average cross sections, and which allows effects of direct reactions to be allowed for in this method.

On the high energy side there was considerable emphasise on photon and lepton interactions although strong interaction physics, particularly at very high energies, also figured strongly. D. H. Perkins (University of Oxford) reviewed the experimental evidence for neutral currents in weak interactions and indicated that the experimental evidence is becoming steadily more convincing and is in good agreement with the Weinberg–Salam–Ward theory as opposed to the long standing Fermi theory which, as for example in beta decay, requires only charged currents.

Two examples of $\bar{\nu}_{\mu} e^-$ scattering have been recorded in the Gargamelle bubble chamber at CERN and many more events of the type ν+hadron $\rightarrow$ ν+hadron at both CERN and the National Accelerator Laboratory, Batavia (NAL).

Storage rings—both electron and proton—are producing results of fundamental interest and several speakers concentrated on data recently produced by these devices which allow observa-

Smittinid ectoprocts from Hawaii



The shallow waters round the Hawaiian Islands seem to be havens for the smittinid family of ectoproct bryzoans—the minute sedimentary aquatic animals which usually live in colonies. D. F. and J. D. Soule have found that the Hawaiian species of Smittinidae seem to be isolated specifically from the Indo-Pacific forms and that there are also species differences between the five major Hawaiian Islands. In a comparative study (*Bull. Am. Mus. nat. Hist.*, **152**, 365; 1973), the Soules recognise twenty-eight taxa of which two are new genera and twenty are new species. This figure shows (*a*) colony with tubular peristomes, (*b*) immersed ovicell and part of developing ovicell and (*c*) aperture with spines of *Parasmittina alanbanneri* n.sp.; (*d*) colony with spines on zooecia of *P. serrula* n.sp.

tion of collision between particles at energies hitherto unattainable by conventional accelerators.

J. Haissinski (University of Paris, Orsay) spoke about electron–positron storage rings where e^+e^- collisions at centre of mass energies of several GeV are studied. From the study of such collisions two main conclusions may be drawn: the first concerns quantum electrodynamics—the fundamental theory which describes the interaction between electrons, muons and photons—which is found to be still valid at distances as short as a few hundredths of the proton radius. Second, electron–positron annihilation processes can lead to the production of hadrons (strongly interacting particles such as pions, protons, neutrons) and production rates have proved to be unexpectedly high. If this trend persists at higher energies it would have important consequences as far as 'elementary' constituents of the hadrons are concerned. These hypothetical fundamental constituents (quarks) are now the object of an intense programme. The storage ring experiments seem to indicate that either these quarks form a rather large family, or that they have a structure of their own. In the latter case, the situation would be quite similar to the one which resulted from the discovery of the internal structure of the nucleons in the 'elementary' constituents of the nuclei.

The experiments currently taking place at the intersections of the Intersecting Storage Rings at CERN were reviewed by M. G. Albrow (CERN). Searches are being made for new exotic particles which may be produced at the very high centre of mass energies available at the ISR (up to ~ 56 GeV). Searches for quarks, magnetic monopoles and intermediate vector bosons have all so far proved negative although antideuterons have been observed. The important discoveries at the ISR of a 10% rise in the total pp cross section and of structure in the angular distribution of elastic scattering are now being measured with higher precision and studies of interactions where particles are produced with high momentum transfer relative to the incident proton direction are also proceeding. The observed high rate of such events would indicate the existence of point like constituents (partons) within the nucleon.

The current interest in nucleon substructure was also reflected in talks by R. G. Moorhouse (University of Glasgow) who dealt with photo-and leptoproduction processes in the context of the quark model and by F. Close (CERN) who described how the number of quarks in a particle can be determined by means of the energy dependance of the elastic scattering cross section at large angles.

Out of the ball park

from a Correspondent

At the Spring meeting of the British Biophysics Society, devoted to nucleation, folding and interaction of biopolymers, held at the University of Newcastle upon Tyne on March 26-28, and organised by Dr R. H. Pain of the Biochemistry Department, the policy was continued of devoting the major part of the programme to long (50 min) papers. In discussion the extent to which the various specialist techniques used to study biopolymers should be regarded as mutually complementary rather than competitive became very clear. A further impression was the way in which improvements in instrumentation and access to computers have facilitated more sophisticated interpretations of data from techniques such as ultracentrifugation and gel chromatography on complex systems of interacting biopolymers, and the rigorous testing of these interpretations by computor modelling of the experimental data.

R. L. Biltonen (University of Virginia) examined the thermodynamics of folding of biopolymers, with particular reference to the thermal cooperative transition to a partially unfolded state on an effective 2-state basis, and to the associated large change in heat capacity ($\triangle C_p$). Large differences in heat capacity between conformational states of biopolymers is now well established, and can be attributed in part to large changes in hydrophobic bonding, and in part to changes in solvent (water) structure due to the rupture of internal hydrogen bonds by partial unfolding, and the formation of new hydrogen bonds between exposed side chains and solvent molecules.

S. Lifson (Weizmann Institute, Rehovot) appealed, with both eloquence and wit, for an increase in the precision of calculation of molecular properties from crystal structure data, arguing that results "in the ball park" were no longer of interest. Conversely, the more precisely that calculations agree with experiment the more interesting the remaining discrepancies become in pointing the way to modification of theoretical parameters. After demonstrating the success of the consistent force field (CFF) method for calculating the properties of the alkanes, Lifson reported a study of the hydrogen bond, starting from crystallographic data on several amides to find the best force field. In the final result charge transfer seems to be unimportant and a 12-6-1 potential, which combines the Lennard-Jones 6-12 potential with partial charges attributed to the participating atoms while keeping the bond electroneutral, properly represents the hydrogen bond. There is a reduction in the effective van der Waals radius of the amide hydrogen, due to shifting of the electron cloud and formation of a large partial positive charge. This allows close approach of the >NH and O=C< groups to form a hydrogen bond of characteristic length, and the resulting associated electrostatic attraction is the main source of the bond energy. Lifson cheerfully conceded that this theoretical picture might have to be drastically modified to explain those features of hydrogen bonds revealed by infrared spectroscopy.

The extent to which the elucidation of hydrophobic bonding is dependent on a theory of water structure (still the subject of much argument) was emphasised by G. Némethy (University of Paris, Orsay) who pointed out that different agencies for changing hydrophobic bonding may alter the properties of proteins in aqueous solution in quite different ways.

B. Robson (University of Newcastle upon Tyne) discussed the application of information theory to estimate the extent to which sequence dictates secondary structure in the folding of a polypeptide chain. It seems that specific single residues can act as nucleation centres for secondary structure, and that the conformation of segments of the polypeptide chain are thus determined locally by the constituent residues. In the refolding process the locally ordered segments may represent metastable intermediates formed early in refolding and surviving in the stable completely folded conformation. Robson noted that Ramachandran conformational energy maps based on Lifson CFF functions were better than most, and also drew attention to map regions for the 4-residue reverse turn, which is now being recognised as an important element of secondary structure.

G. C. K. Roberts (National Institute for Medical Research, Mill Hill) described how NMR spectroscopy can be used to study protein unfolding, and referred to work on pancreatic ribonuclease (RNase). Subject to the difficulties of assigning NMR resonances to protons in individual residues, which is currently possible only for a small number of particular residues in the proteins studied in detail, it is possible in principle to follow the participation of individual residues in unfolding processes in reversible conditions. For RNase, the NMR studies indicate that the thermal transition at *p*H 5 proceeds cooperatively to a still partly ordered state, and that two of the four histidine C_2 proton resonances are affected. With denaturants such as guanidine hydrochloride, the NMR spectra at low denaturant concentration

indicate changes not revealed by optical methods. From resonance peak areas as a function of denaturant concentration, it seems that the order in which the histidine residues participate in the unfolding process is 12, 119, 48, 105. This order can be rationalised in terms of the known tertiary structure of RNase, and is therefore also relevant to the folding process.

The possible methods for revealing the existence of intermediate partially unfolded states were noted by R. H. Pain who pointed out that although some earlier equilibrium and kinetic studies on, for example, lysozyme unfolding in guanidine hydrochloride, indicate a 2-state process with first-order kinetics up to about 90% reaction, this situation is not general. The analysis of bimodal kinetics can reveal the presence of intermediate states, or of alternative states off the main folding-unfolding path. Pain reported his investigation of the pencillinase of *S. aureus*, a protein with no sulphydryl or disulphide groups, which was specifically selected for unfolding studies, and summarised the evidence for the existence of a highly expanded state, with the same α-helix content as the native protein, formed by a cooperative transition at low denaturant concentrations.

L. W. Nichol (Australian National University, Canberra) discussed ligand binding in associating protein systems, and showed how the form of the binding curve could be discussed in terms of regions of sigmoidality defined by the interaction parameters. He described how ultracentrifugal sedimentation equilibrium measurements can be used to evaluate these parameters. G. A. Gilbert (University of Birmingham) emphasised the central role of protein–protein interactions in the conformational control of protein function mediated by allostery, and pointed out that in the so-called non-equilibrium methods (for example, chromatography, electrophoresis, velocity centrifugation) the progress of the moving boundary reflects the equilibrium situation in the following plateau region, as well as the non-equilibrium situation within it.

The topical subject of membrane-bound proteins and protein–lipid interactions was discussed by C. Tanford (Duke University). Experimental data on protein–detergent associations (much used as a contemporary model) were described. The behaviour of cytochrome *b5* and a phage protein in detergent systems as consistent with the idea that membrane proteins possess distinct regions around which true micellar structures can organise. The discussion included a brisk exchange on the problems of measuring protein molecular weights by ultracentrifugation in the presence of detergents.

Proton NMR studies on histones were discussed by E. M. Bradbury (Portsmouth Polytechnic). With increasing ionic strength structure is induced through interchain interactions between regions of apolar residues, and the basic segments interact with DNA, leading to the formation of chromosome superstructure. The lysine-rich F1 histone, however, seems to be involved specifically in salt-induced contraction of chromatin gel, and in chromosome condensation through a phosphorylation process.

The programme included two parallel sessions of shorter papers on various aspects of protein unfolding and folding processes, ribosome reconstitution, RNA-protein complexes, and mucoprotein. Noteworthy was the study by Creighton, Dyckes and Sheppard (MRC Laboratory of Molecular Biology, Cambridge) on the refolding of reduced pancreatic trypsin inhibitor, in which the different species existing at the 1-disulphide bond stage were detected and characterised. The refolding of this protein proceeds with eventual complete recovery of native structure, and the pathway for refolding indicated by this work is consistent with the view that folding is directed by segments which have acquired the correct secondary structure.

Chlorophyll and primary productivity

from our Plant Ecology Correspondent

SINCE chlorophyll is essential for photosynthetic energy conversion, it is not unreasonable to suppose that it might provide a rough estimate of the potential gross primary productivity of an ecosystem. Early in this century the work of German plant physiologists suggested a close relationship between chlorophyll and photosynthetic rate. Later it became apparent that the chlorophyll density of vegetation expressed as g m^{-2} of ground area was remarkably similar in a variety of ecosystems and this led Odum (*Fundamentals of Ecology*, Saunders, 1959) to propose that there is a community homeostasis in which chlorophyll levels are maximised within the limiting physical constraints of the environment, leading to similar chlorophyll densities in most ecosystems.

Such a tenuous hope was shattered by Bray (*Can. J. Bot.*, **38**, 313; 1960) who concluded that chlorophyll densities in a wide variety of vegetation types might vary by as much as twenty times in natural ecosystems. He found that chlorophyll densities were higher in climax ecosystems than in seral ones and were greatest in highly stratified systems such as forests. These conclusions were largely confirmed by Ovington and Lawrence (*Ecology*, **48**, 515; 1967) who made detailed studies of four contrasting ecosystems—a prairie grassland, a savanna, an oakwood and a maize field.

Although Odum's simple homeostasis proposal was no longer tenable, there remained the possibility that chlorophyll might provide an index of gross primary productivity. Ovington and Lawrence's data in no way contradicted such a proposal. The underlying presupposition, however, is that all of the chlorophyll present must be active in photosynthesis whenever light levels permit; physiologists have always regarded this as extremely unlikely.

One rather startling observation which Ovington and Lawrence made was that a large proportion of the total chlorophyll content of their oak woodland was contained in the branches of the trees. In the month of August the trees themselves contained 29×10^3 g ha^{-1} chlorophyll, of which 12×10^3 g ha^{-1} (41%) was contained in the older branches. The bulk of this branch chlorophyll remained after leaf fall in September/October. Immediately the question must be raised as to whether this large reserve of chlorophyll is active and, perhaps even more important, whether it could result in winter photosynthesis in these deciduous trees.

Although only relatively simple experiments were required to settle this question, they have been conducted only very recently. Much is known about the winter photosynthesis of evergreens (see, for example, Kozlowski and Keller, *Bot. Rev.*, **32**, 293; 1966) but very little work has been done on deciduous trees. Keller (*Photosynthetica*, **7**, 320; 1973) has now examined rates of photosynthesis and respiration in the bark of ash (*Fraxinus americana*), aspen (*Populus tremula*) and larch (*Larix decidua*) during the winter. In his experiments plants were taken from their growing sites where they had been exposed to normal winter temperatures, they were kept at 16°C for 16-20 h and their carbon dioxide exchange rates were then measured by infrared gas analysis at 40, 10 and 0 klx respectively. In all species at all light intensities the carbon dioxide emission exceeded absorption; in other words, respiration exceeded photosynthesis. Thus, despite the presence of abundant chlorophyll, these species are likely to be experiencing a negative carbon balance through the winter months.

These experiments should serve as a salutary warning to production ecologists of the dangers inherent in the use of chlorophyll level as a productivity index, despite the general correlation between chlorophyll density and the gross primary productivity of ecosystems.

DNA-mediated genetic correction of thiamineless *Arabidopsis thaliana*

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Thiamineless mutants of Arabidopsis *were corrected by treating the seeds with DNA preparations from various bacteria. Possible mechanisms for this are discussed.*

TRANSFORMATION of prokaryotes by addition of exogenous genetic information has been possible since the classical experiments of Avery *et al.*[1] and since the demonstration that bacteria can take up and integrate DNA into their genome by genetic recombination.

Until recently, such 'cell manipulation' with higher organisms seemed almost impossible, presumably because of the inadequate means available for critical studies. The necessary biochemical background had to be obtained: good methods to prepare nucleic acids and to recognise them in a population of similar molecules had to be developed. The scarcity of mutants with defined biochemical lesions precluded (and still limits) genetic experimentation[2,3].

Nevertheless, DNA-mediated corrections in higher organisms were reported: with mammalian cells in tissue culture[4–12], with *Ephestia*[13] and with *Drosophila*[14,15], with *Petunia*[16–19] and with *Arabidopsis thaliana*[20–25]. Finally, phage-mediated corrections were reported with animal cells[11], with haploid tomato and *Arabidopsis* calluses[26–28] and with sycamore cells[29].

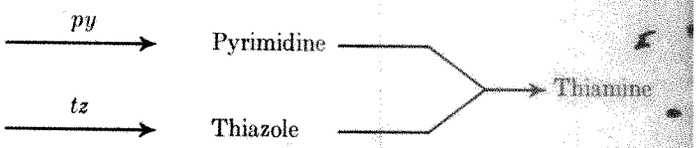
Extensive use of the CsCl gradient technique has led to the unexpected results that foreign DNA can be translocated to large distances across cell membranes[30,31] and seems to survive several mitotic cycles in different animal or plant systems[2,30,32]. In *A. thaliana*, it seems to survive at least one round of meiotic division[23,25,33]. More surprisingly, it seemed that the foreign DNA became covalently linked to the recipient DNA[21,23,25,30] as double-stranded material (a phenomenon similar to the incorporation of the λ DNA in the *E. coli* genome).

As already indicated in preliminary reports[20,25], correction of nutritional mutants of *A. thaliana* by exogenous DNA was attempted. We present here an overall picture of the results so obtained. More extensive genetic data will be presented elsewhere.

Nutritional mutants of *Arabidopsis thaliana*

Described auxotrophs are rare in *A. thaliana*, with the exception of those requiring thiamine[34]. They can be obtained by X rays or chemical mutagens. They are also conditional obligate auxotrophs with a strict phenotype, whilst other auxotrophs exhibit bradytrophy.

Mutants at one locus (*py*) control the synthesis of the pyrimidine moiety of thiamine[34,35], locus *tz* controls the synthesis of the thiazole half of the vitamin and two other genes are concerned with some intermediate steps between these two precursors and thiamine[34]. These mutants can be used for strictly genetic studies such as allelic complementation or recombination or reverse mutation because of the built-in selective mechanisms[34]. The mutability of the *py* locus is apparently the highest[34,35] and is close to 2×10^{-4}. The frequency of spontaneous reversion has been estimated to be lower than 5×10^{-6}.



Treatment with ethyl methanesulphonate produced apparent reversions at the *py* locus, at a frequency of about 4×10^{-5}. These revertants were nonhomozygous and segregated in the following generations. In test crosses, they also yielded heterozygotes and lethal mutants in approximately equal frequencies[36]. No homozygous wild types were thus obtained by back mutation.

The monogenic conditional lethal mutants used here are normally propagated in the presence of thiamine. When sown on mineral medium, the seeds germinate normally and develop their cotyledons which appear normal green. The first leaves progressively bleach out completely and the plant soon dies. Thiamine (10^{-6} M–10^{-5} M) promotes a normal growth of all these mutants.

Mutants used in this study were the following: *thi*51 from Rédei, *thi*V447 and *py*V131 from Feenstra, and *py*431 and *tz*432 from Jacobs. They bear the side markers indicated in Table 1; the only wild-type *Arabidopsis* used in the laboratory was the Wilna *g*1.

Treatment of seeds

Seeds were disinfected with a 95% ethanol and 3% H_2O_2 mixture (1:1) or with a 5% calcium hypochlorite solution[34]. The seeds were treated for 5–8 min and rinsed with a few changes of sterile distilled water. About 50 seeds were then immediately placed in a well of a spot plate and immersed in 25 μl of a DNA solution (see below) or of 0.01 M NaCl. Spot plates were placed in Petri dishes over wet sterile filter paper to limit evaporation of the solvent. During the three following days, 25 μl of the DNA solution were added to the seeds to keep them moistened. Petri dishes were kept at 24° C under continuous illumination for 4 d. Both DNA and NaCl treated seeds germinated during the incubation period. After incubation seedlings were laid out (a) in tall Petri dishes containing perlite moistened with the mineral nutrient solution of Jacobs[37] or (b) in numbered test tubes containing 5 ml 0.78% agar dissolved in the mineral nutrient solution

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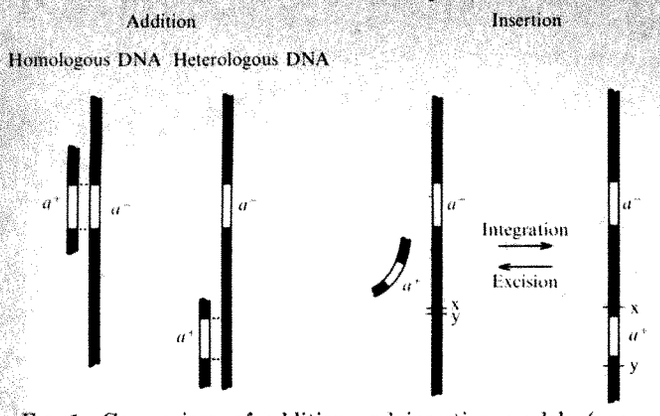


FIG. 1 Comparison of addition and insertion models (one of two homologous chromosomes is shown). In *Drosophila* transformed by homologous DNA Fox's exosome model implies a close association of the foreign gene with its homologous chromosome locus. This exosome is not integrated but replicates in step with the chromosome and can be lost or transmitted with it at the time of cell division. Either the exosomal gene or the chromosomal one is transcribed, leading to phenotypic mosaicism. When heterologous DNA is used for correction, as in our case, the necessity of a close association with the mutated site is less mandatory and the heterologous exosome could become associated to another region, where the actual base sequence is in favour of such an association. Here too, the exosome could replicate in step with the chromosome and be lost or transmitted with it at the time of cell division. The probability of a loss would however be high due to the imperfect homology. In the insertion model, the foreign gene is integrated in the recipient DNA, like episomal DNA, through the homology of a few base pairs. Once integrated, it would be hard to lose it, except on pairing with a chromosome homologous for the rest of the structure but lacking the inserted foreign piece. Although gene conversion could interfere at that step, chromosome aberration is an obvious alternative, possibly leading to the loss of the foreign gene.

of Miksche and Brown[38]. The tubes were placed in wooden racks, kept under continuous illumination (6,000 lux) at 24° C and examined each second day.

All mutant seedlings on mineral medium died within 15–20 d; they showed chlorophyll deficiency in the cotyledons and in the rosette leaves. Corrected plants grew slowly, with a more or less normal green colour. About one-third produced fertile siliques (see below). These siliques were collected and kept separately in cellophane bags. The thiamine concentration necessary to provide complete growth when seeds were soaked in it for 3 d, then washed and allowed to develop in the absence of thiamine, was found to be about 10^{-3} M.

DNA preparations

DNA preparations were obtained from frozen or fresh bacteria (or from isolated and CsCl-purified bacteriophages) by the technique of Marmur[39] or by CsCl gradient preparative ultracentrifugation, after treatment with pronase and RNase[40]. The DNA was precipitated with 2 volumes of ethanol, kept in 70% ethanol, and dissolved in sterile 0.01 M NaCl just before use. The concentration used was about 0.5–1.0 mg ml^{-1}. We successively tried and rejected the use of chloroform or phenol for deproteinisation: both are toxic to the plant. They may also selectively remove the protein or membrane bound DNA molecules from the bulk of the DNA preparation.

Our study of the biochemical fate of labelled DNA in various systems led us to recognise that the DNA has to be freshly prepared, of high molecular weight ($> 10^7$ daltons), very clean and intact. When single-strand breaks in a DNA preparation could be detected from the depolymerising effects of alkali[40], the preparation was discarded. Finally, the DNA had to be given during the first 24 h of imbibition of the seeds; otherwise it was not integrated into the recipient material (unpublished data).

In general, we used small numbers of seeds per batch of DNA and preferred to repeat the treatment with freshly prepared batches. Perhaps because of fragmentation of the DNA molecules during their isolation and purification, some batches of a given DNA species were ineffective with the mutant plants tested, whereas others were quite effective. Yet these DNAs were undistinguishable by the physiochemical methods used. Even if it is tempting to blame the DNA itself, it is of course difficult to eliminate uncontrolled parameters related to plant growth as the source of these discrepancies.

DNA was prepared from the following strains: *Escherichia coli* K12, wild type; *Agrobacterium tumefaciens* B6, obtained from Dr A. Kurkdjian; *Bacillus subtilis*, 168 T^-; *Micrococcus lysodeikticus*, ATCC 4698; *Streptomyces coelicolor* AS/2 obtained from Dr H. Hopwood; *E. coli* P678 (*thia*A). Phage T7 (with normal bases) and phage 2C (with hydroxymethyl uracil replacing thymine) were also used. They were isolated and purified following the technique of May *et al.*[41]

Correction in DNA-treated populations

When seeds of the different mutant types were treated with different bacterial DNAs, corrections could be obtained with a relatively high frequency (10^{-2} to 10^{-4}) comparable with that observed with bacterial transformation. Table 1 shows an overall picture of the data gathered during the last 3 yr with a total of 27 different batches of DNA tested with five different mutants. These data include DNA extracted from wild-type bacteria, from bacteria with a thiamine deficiency (corresponding to the thiazole pathway) and from phages containing normal (T7) or abnormal (2c) bases.

Further controls were obtained by treating mutant seeds with 0.01 M NaCl, the solvent used for the DNA assays. After DNA treatment, a few mutant seedlings, which normally died within 15–20 d of sowing, displayed better growth, formed small rosettes and eventually flowered. Only a few of them were fertile, however. Table 1 takes into account only the restored plants which produced seeds, thus allowing a study of their progeny. Table 1 also indicates, for each case, the number of germinating seeds and the total number of seeds used. Contamination by wild-type seeds is highly improbable as strict precautions were taken in treating and growing the plants. Furthermore, most of the mutants used bear additional markers which make them easily recognisable from the only wild-type strain used in the laboratory. These results indicate that the percentage of correction obtained with the group of DNAs from wild-type bacteria is similar for all mutants: 0.7%, 0.85% and 0.49% respectively for thiamine, thiazole, and pyrimidine-requiring mutants. On the other hand, the percentages of correction obtained for five mutants seem to vary with the source of DNA, *E. coli* being at the moment the most effective (0.89%) and *S. coelicolor* the less effective source of DNA (0.19%). Negative results were obtained with DNA from phage T7 or phage 2C. DNA from *E. coli thia* did not correct thiazole mutants but, as expected did correct pyrimidine ones. It should also be noted that in all cases, the DNA-treated plants, growing in the absence of thiamine showed a delay in growth and development, as compared with the wild type or to mutants supplemented with thiamine (5×10^{-5} M).

Progeny obtained by selfing corrected types

Progenies of various corrected types obtained after DNA treatment were sown on perlite soaked with mineral medium or on soil and observed during the entire growth of the plant. The number of F1 plants tested varied as a function of the number of seeds present in the fruits of the corrected plant growing on a thiamineless medium. In a very few cases, thiamine was provided at the flowering stage to increase vigour and thus seed production. F2 and F3 progenies in-

TABLE 1 Corrections observed in DNA-treated populations

DNA	Lethal mutants (additional markers) *thi*51 (gl)	*thi*V447 (er–gl)	*tz*432 gl	*py*V131 (er, gl)	*py*431 (–)	Total
a.						
E. coli	6/355/685	2/393/525	2/284/550	1/384/400	4/270/477	15/1686/2637
Agrobacter tumefaciens	2/182/420	0/100/100	3/230/480	1/100/200	0/130/250	6/742/1450
Bacillus subtilis	0/128/288		3/157/328	0/28/100	0/162/420	3/497/1136
Micrococcus lysodeikticus	0/176/450		0/122/120		2/428/705	2/428/1375
Streptomyces coelicolor	0/145/274	1/75/75	0/152/272	0/22/100	0/120/410	1/524/1131
b.						
E. coli P678*(*thi*A⁻)			0/263/350	1/254/350		1/517/700
Phage T7 (*E. coli*)	0/425/542		0/440/500		0/356/520	0/1221/1562
Phage 2C (*B. subtilis*)	0/189/252		0/182/220			0/371/472
c.						
NaCl 0.01 M	0/395/642	0/233/291	0/467/669	0/214/262	0/1580/2960	0/2889/5024

Figures show no. of corrected plants with a progeny/no. of germinated seeds/no. of treated seeds.
* Requiring thiazole for growth.

cluded at least fifty plants for each experiment. The majority of the corrected plants were studied up to three successive generations and fresh and dry weights for 5 corrected mutants were determined.

The most striking result of this study concerns the lack of segregation on selfing in the progenies of the various corrected types. All F1 to F3 plants originating from each corrected plant look phenotypically alike and grow reasonably well on mineral medium. This was observed also in one case where the selfing was pursued up to the seventh generation. They show, however, in most but not all cases, a variegated pattern of chlorophyll pigmentation with light discolouration at the base of the leaf and along the main vein. In practically all cases, the average fresh weight (and particularly the average dry weight) of the corrected types grown on mineral medium, were lower than those of plants from the same progeny, supplemented with 10^{-4} M thiamine (37 to 92%). Each of these types thus display sensitivity to the addition of thiamine. Other experiments showed that the corrected 'pyrimidine' or 'thiazole' mutants specifically responded to the addition of pyrimidine or thiazole and showed an improved growth. On the other hand, and contrary to the situation for wild-type control plants, the progeny of some of the corrected plants exhibit thermosensitivity; at 16° C they showed signs of thiamine deficiency, which disappeared at a higher temperature.

In selfing experiments, the corrected plants behaved as homozygotes breeding true, in contrast to what is found with mutagen-induced revertants. This is also in contrast to what is expected in the case of a suppressor mutation introduced into a homozygous diploid. Indeed, in the seed, the germline is represented by two diploid cells[42,43]. These cells, at least, should contain the thiamine information to explain the lack of segregation after selfing. In F1 the ratio of mutants: wild types should vary from 1:3 to 1:7. The fact that correction results in a homozygous corrected plant suggests that there has been continuous selection for the corrected germ cells and, presumably a meiotic drive at the time of meiosis favouring those gametes which harbour the thiamine information. Indeed, when selection pressure is avoided by giving thiamine supplementation to plants treated with DNA which show signs of abortive flowering, a high percentage of lethal mutants is observed in the F1 progeny.

Offspring of out-crosses

We have analysed, in parallel, the offspring of crosses made between corrected plants and either the wild or the original mutant type (back or test crosses). Table 2 shows the results obtained in the case of tz^c a homozygous 'thiazole' mutant corrected by a treatment with *B. subtilis* DNA. (Similar data were and are being gathered for other corrected types such as py^c, th^c or tz^c corrected with other DNA. They will be described elsewhere together with the results of crosses between mutants corrected with different DNA). The phenotypes presented by progenies of successive self fertilisation were analysed.

The first generation of the outcrosses (x) does not segregate. The results of the test cross ($tz^c \times tz^-$) implies that the correction is dominant and that the corrected plants behave as true homozygotes. Reciprocal crosses between corrected mutants and lethal mutants indicated that the correction can be transmitted through the male as well as through the female and therefore does not seem to correspond to a maternal cytoplasmic factor.

Leaky mutants

Segregation is observed in the offspring of the corrected plants outcrossed to the wild type (xF1 → xF2). Lethal mutant types are recovered (although in low frequencies) together with plants exhibiting a new phenotype called here 'leaky mutant'. This is characterised by a weaker pigmentation, white patches of discolouration on the leaves, sometimes white sectors on the stem and relatively poor growth and fertility, in contrast with the normal looking plants of the same progeny. A whole range of these phenotypes, intermediate between the normal green plants, and the lethal chlorophyll deficient plants were classified under the name 'leaky mutant'. These mutants are obtained in crosses between the corrected type and the wild type, as well as in crosses between the corrected type and the lethal mutant (in the latter case, a high frequency of lethal mutant being obtained). The leaky types keep their sensitivity to supplementation with thiamine, thiazole or pyrimidine respectively. This indicates that the correction has been added to the mutation and has not been substituted by it. Correction being dominant, the recessive mutation is not expressed unless the correction is masked or lost, for instance by crossing with an uncorrected plant.

The leaky mutants are found at equal frequencies in the XF1 offsprings from $tz^c \times tz^+$ (9.2%) and $tz^c \times tz^-$ (6.1%). Their offspring includes high frequencies of leaky and lethal types and also normal looking plants. This normal phenotype can be recovered at frequencies (21.7% and 24.7%) suggesting that the 'leaky mutants' behave as heterozygotes. They should however, also segregate into mutant types with similar frequencies. The correspondingly lower values obtained for the lethal mutants and the reproducibility of the phenotype distributions could be acounted for by various mechanisms.

We expected that this study would help to correlate genetic and biochemical data. This was in fact the main reason why bacterial DNA was used in the biological studies. We have not used homologous *Arabidopsis* DNA because it

TABLE 2 Results of outcrosses and control crosses

Cross	Progeny tested	No. growing plants	No. ungerminated plants	% Phenotypes Normal (N)	Leaky (Lk)	Lethal
$tz^e \times tz^+$	× → ×F1	11	20	100		
	×F1 → ×F2	163	15	90.1	9.2	0.7
	×F2N → ×F3	198	5	95.5	2.0	2.5
	×F2Lk → ×F3	198	13	21.7	70.2	8.1
$tz^e \times tz^-$	× → ×F1	20	5	100		
	×F1 → ×F2	116	15	75.8	6.1	17.3
	×F2N → ×F3	131	4	76.3	0	23.7
	×F2Lk → ×F3	202	21	24.7	65.0	10.3
$tz^- \times tz^+$	× → ×F1	15	0	100		
	×F1 → ×F2	106	7	72.6	0	27.4
	×F1 → ×F2	63	4	73.0	0	27.0

is difficult to prepare from this material the clean, intact DNA of high molecular weight which we consider a prerequisite for efficient uptake and integration. On the other hand, although we wanted to use specialised transducing phages to introduce selected genes only, we were not in a position to do so, because of the lack of phages carrying thiamine information. (The amount of DNA which can be taken up by a cell is limited as are the size of the populations to handle. It therefore seems reasonable to try to increase the dosage of the interesting gene in the DNA preparation used. This can be achieved by using DNA from bacteria, transducing phages or episomes.) This kind of difficulty has recently been overcome by using DNA from thiamineless bacteria (ineffective as such for correction) harbouring a F′ factor bearing the thiamine information (KLF10). These results will be published elsewhere. To what type of genetic model do these results lead?

Transformation models

Different models have been discussed by Fox *et al.*[15] to explain their results showing genetic transformation in *Drosophila* with homologous DNA. They argue in favour of the exosome model, also considered by Hess to interpret *Petunia* transformation. The exosome model (Fig. 1 of ref. 22) is the only one which does not require an integration of the exogenous information into the linear structure of the chromosome. Such an integration is rejected by Fox because of the absence of whole-body transformants among the transformed *Drosophila* and to the instability of the correction. In the *Arabidopsis* system the nonautonomous type of biochemical lesions arising from the possible diffusion of thiamine makes it difficult to ascertain the presumably mosaic nature of the corrected type. Its progeny is, however, easier to analyse. It therefore seems that the corrected plants do behave as homozygotes as they do not segregate in their offspring (they do not segregate 1:1 in test crosses). As the correction is stable in subsequent offspring, it must correspond to a surprisingly stable form of exosome.

The effect of the exogenous DNA seems to be associated with the gene concerned (thiamine, thiazole or pyrimidine loci). This leads to the concept of an association of the bacterial information with the *Arabidopsis* genome. Such a close association is also indicated by the absence of significant differences between progenies of reciprocal crosses implying corrected types; 100% normal plants are obtained upon crossing corrected either male or female to the mutant type. The thiamine mutation remains in the genome of the corrected type; by crossing the corrected plants with the wild type, a resurgence of the original mutant type does occur in F2 although at very low frequencies. In fact, the passage through meiosis for the plants arising from a cross with one of the corrected types seems to increase the instability of the exogenous information. This seems to be the consequence of the appearance of the 'leaky mutant' phenotype (which could eventually be considered as functional mosaics).

The regularity in the transmission of the genetic information as well as the survival of the mutated site in the corrected plant could be explained if we assume that the necessary amount of information has been added to the genome (and not substituted for the mutant gene) and can be removed by crossing the plant with the wild type or with the mutant, but not on selfing. Perhaps difficulties in chromosome pairing, because the correction is present in only one of the two homologous chromosomes forming the bivalent, could be responsible for removing this correction. Such a picture fits well with the 'episomal DNA' type of interpretation (Fig. 1 of ref. 22) emerging from the biochemical analysis. This model also allows an explanation for the variability in the functioning of the bacterial thiamine gene in the recipient plant, possibly related to its relative position. These differences in the phenotypic expression of the thiamine locus do not seem to result from a lack of integration but from a change in the gene environment that is able to influence its genetic transcription and therefore its expressivity.

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1 Avery, O. T., McLeod, C. M., and McCarty, M., *J. exp. Med.*, **79**, 137 (1944).
2 Ledoux, L., in *Progress in Nucleic Acid Research and Molecular Biology* (edit. by Davidson, J. N., and Cohn, W. E.), **4**, 231 (Academic Press, New York, 1965).
3 Bhargava, P. M., and Shanmugan, G., in *Progress in Nucleic Acid Research and Molecular Biology* (edit. by Davidson, J. N., and Cohn, W. E.), **11**, 103 (Academic Press, New York, 1971).
4 Szybalski, E. H., and Szybalski, W., *Proc. natn. Acad. Sci. U.S.A.*, **48**, 2026 (1962).
5 Glick, J. L., and Salim, A. P., *J. Cell. Biol.*, **33**, 209 (1967).
6 Majumdar, A., and Bose, S. K., *Br. J. Cancer*, **22**, 603 (1968).
7 Fox, M., Fox, B. W., and Ayad, S. R., *Nature*, **222**, 1086 (1969).
8 Ottolenghi-Nightingale, E., *Proc. natn. Acad. Sci. U.S.A.*, **64**, 184 (1969).
9 Borenfreund, E., Honda, Y., Steinglas, M., and Bendich, A., *J. exp. Med.*, **132**, 1071 (1970).
10 Roosa, R. A., in *Informative Molecules in Biological Systems* (edit. by Ledoux, L.), 67 (North-Holland, 1971).
11 Merril, C. R., Geier, M. R., and Petricciani, J. C., *Nature*, **233**, 398 (1971).
12 Hill, M., and Hillova, J., *Nature*, **273**, 35 (1972).
13 Nawa, S., Sakaguchi, B., Yamada, M. A., and Tsujita, M., *Genetics*, **67**, 221 (1971).
14 Gershenson, S. M., *Genet. Res.*, **6**, 157 (1965).
15 Fox, A. S., Yoon, S. B., Duggleby, W. F., and Gelbart, W. M., in *Informative Molecules in Biological Systems* (edit. by Ledoux, L.), 313 (North-Holland, 1971).

[16] Hess, D., *Z. Pflanzenphysiol.*, **60**, 348 (1969).
[17] Hess, D., *Z. Pflanzenphysiol.*, **68**, 432 (1973).
[18] Hess, D., *Naturwissenschaften*, **58**, 366 (1971).
[19] Hess, D., *Naturwissenschaften*, **59**, 348 (1972).
[20] Ledoux, L., and Jacobs, M., *Arch. int. Physiol. Biochim.*, **77**, 568 (1969).
[21] Ledoux, L., and Huart, R., in *Barley Genetics* (edit. by Nilan, R. A.), **2**, 254 (Washington University Press, 1970).
[22] Ledoux, L., Huart, R., and Jacobs, M., *Arch. int. Physiol. Biochim.*, **78**, 591 (1971).
[23] Ledoux, L., Huart, R., and Jacobs, M., in *Informative Molecules in Biological Systems* (edit. by Ledoux, L.), 159 (North-Holland, Amsterdam, 1971).
[24] Ledoux, L., Huart, R., and Jacobs, M., in *The Way ahead in Plant Breeding* (edit. by Lupton, F. G., Jenkins, G., and Johnson, R.), 165 (Adlard and Son, Dorking, 1972).
[25] Ledoux, L., Brown, J., Charles, P., Huart, R., Jacobs, M., Remy, J., and Watters, C., in *Advances in the Biosciences* (edit. by Raspé, G.), **8**, 347 (Pergamon Press, 1972).
[26] Doy, C. H., Gresshoff, P. M., and Rolfe, B. G., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 723 (1973).
[27] Doy, C. H., Gresshoff, P. M., and Rolfe, B. G., in *The Biochemistry of Gene Expression in Higher Organisms* (edit. by Pollak, J., and Wilson, J.), 21 (Australia and New Zealand Book Co., 1973).
[28] Doy, C. H., Gresshoff, P. M., and Rolfe, B. G., *Nature*, **244**, 90 (1973).
[29] Johnson, C. B., Grierson, D., and Smith, H., *Nature new Biol.*, **244**, 105 (1973).
[30] Ledoux, L., and Huart, R., in *Uptake of Informative Molecules by Living Cells* (edit. by Ledoux, L.), 254 (North-Holland, 1972).
[31] Ledoux, L., Huart, R., and Jacobs, M., *Eur. J. Biochem.*, **23**, 96 (1971).
[32] Ledoux, L., and Charles, P., in *Uptake of Informative Molecules by Living Cells* (edit. by Ledoux, L.), 397 (North-Holland, 1972).
[33] Brown, J., Huart, R., Ledoux, L., and Swinnen-Vranckx, J., *Arch. int. Physiol. Biochim.*, **79**, 820 (1971).
[34] Rédei, G. P., *Biblphia genet.*, **20**, 20 (1970).
[35] Feenstra, W. J., *Arabid. Inf. Serv.*, **2**, 25 (1965).
[36] Feenstra, W. J., and Van den Berg, B. I., *Proc. XII int. Congr. Genet.*, **1**, 26 (1968).
[37] Jacobs, M., *Arabid. Inf. Serv.*, **1**, 36 (1964).
[38] Miksche, J. P., and Brown, J. A. M., *Am. J. Bot.*, **52**, 533 (1965).
[39] Marmur, J., *J. molec. Biol.*, **3**, 208 (1961).
[40] Ledoux, L., and Charles, P., in *Uptake of Informative Molecules by Living Cells*, (edit. by Ledoux, L.), 29 (North-Holland, 1972).
[41] May, P., May, E., Granboulan, P., Granboulan, N., and Marmur, J., *Ann. Inst. Pasteur*, **115**, 1029 (1968).
[42] Li, S. I., and Rédei, G. P., *Biochem. Genet.*, **3**, 163 (1969).
[43] Nikolov, C. V., and Ivanov, V. I., *Genetika*, **5**, 166 (1969).

LETTERS TO NATURE

PHYSICAL SCIENCES

Perturbations of Pioneer 6 telemetry signal during solar occultation

ATTENTION has been drawn recently to unexplained perturbations in the telemetry signal of Pioneer 6 (2,300 MHz) during solar occultation. The results[1] shown in Fig. 1 present the following odd features:

(1) An anomalous redshift is added to a normal linear redshift due to the spacecraft oscillator. This residual redshift which is symmetrical with respect to the centre of the Sun is of the order of $z = 5.18^{-8}$ at four solar radii.

(2) The bandwidth increases sharply when the telemetry signal grazes the Sun.

(3) There are some extremely sharp pulses in the bandwidth. In Fig. 2 we show that these pulses are clearly associated with a sharp increase of the redshift and correspond to solar flares.

The existence of the redshift is particularly puzzling because it cannot be attributed to gravitational effects nor to the usual Doppler effect.

Various explanations of those phenomena have been attempted:

Photon-photon interaction: P. Merat and colleagues[8] (unpublished work) have presented an explanation based upon previous work by Pecker, Roberts and Vigier[2,28]. Assuming an hypothetical photon-photon interaction, they supposed that each photon passing through a photon bath loses energy according to the semi-empirical law:

$$z = -\delta\nu/\nu = C \int U(s)\, ds$$

where $U(s)$ represents the background photon density, s the distance travelled and C a constant whose value lies around 10^{-30} cm^2 erg^{-1}. Their hypothesis gives a good account of the redshift. But the width of the signal remains unexplained, as does the correlation with solar flares; indeed the photon density $U(s)$ remains constant during the flare. And the

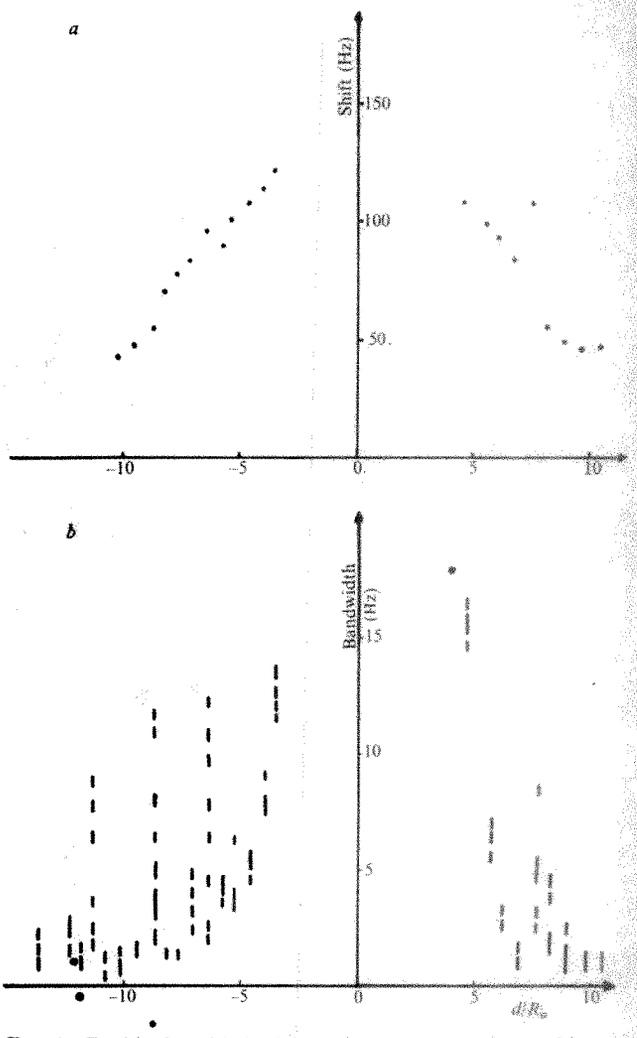


FIG. 1 Residual redshift (*a*) and bandwidth (*b*) in Hz as functions of the minimum distance of Pioneer 6 telemetry signal to the centre of the Sun.

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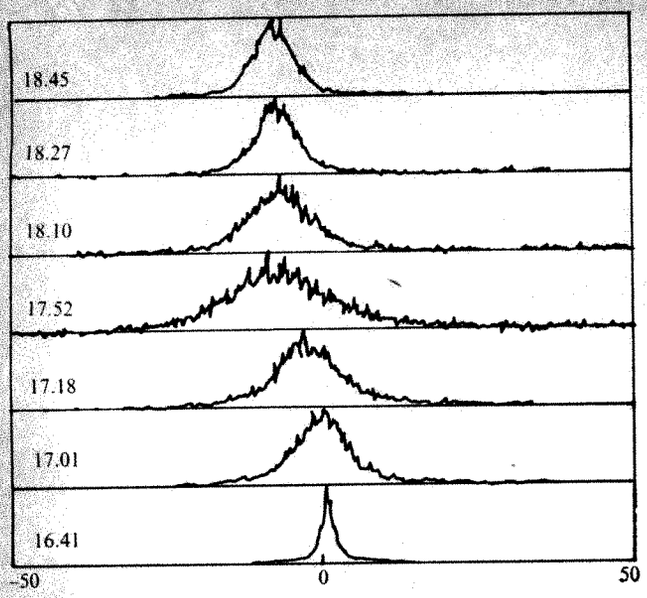


FIG. 2 This sample spectrogram taken on November 8, 1968, shows the effects of a solar event and demonstrates the association between the broadening of the line (12 Hz · h^{-1}), the anomalous redshift (4 Hz h^{-1}) and the solar flares.

Theoretical foundation of their formula is, in our opinion, unclear. We have therefore considered more 'classical' explanations.

Doppler-type effect: The optical index n varies when the signal passes through the solar corona. But it is easy to see that this effect is too small (of the order of 2.5×10^{-9}) and asymmetrical with respect to the Sun.

Induced scattering: It is well known that induced scattering gives rise to a redshift of intense electromagnetic radiation if the scatterers have a decreasing energy spectrum. This is true for ions and electrons. But calculations including an energetic tail in the distribution function of the electrons indicate that the effect is much too small. Scattering which might change the nature of the waves, in particular Langmuir into electromagnetic waves, is inefficient at this frequency.

Non-linear wave interactions: The combination and decay of waves or scattering of waves by waves must be considered. A plasma can support electromagnetic waves, space-charge waves and low frequency waves of the acoustic type. But interaction of electromagnetic waves with high frequency (Langmuir) waves leads to the build-up of a system of satellite lines, at a distance from the central lines equal to the plasma frequency ω_e that is much larger than the frequency window in Goldstein's experiment. Similarly, scattering of these waves would introduce both a large angular scattering and a frequency broadening of the order of the plasma frequency.

Therefore, low frequency waves are the only ones that may give rise to the perturbations.

We emphasize that if such processes are responsible for the perturbations of the signal, it is simple to explain why these perturbations are correlated with solar flares, which are known to generate strong electronic perturbations in the solar corona. Low frequency waves in the solar corona can be either ion-sound waves or magneto-sound waves depending on the magnetic field.

Evidence for the existence of ion-sound waves in the solar corona are of three types: (1), Gordon[3] explained strong radar backscatter from the Sun using them; (2), scintillations of radio sources allow us to study the density fluctuation and therefore the level of turbulence $W_{turb}/W_{th.e}$ in the corona, at least at high altitudes; (3), broadening of sources of solar origin (such as Type I and Type III bursts[4]) indicates that density fluctuations are certainly present.

From all these data we estimate that at four solar radii the level of turbulence is of the order of a few per cent while the frequency ω_s of the ion-sound waves could be of the order of 0.1 Hz.

Our calculations have shown that induced processes are negligible and that the decay process (e.m. wave → e.m. wave + ion-sound wave[6]) gives essentially a broadening of the line:

$$\Delta\sigma^2 = \frac{1}{512\pi} \frac{\omega_e^4 \omega_s}{\omega^2} \frac{L}{c} \frac{c_s}{c} \frac{W_{turb}}{W_{th.e}}$$

where $W_{turb}/W_{th.e}$ is the level of turbulence and L the interaction length between the solar plasma and the telemetry signal. At four solar radii, ω_e and c_s/c have to be taken respectively as 10^7 Hz and 3×10^{-4}, leading to a broadening of the line of the order of 10 Hz as observed. Further, the scattering process (e.m. wave + ion-sound wave → e.m. wave + ion-sound wave[7]) could very well produce a redshift. Detailed calculations of this effect will be presented elsewhere.

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1 Goldstein, R. M., *Science*, **144**, 598 (1969).
2 Pecker, J.-C., Roberts, A. P., and Vigier, J.-P., *Nature*, **237**, 227 (1972).
2a Pecker, J.-C., Tait, W., and Vigier, J.-P., *Nature*, **241**, 338 (1973).
3 Gordon, I. M., *Astrophys. Lett.*, **2**, 49 (1968).
4 Aubier-Giraud, M., Boichot, A., Leblanc, Y., and Steinberg, J. L., *Astr. Astrophys.*, **10**, 362 (1971).
5 Little, L. T., *Astr. Astrophys.*, **10**, 301 (1971).
6 Kovrijnikh, L. M., *Soviet Phys. JETP*, **21**, 744 (1965).
7 Tsytovitch, V. N., *Soviet Phys. Uspekhi*, **9**, 805 (1967).
8 Merat, P., Pecker, J.-C., and Vigier, J.-P., *Astr. Astrophys.*, **30**, 167–174 (1974).

Speeding up astronomical photography

VARIOUS methods have been used for increasing the sensitivity of photographic emulsions prior to exposure in the telescope. For example, washing red sensitive (panchromatic) emulsions in ammonia or water, and latterly baking blue sensitive (orthochromatic) emulsions at 50° C have given reductions in the exposure times required to reach a given density, although at the cost of greater intrinsic fog and non-uniformities, and poorer photometric properties. More recently, placing the photographic plates in vacuum for several hours, with or without simultaneous baking, has come into common use for some emulsions, and this has been followed by baking in a dry nitrogen atmosphere (see ref. 1 and references therein).

The object of baking or vacuum treatment is to remove from the emulsion water vapour and oxygen, which act as desensitising agents. Both techniques depend on the principle of excess partial pressures to drive out the unwanted gas or vapour, in the first by increasing the partial pressures in the emulsion and in the second by reducing them in the ambient atmosphere. Both produce undesirable side effects, the first an increase of intrinsic fog, often non-uniform, the second the removal of all gases and a shrinkage and hardening of the emulsion.

It seemed to us that the objective could be achieved on the basis of excess partial pressures but without introducing undesirable side effects, by prolonged soaking of photographic plates in oxygen-free dry nitrogen of high purity, at room temperature and pressure. So we carried out experiments in the laboratory at Edinburgh in mid-1972, photographic plates being stored in a gas tight tank which

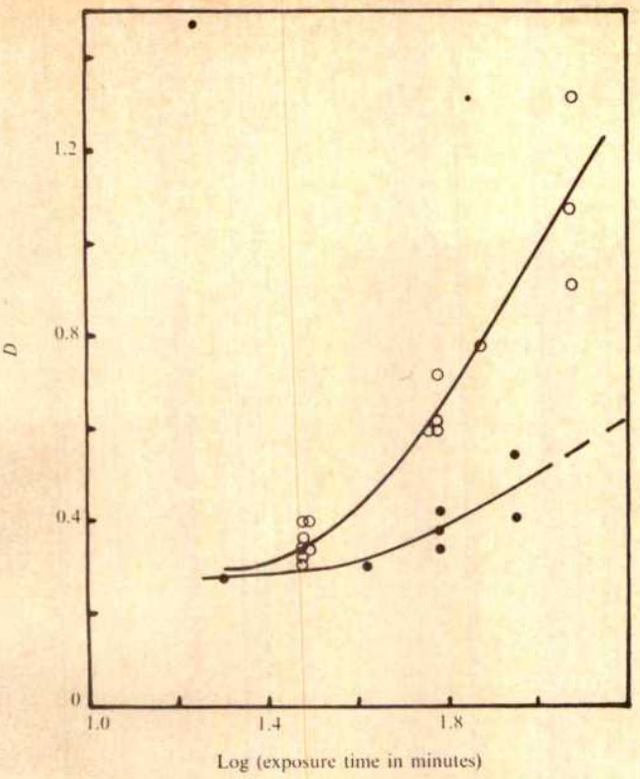


FIG. 1 Photographic diffuse density against logarithm of exposure time in minutes. Open circles, nitrogen sensitised IIIaJ emulsion; filled circles, unsensitised.

was flushed with high purity nitrogen for several minutes each day. These experiments showed that, in the case of the Kodak IIIaJ emulsion, nitrogen soaking for a month was necessary and gave reductions by factors of between two and five in the exposure times needed to reach moderate density—a similar result to that obtained by vacuum baking but without the undesired increase in intrinsic fog.

Prolongation of the soaking gave no further gain but no deleterious effects resulted. Delaying exposure of the plate for 24 h after removal from the nitrogen showed no significant fall in the sensitivity that had been gained—a not unexpected improvement over the alternative baking and vacuum techniques.

These preliminary results were communicated privately to astronomers in Britain, at the European Southern Observatory and at Palomar, and to Eastman Kodak.

The nitrogen soaking technique has been in routine use with the *f*2.5 UK 48-inch Schmidt Telescope at Sliding Spring, Australia since the telescope was commissioned in July/August 1973. Here we report on the efficacy of the technique in operating conditions; this is important because it is well known that the photometric properties of photographic emulsions generally depend on the temperature and humidity prevailing during exposure in the telescope. The results refer to Eastman Kodak IIIaJ plates (batch 2C3) since this emulsion type is to be used for the Science Research Council's southern sky survey.

Figure 1 shows the densities reached with given exposure times in the telescope for 15 sensitised and 7 unsensitised plates. The sky brightness during the exposures was $B = 22.25 \pm 0.1$ mag (arc s)$^{-2}$, measured by a photoelectric photometer. The densities measured by a Macbeth densitometer are close to ASA diffuse values.

There were considerable reductions in the exposure times required to reach given densities, by a factor 2.5 at $D = 0.6$ and increasing with density (Fig. 1). Such a gain would, however, be spurious if the photometric properties of the emulsion had shown a corresponding deterioration. It is therefore important to compare the density noise before and after sensitising, and this is done through the factor

$$(D/\sigma_D)^2 \qquad (1)$$

where D is the density and σ_D is the r.m.s. density noise for the sampling aperture used, in this case 25μm × 35μm at the plate. The quantities were measured for nine sensitised and six unsensitised plates and show no significant difference between the two sets.

It is notable that any increase of intrinsic fog density due to the sensitising was ≤ 0.05.

A measure of efficiency sometimes used in the USA is

$$\text{overall efficiency} = \left(\frac{dD/dE}{\sigma_D}\right)^2 S \qquad (2)$$

where E denotes exposure and S the reciprocal exposure required to reach a specular density of 1.0 (probably close to $D = 0.6$ in this paper). The results produced here for the nitrogen soaking technique show a gain by a factor of about 20 in this measure at $D = 0.6$, about equal to the gain reported for baking in nitrogen.

Using sensitised IIIaJ plates, limiting magnitudes have been determined by photographing the star cluster Kron 3 which contains a photoelectronographic sequence to $B = 22.7$ by Walker[2]. These show that $B = 22.3$ is detected in 30 min, $B = 22.7$ is visible in 1 h, and easily visible in 2 h. A reproduction of a 2 h exposure is shown in Fig. 2. For comparison, an optimum 40 min exposure on IIaO emulsion reaches $B = 21.5$.

The nitrogen soaking technique has been tested on Kodak IIaO emulsion by B. A. Peterson and J. McKinlay at the

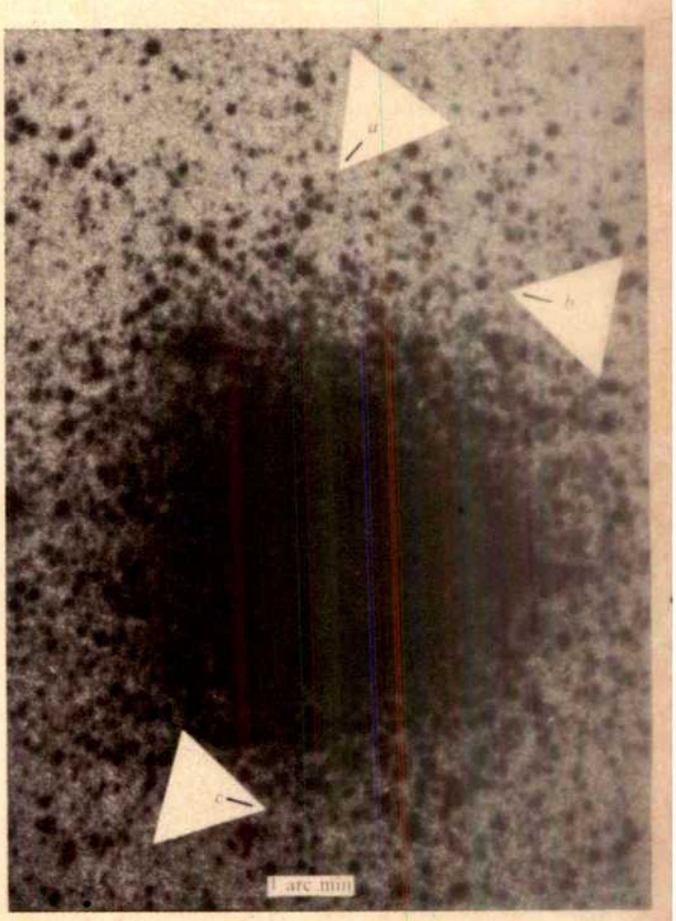


FIG. 2 Enlargement of the star cluster Kron 3 in the field of the Small Magellanic Cloud. Exposure 2 hours on nitrogen sensitised IIIaJ plate with the UK 48-inch Schmidt telescope. According to Walker's photoelectronographic measurements, the stars marked *a*, *b* and *c* are respectively of magnitudes $B = 22.7$, 22.3 and 21.7.

Mt Stromlo Observatory, and they report the same gain as for baking but without the undesirable increase in intrinsic fog.

The large gain in efficiency obtained by nitrogen soaking of the Kodak IIIaJ emulsion has led to it being used for the Science Research Council's southern sky survey which will consequently have a limiting magnitude $B = 23$. This is two magnitudes fainter than the Palomar survey of the northern sky which was caried out before this emulsion was developed.

Another emulsion with the high signal to noise characteristic of the Kodak IIIaJ is the Ilford R40. This panchromatic process emulsion was brought into use by one of us (V. C. R.) at the Royal Observatory Edinburgh in 1956 and has since been used regularly there and by the UK 48-inch Schmidt Telescope in Australia. It has good photometric characteristics, low noise and high uniformity. Nitrogen soaking does not affect its sensitivity. It is difficult to compare its performance with that of the IIIaJ because of the three times wider bandwidth of the R40 which has a peak sensitivity in the red, and because the telescope corrector plate is optimised for the blue waveband giving better images on the IIIaJ, but one hour exposures of the same field taken with sensitised IIIaJ and unsensitised R40 plates show that most objects occurring on either plate occur on the other.

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[1] Hoag, A. A., and Schoening, W. E., *Emulsion Bake Tests*, (Kitt Peak National Observatory preprint, 1973).
[2] Walker, M. F., *Astrophys. J.*, **161**, 835 (1970).

Molecular hydrogen in X-ray astronomy

There is a serious discrepancy between determinations of the column density of interstellar matter in the direction of the Crab Nebula. Virtually every soft X-ray observation[1–5] with sufficient energy resolution to derive this column density yields a result substantially in excess of determinations based on 21-cm absorption[6–8]. The quality of the X-ray and radio data is now such that this disagreement must represent a genuine physical effect rather than an artefact of the data analysis.

It seems likely that this situation also applies to many or all galactic X-ray sources. The discrepancy is easily recognised in the Crab as it is a strong source with a well defined, nonvariable, power-law spectrum extending over many decades, but many other low latitude X-ray sources show a similar effect[9], as does a recent detailed study of the Tycho supernova remnant[10]. If the cause of this anomalous absorption is interstellar, it will affect all distance estimates of X-ray sources based on correlated 21-cm and soft X-ray observations.

So far there is no satisfactory explanation of this phenomenon. An increased abundance of heavy elements over that assumed in the X-ray photoelectric absorption cross section[11,12] has been considered[1,2,4], but the range of elements responsible for absorption in the 0.5–1.5 keV band is limited. Oxygen is eliminated[1] as the required absorption edge does not appear prominently in X-ray spectra. Helium cannot be solely responsible[4] since the required abundance increase would be hopelessly inconsistent with all other data. A substantial increase in the Brown and Gould[12] neon abundance coupled with a modest increase in helium could reconcile the data[4] but the latest estimates of the cosmic neon abundance[13–16] are at variance with this explanation.

Absorption due to interstellar grains with an extreme superabundance of carbon has also been examined[1]. An increase of the cosmic carbon abundance by an order of magnitude above currently accepted values is required. Carbon is one of the few reasonably well-determined heavy element abundances, so this seems unlikely.

I suggest that interstellar molecular hydrogen is responsible for a significant component of the necessary opacity. The predominance of molecular hydrogen in dark interstellar clouds is well understood theoretically and established observationally, and the very low abundance of H_2 in regions of negligible optical extinction also presents a consistent theoretical and observational picture. But the intermediate case of the fractional H_2 abundance in interstellar clouds of low to moderate optical extinction is not nearly so well studied or understood, and this is in fact the case appropriate to the Crab Nebula.

The optical extinction in the line of sight to the nebula has been determined by several methods[17–19] whose results agree quite well and centre on $A_V = 1.8$ mag. In this region of moderate extinction there have been detections of both hydroxyl[20] and formaldehyde (C. Heiles and T. Troland, personal communication; R. Davies, personal communication) in absorption against the Crab continuum source. These observations indicate two distinct molecular clouds which correspond well with neutral hydrogen features in both velocity and profile[6,7].

The chief problem is whether clouds of such moderate optical depth provide sufficient shielding from the interstellar ultraviolet radiation field so that any significant H_2 abundance may be expected in an equilibrium situation. I examine the feature[6] at 11 km s^{-1} and, since two molecular clouds are observed, assume that it provides about half the observed visible extinction, implying an optical depth of the cloud centre in visible light[21] of 0.4. It could be argued instead that the extinction increases in a completely linear fashion over the distance of 2 kpc, although the available data are against this possibility[18]. This, then, is an assumption of the model, although hardly an extreme one, and the X-ray data itself is evidence in its favour. Using the model parameters of ref. 6 and the shielding criterion of Hollenbach *et al.*[22], I find that H_2 can be formed for fractional molecular abundance of $f \gtrsim 0.8$. This is in fact close to the abundance that is observed by OAO for some stars with comparable visual extinction[23]. Under these assumptions, this cloud could contain $N(H_2) \geq 1.4 \times 10^{21}$ molecules cm^{-2}. Although interferometric studies[24] have shown small gradients in optical depth across the face of the radio source, the X-ray source is of much smaller spatial extent[25] and so all this material should be available to attenuate the X-ray spectrum.

This argument is conservative in that the direct observation of hydroxyl and formaldehyde is alone compelling evidence for the presence of a substantial amount of H_2. The nominal lifetime of these molecules in clouds of moderate optical depth is extremely short, which strongly implies a deficiency in the theoretical calculations of molecular formation or destruction rates, or both, in the sense that larger fractional abundances than predicted are invariably observed[7,26]. In particular, Herbst and Klemperer[27] have noted that all reactions which effectively produce formaldehyde require molecular hydrogen.

Can the inferred amount of H_2 provide the missing opacity? Unfortunately the photoelectric absorption cross-section of H_2 at soft X-ray energies is not well established, with theory[12] and experiment[28] in some disagreement between 0.1 and 10 keV, but calculations and experiments agree on the somewhat surprising result that the H_2 photoelectric cross section substantially exceeds twice the atomic cross section, apparently because of molecular bonding effects. There are no measurements at precisely the wavelengths of interest but Brown and Gould[12] estimate a cross section

of 180 b per molecule at 1 keV, giving the cloud under consideration an X-ray optical depth at this energy of $\tau \geq 0.25$. The amount of 'missing matter' generally derived by most experimenters[4,5] is of order 1×10^{21} atoms cm^{-2} or an optical depth $\tau = 0.3$. Thus the H_2 in this cloud alone may make a substantial contribution to the necessary opacity. Since both the theoretical and experimental values of the cross section increase more rapidly than that of the overall interstellar medium as one considers energies < 1 keV, even the smallest molecular hydrogen cross section recently suggested[28] will supply sufficient opacity at 0.5 keV to explain the entire discrepancy.

This sharp increase of the cross section at longer wavelengths also enables us to predict that the apparent opacity discrepancy should become worse for observations at softer energies. Thus a positive detection of the Crab source at 0.25 keV, which may be within present capabilities, would provide a test of this proposal. It may also be possible to observe the H_2 directly in absorption against bright stars with small angular displacements from the Crab using satellite-borne equipment. The colour excesses of the closest such stars, Zeta Tau and 114 Tau, indicate however that they are probably not behind the same cloud as the nebula itself.

There are several problems using correlated 21-cm and soft X-ray data to set distances to galactic X-ray sources[29]. Intrinsically variable and self-absorbed X-ray spectra, uncertain cross sections, abundances, spatial abundance gradients and interstellar medium excitation temperatures all contribute errors. If we now include the possible presence of non-negligible amounts of H_2 in the direction of many galactic X-ray sources it seems that, contrary to the conclusions of several previous investigations, such correlations may not be useful as accurate distance indicators.

The hypothesis presented here may have possible implications for observations of the soft component of the diffuse X-ray background. These molecular clouds of low-to-moderate optical depth are most easily recognised in absorption against a few strong radio sources but if they are present elsewhere in any significant number they could cause substantial small-scale granularity in the observed X-ray flux. This granularity will not necessarily be well correlated with 21-cm data over small regions of sky and could lead to the erroneous conclusion[30] that the observed flux is not of extragalactic origin.

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1 Fritz, G., Meekins, J. F., Chubb, T. A., Friedman, H., and Henry, R. C., *Astrophys. J. Lett.*, **164**, L55 (1971).
2 Henry, R., Fritz, G., Meekins, J., Chubb, T., and Friedman, H., *Astrophys. J.*, **174**, 389 (1972).
3 Clark, G. W., Bradt, H. V., Lewin, W. H. G., Markert, T. H., Schnopper, H. W., and Sprott, G. F., *Astrophys. J.*, **179**, 263 (1973).
4 Charles, P. A., Culhane, J. L., and Tuohy, I. R., *Mon. Not. R. astr. Soc.*, **165**, 355 (1973).
5 Hill, R. W., Burginyon, G. A., Seward, F. D., Stoering, J. P., and Toor, A., *Astrophys. J.*, **187**, 505 (1974).
6 Clark, B. G., *Astrophys. J.*, **142**, 1398 (1965).
7 Davies, R. D., and Matthews, H. E., *Mon. Not. R. astr. Soc.*, **156**, 253 (1972).
8 Hughes, M. P., Thompson, A. R., and Colvin, R. S., *Astrophys. J. Suppl.*, **23**, No. 200 (1971).
9 Ilovaisky, S. A., *Astr. Astrophys.*, **11**, 134 (1971).
10 Coleman, P. L., Bunner, A. N., Krauschaar, W. L., McCammon, D., Williamson, F. O., Kellogg, E., and Koch, D., *Astrophys. J. Lett.*, **185**, L121 (1973).
11 Bell, K. L., and Kingston, A. E., *Mon. Not. R. astr. Soc.*, **136**, 241 (1967).
12 Brown, R. L., and Gould, R. J., *Phys. Rev. D.*, **1**, 2252 (1970).
13 Dalgarno, A., and McCray, R. A., *A. Rev. Astr. Astrophys.*, **10**, 375 (1972).
14 Tucker, W. H., and Koren, M., *Astrophys. J.*, **168**, 283 (1971).
15 Davidson, K., *Astrophys. J.*, **171**, 213 (1972).
16 MacAlpine, G., *Astrophys. J.*, **175**, 11 (1972).
17 O'Dell, C. R., *Astrophys. J.*, **136**, 809 (1962).
18 Brodskaya, E. S., *Bull. Crimean Astrophys. Obs.*, **30**, 126 (1963).
19 Miller, J. S., *Astrophys. J. Lett.*, **180**, L83 (1973).
20 Goss, W. M., *Astrophys. J. Suppl.*, **15**, No. 137 (1968).
21 Spitzer, L., *Diffuse Matter in Space* (Interscience, Princeton, 1968).
22 Hollenbach, D. J., Werner, M. W., and Salpeter, E. E., *Astrophys. J.*, **163**, 165 (1971).
23 Spitzer, L., Drake, J. F., Jenkins, E. B., Morton, D. C., Rogerson, J. B., and York, D. G., *Astrophys. J. Lett.*, **181**, L116 (1973).
24 Greisen, E. W., *Astrophys. J.*, **184**, 379 (1973).
25 Bowyer, S., Byram, E. T., Chubb, T. A., and Friedman, H., *Science*, **146**, 912 (1964).
26 Heiles, C., and Gordon, M. A., in *Interstellar Dust and Related Topics, IAU Symp. No. 52*, (edit. by Greenberg, J. and van de Hulst, H.), (Reidel, Dordrecht, 1973).
27 Herbst, E., and Klemperer, W., *Astrophys. J.*, **185**, 505 (1973).
28 Cruddace, R., Paresce, F., Bowyer, S., and Lampton, M., *Astrophys. J.*, **187**, 497 (1974).
29 Hayakawa, S., in *X- and Gamma-Ray Astronomy, IAU Symp. No. 55*, (edit. by Bradt, H., and Giacconi, R.), 235 (Reidel, Dordrecht, 1973).
30 Gorenstein, P., and Tucker, W. H., *Astrophys. J.*, **176**, 333 (1972).

Kilometre and centimetre Earth strainmeters

IT has been customary to construct strainmeters within a length range of 5–500 m (ref. 1). Prior to the instruments reported here the longest instrument was a laser strainmeter 1,020 m long[2] and the shortest also a laser instrument 1 m long[3].

It has been recognised for many years that long strainmeters are desirable for the measurement of strains near the Earth's surface. There are two principal advantages: first, a long strainmeter averages out short wavelength variations in the strain field; second, the signal to be measured increases proportionately with instrument length whereas the 'attachment noise', caused by instabilities in coupling the instrument to the ground, remains constant. The long instrument approach using a laser system has been successfully operated above the Earth's surface[4].

The advantages of short instruments lie in their portability and ease of installation if suitable low noise methods of mounting them to the rock can be used.

The instruments described here are mechanical, remarkably simple and inexpensive. The long instrument is 1,025 m long (thus the longest strainmeter ever built) and the short instrument is 45 cm long and is the shortest ever built. The

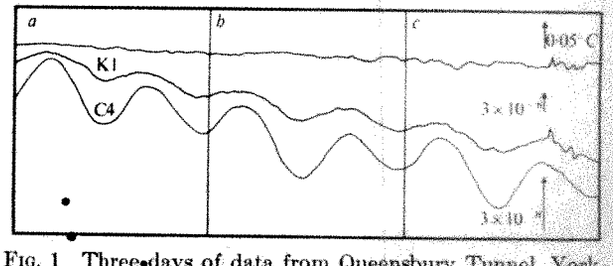


FIG. 1 Three days of data from Queensbury Tunnel, Yorkshire. *a*, September 13; *b*, September 14; *c*, September 15, 1973. K1 is a 1.025 km wire strainmeter and C4 a buried 10 m wire strainmeter. The upper trace is from a thermistor thermometer. Earth strain extension upwards.

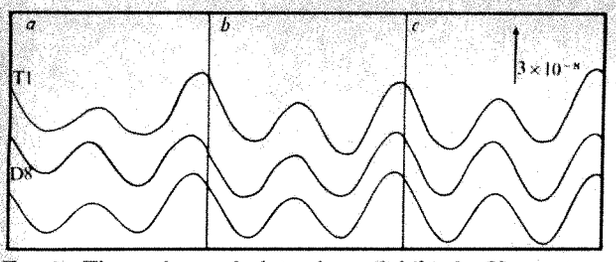


FIG. 2 Three days of data from Schiltach Observatory, Schwarzwald. *a*, January 15; *b*, January 16; *c*, January 17, 1973. The upper trace is from a 45 cm strainmeter (N20E) and trace D8 is from a nearby 10 m wire strainmeter (N2E). The lower trace is the predicted tidal strain (N2E). A linear trend of 3×10^{-8} strain contraction per day has been removed from the observed data.

two strainmeters have operated well for 6 and 12 months respectively.

As a length standard the 1,025 m strainmeter uses a 0.38 mm diameter Invar 36 wire held under constant tension. The wire is supported at approximately 9 m intervals along its entire length by thin wires which constrain vertical and transverse motions but allow longitudinal movements. At each end the strainmeter is attached to the gravel floor of the tunnel by a 20 kg steel plate resting on a thin bed of moist sand. At one end the wire attaches directly to the plate and at the other end a simple weight and lever system provides a constant tension of approximately 15 N. The principle of the tensioning system is the same as for other wire strainmeters developed at Cambridge[5,6]. The resonant modes of the instrument are complex; the amplitude of the fundamental resonant frequency (approximately 0.05 Hz) is reduced by mechanical damping and electronic filtering. A micrometer at one end provides a means of calibrating the instrument but this is only to within 20% due to uncertainties in the restoring force produced at each support point.

We made no attempt to protect the length standard from its environment. Consequently, some parts of the wire are subject to water dripping from the roof and others are exposed to air currents near imperfectly sealed ventilation shafts. Figure 1 shows three days of data. The upper trace records air temperature near the end of the long strainmeter. The lower trace is from a standard 10 m wire strainmeter enclosed in a pipe 50 cm below the tunnel floor. The remaining record is from the long wire strainmeter. Solid Earth tides are clearly seen on this instrument and it is obvious that some of the noise correlates with temperature changes. The higher noise level of the long instrument is not unexpected in view of its exposure to the air, an aspect of the design which clearly can be improved.

An interesting feature of multiple-support wire strainmeters is that within reasonable limits the instrument need not be straight. This ability to tolerate slight lateral or vertical offsets avoids the rigorous site constraints associated with other forms of long strainmeter.

The 45 cm strainmeter uses a 1.5 mm diameter Invar 36 wire as a length standard which is sufficiently stiff to support itself horizontally. The instrument spans a block joint in granite within the Geowissenschaftliches Gemeinschaftsobservatorium at Schiltach in the Schwarzwald, Germany. The purpose of the installation was to observe any anomalous tidal movements between the granite blocks but it has been found that tidal amplitudes are normal. The instrument is attached at each end by an expanding rock bolt. At one end the wire is bolted directly to the granite and at the other end the wire is fastened to an electromagnetic displacement transducer. The calibration of the system is estimated to be not better than ±10%. The output is recorded on a multi-channel chart recorder together with four standard 10 m strainmeters. Three days of record are shown in Fig. 2, together with the predicted tidal strain for Schiltach.

The 45 cm instrument gives a clear tidal record. Its azimuth is 18° different from the 10 m instruments and data from all of these instruments have been spectrally analysed[7]. The amplitude agreement between instruments is within 10% at both diurnal and semi-diurnal periods. The close agreement of the 45 cm strainmeter with the 10 m instruments is surprising since alignment and attachment errors are correspondingly larger in the small device. In some circumstances it is conceivable that an array of colinear small instruments may provide a more representative value than a single larger instrument.

It is possible to use inexpensive mechanical devices as strainmeters with both very short and very long spans. There is no reason to suppose that it is impractical to build strainmeters with dimensions beyond the limits of those described. Whether this ability will prove useful or not is a separate and complicated problem which will only be resolved by experiments with large numbers of instruments.

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1 Bilham, R. G., *Phil. Trans. R. Soc.*, **A274**, 429 (1973).
2 Vali, V., and Bostrom, R., *Earth planet. Sci. Lett.*, **4**, 436 (1968).
3 Van Veen, H. J., Savino, J., and Alsop, L. E., *J. geophys. Res.*, **71**, 5478 (1966).
4 Berger, J., and Lovberg, R. H., *Rev. scient. Instrum.*, **40**, 12, 1569 (1969).
5 Bilham, R. G., and King, G. C. P., *Observatoire Royale de Belgique, Comm. A Series Geophysics*, **101**, 258 (1971).
6 King, G. C. P., and Bilham, R. G., *Phil. Trans. R. Soc.*, **A274**, 209 (1973).
7 Bilham, R. G., Emter, D., and King, G. C. P., *Seventh International Symposium on Earth Tides.* (Sopron, 10–15 Sept., 1973).

Production of secondary ice particles during the riming process

ICE crystals play a vital part in the formation of precipitation, particularly outside the tropics, so it is important to know the concentrations of ice particles in natural clouds and to attempt to predict them from measurements made, for example in laboratory cloud chambers, at the same temperature.

There are sometimes large discrepancies, however[1]; for example[2] in maritime cumuli the concentration of crystals can exceed the concentration of ice nuclei as measured in a cloud chamber by a factor of 10^4 at −10° C. One of the oldest explanations[3] for this, revived at intervals[2,4], is that ice particles growing by sweeping up supercooled drops might throw off secondary 'splinters' of ice. Recent laboratory experiments have, however, failed to show any appreciable splinter production in the riming process (ref. 5 and S.C.M., J. L. Brownscombe and G. J. Collins, to be published). We now present new experimental evidence that copious secondary ice particles are thrown off when ice particles grow by riming in clouds at about −5° C. This phenomenon, which occurs under a narrow range of conditions not covered by previous investigators, will probably explain the anomaly.

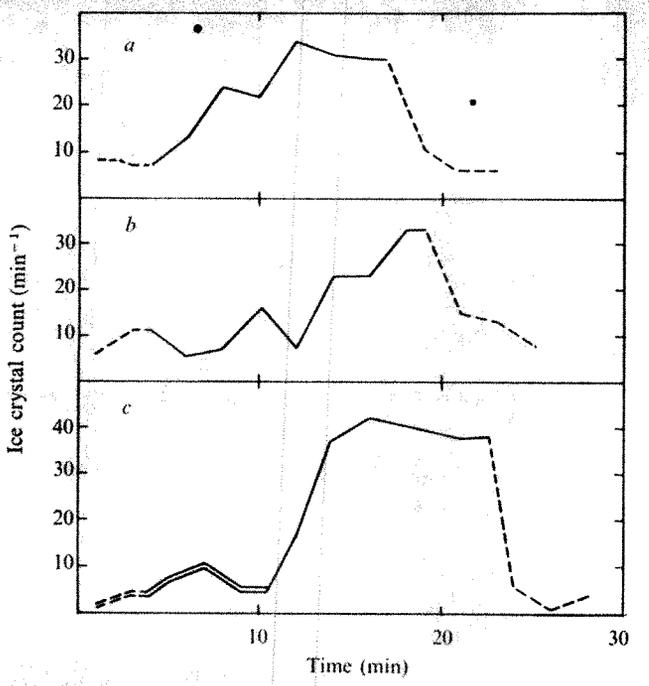


FIG. 1 Production of ice crystals by riming at $-5\pm1°$ C - - -, Target rod stationary; ———, target rod moving at 2.7 m s^{-1}. *a*, Typical experiment with target rod in normal condition; *b*, Target rod coated with glycerin; *c*, Target rod heated above 0° C during period when curve is shown double.

Our experiments were carried out in a plastic walled chamber 1.5 m × 1.5 m × 1.7 m high, enclosed in a cold room. A supercooled cloud of reproducible characteristics was made by injecting steam into the chamber from a boiler run at constant power. Rime was grown on a vertical metal rod 30 cm long and 0.24 cm in diameter which could be moved in a circular path of diameter 30 cm about a vertical axis, at velocities up to about 3 m s^{-1}, in the cloud. The bottom of the riming rod was 64 cm away from the steam inlet at closest approach. Ice crystals could readily be detected by eye in a light beam which traversed the chamber below the riming rod, provided one looked towards the light source at about 10° above or below the beam. With the rod stationary a few crystals per minute appeared in the beam. When the rod was rotated so as to sweep up water droplets which froze and built up as rime on the leading edge, the rate at which crystals appeared increased, to reach an approximately constant value after 4 min. When rotation was stopped, the count of crystals per minute returned to the 'background' value within 4 min. A typical experiment at a cloud temperature of −4.5° C is illustrated in Fig. 1*a*.

Two types of experiment demonstrated that the increase in ice crystal count was indeed caused by the riming and not by some other process, such as the detachment of ice fragments from the walls of the cloud chamber. In the first type of experiment, illustrated in Fig. 1*b*, the target rod was coated with glycerin. The ice crystal counts stayed small for the first 8 min of rotation, then increased when the glycerin had been sufficiently diluted for the supercooled drops to freeze and build up a layer of rime on the rod. In the second type of experiment, illustrated in Fig. 1*c*, the target rod could be heated by an internal electric heater to a temperature above 0° C. No rime grew on the moving rod and the crystal counts increased only slightly above the background value. When the heater was switched off and rime grew on the rod, the counts increased in a way similar to those shown in Fig. 1*a*.

The relationship between the rate of appearance of ice crystals in the light beam and the total rate of production of crystals was established by placing trays of supercooled sugar solution at various positions on the floor of the cloud chamber and counting the number of crystals appearing in the sugar in a given time. A rate of appearance of one crystal in the beam per minute corresponded to a total production rate of 12 crystals per second. The rate of production of secondary crystals (after subtracting the background) was then divided by the rate of accretion of rime to give the number of crystals per milligram of rime.

Figure 2 indicates how the rate of production of ice particles varies with cloud temperature and shows that splinter production is virtually confined to the temperature range −3° C to −8° C. These experiments were carried out at a target velocity of about 2.7 m s^{-1}. As the velocity was decreased, the rate of production of splinters per unit weight of rime increased to a maximum and then fell away to zero at about 0.7 m s^{-1} (Fig. 3).

At a cloud temperature of about −5° C the density of the rime increased with velocity from 0.1 g ml^{-1} at 0.7 m s^{-1} to 0.5 g ml^{-1} at 2.7 m s^{-1}.

By attaching a glass slide, 3 mm wide and coated with MgO, to the riming rod and rotating it at 2.7 m s^{-1} in our experimental cloud at −5° C, we have established that the maximum drop diameter is 35 μm and that such drops are found in a concentration of 0.2 cm^{-3}.

The liquid water content of our experimental cloud was about 1 g m^{-3}, as indicated by an instrument in which the liquid water was evaporated and the resultant dew point measured.

Present experiments shed little light on the physical mechanism by which splinters are produced. The greatest rate of production occurs when the rime surface, being slightly warmer than the cloud, is at about −4.5° C. This is close to the temperature at which ice crystals growing by diffusion show a maximum in the growth rate along the *c* axis and grow as needles or hollow columns[6]. Drop interaction with these extreme crystalline forms may be responsible for splinter formation.

These experiments simulate the growth of graupel particles falling at constant orientation in a supercooled cloud. During the accretion of 1 mg of rime (corresponding to a graupel particle of diameter about 2 mm) several hundred daughter particles may be shed. If each secondary ice particle grows and rimes in its turn, there should be no difficulty in provid-

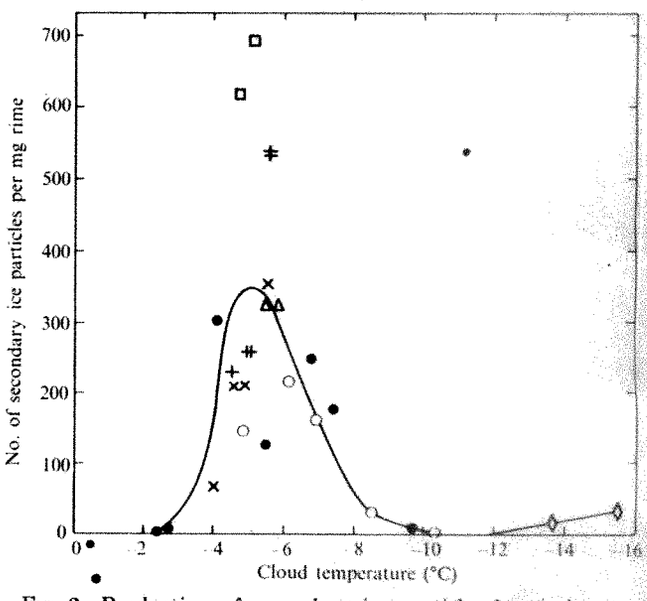


FIG. 2 Production of secondary ice particles by riming as a function of temperature at a target velocity of 2.7 m s^{-1}. Different symbols indicate different days. The curve was drawn by averaging the points over narrow temperature intervals.

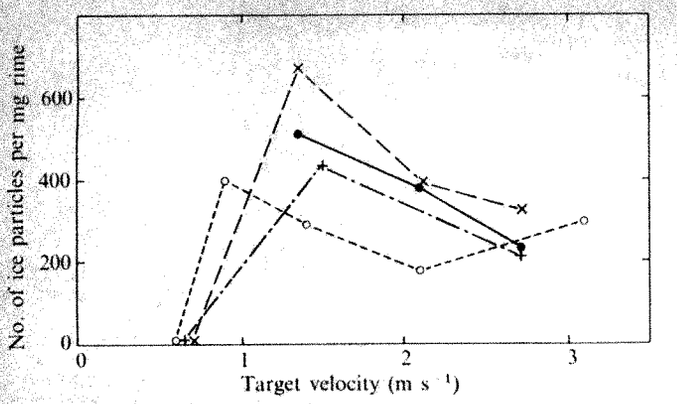


FIG. 3 Production of secondary ice particles by riming as a function of target velocity at a temperature of about −5° C. ●, November 5, 1973; −4.5° C. ×, November 9, 1973; −5.5° C. ○, December 3, 1973; −5.7° C. +, December 5, 1973; −5.2° C.

ing, within a reasonable time, the observed 'multiplication' factor of about 10^4 by which ice crystals sometimes exceed ice nuclei.

We have still to explain why the multiplication process is absent in some natural clouds which span the critical temperature range −3° C to −8° C. In the shallow stratiform clouds sampled by Mossop *et al.*[7], this can be readily explained by the absence of rimed ice particles. The same argument cannot, however, be applied to the winter cumuli over Israel studied by Gagin[8]. These clouds contained plenty of rimed ice particles and also drops up to 36 μm in diameter. Yet there is little evidence of splintering, since the concentrations of ice crystals and ice nuclei agree to within a factor of 10. It may be possible to explain this on dynamical grounds. Maritime cumuli which show comparatively large crystal concentrations are usually wholly below the −10° C level[2], so that much of the graupel growth occurs at temperatures favourable for splintering. In the more vigorous clouds over Israel where the tops reach much colder temperatures, the initial riming growth of graupel particles takes place mainly at temperatures below −10° C. These particles may then fall rapidly, with surface temperature raised by latent heat release. Splinter production apparently requires a surface temperature of about −5° C and therefore depends not only on the ambient temperature of the cloud but also on the fall velocity of the particle and the liquid water content of the cloud[9]. In the Israel clouds the time for which the surface temperature of the graupel particles is close to −5° C may be so short that few secondary ice particles are produced.

We see that the high ice crystal concentrations observed in some cumuli can be explained without postulating earlier growth of the clouds to higher levels[10]: such growth may actually produce smaller concentrations.

The electrical effects accompanying splintering have still to be investigated; they may be important in charge generation in thunderstorms.

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1 Dobson, G. M. B., *Q. Jl R. met. Soc.*, **75**, 117 (1949).
2 Mossop, S. C., Ono, A., and Wishart, E. R., *Q. Jl R. met. Soc.*, **96**, 487 (1970).
3 Brewer, A. W., and Palmer, H. P., *Nature*, **164**, 312 (1949).
4 Mossop, S. C., *Bull. Am. met. Soc.*, **51**, 474 (1970).
5 Aufdermaur, A. N., and Johnson, D. A., *Q. Jl R. met. Soc.*, **98**, 369 (1972).
6 Hallett, J., and Mason, B. J., *Proc. R. Soc.*, **A247**, 440 (1958).
7 Mossop, S. C., Cottis, R. E., and Bartlett, B. M., *Q. Jl R. met. Soc.*, **98**, 105 (1972).
8 Gagin, A., *Proc. int. Conf. Weather Modification, Canberra, September 1971*, 5 (1971).
9 Ludlam, F. H., *Nubila*, **1**, 12 (1958).
10 Mason, B. J., *Nature*, **245**, 451 (1973).

Ultra-thin conducting films of gold on platinum nucleating layers

WE report a method for obtaining highly conducting films of noble metals between 11 and 50 Å thick on mica substrates. At the lower limit, the ultra-thin film (11 Å) may consist only of two or three monolayers each of gold and platinum. The minimum thickness at which pure gold films start to conduct is known to be 30 Å (ref. 1). Before this we had found that a pre-nucleating layer of silver, 5 to 7 Å thick, on mica yielded a highly conducting gold overlayer above a total (Au + Ag) thickness of about 32 Å (ref. 2). It was concluded that nucleating layers of suitable materials, of a thickness below which island formation commences, might lead to a higher conductivity in films of total thickness less than 50 Å. In this range, irregularities in the surface of the substrate, for example cleavage steps, become comparable to, or even greater than the film thickness. Cleaved mica surfaces are known to have hill-and-valley type microstructure. The cleavage step heights are about 20 Å or integer multiples thereof[3]. As film thickness decreases to these values, d.c. measurements of conductivity become progressively less accurate. To alleviate this difficulty, we have measured microwave sheet resistance (R_μ in Ω per square) at 9.7 GHz by Slater's method[4]. The corresponding wavelength is more than six orders of magnitude greater than any irregularity in the surface of the mica. We have used this method previously to estimate the conductivity of gold films thicker than 50 Å (ref. 5).

For the work reported here, platinum was selected as the nucleating material; adhesion of platinum to substrates is known to be better than that of silver[6,7]. The films were deposited on to air-cleaved and clear mica sheets less than 0.001 inch thick, from a multi-crucible electron-beam evaporator. The substrates were kept at room temperature (~ 22° C). Eight targets were placed around the axis of the vapour beam at 50 cm from the source in a manner calculated to deposit films of equal thickness[8]. The mass-thickness of the films was monitored by a quartz crystal microbalance with the AT-cut, 5 MHz crystal placed in the centre of the targets. The crystal plate (0.75 inch-square, 1 mm thick) has a mass-frequency proportionality constant, C_f, of 57.6 Hz cm² μg⁻¹ (ref. 9) giving a sensitivity of 11.13 Hz Å⁻¹ for gold and 12.36 Hz Å⁻¹ for platinum; the bulk and film densities were assumed to be the same. The oscillator frequency was measured by a digital frequency counter and recorded on a chart through a digital-to-analogue converter to ascertain frequency stability. With the crystal seat temperature stabilised by flowing water, the maximum frequency drift recorded over a period of 12 h was ± 5 Hz. At an electron-beam power between 500 to 600 W, the deposition rate was typically 0.5 Å s⁻¹ for both metals. Films were thus deposited in a few seconds but, because the effects of thermal shock[10] lasted more than 5 min, continuous monitoring of rate and

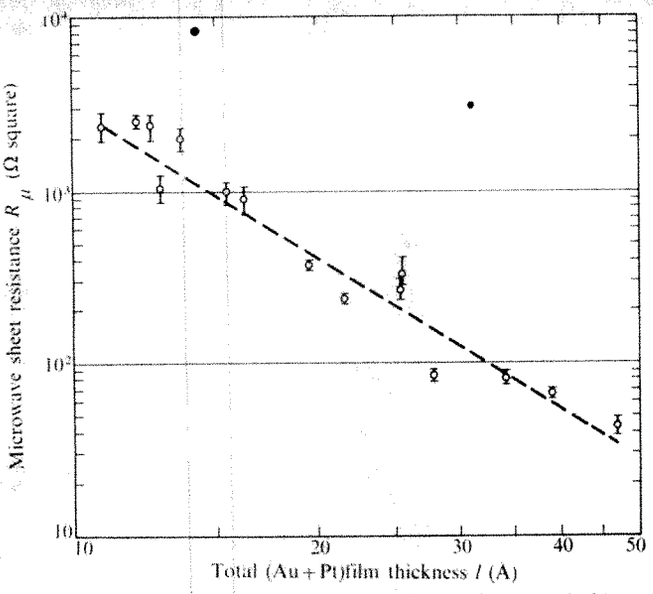


FIG. 1 Microwave sheet resistance, R_μ, against total (Au + Pt) thickness, l. ———, $R_\mu = 2.77 \times 10^6\ l^{-2.944}$ with a correlation coefficient of 0.96.

thickness was not possible. Deposition times were estimated and the difference between the stable pre- and post-deposition frequencies was used to compute the actual film thickness. The starting vacuum was $< 10^{-7}$ torr; the pressure rose to $\sim 5 \times 10^{-6}$ torr during deposition.

Using this method, gold films of varying thicknesses were deposited on to platinum nucleating layers ranging in thickness from 3 to 10 Å. A mean value of R_μ for sets of eight films deposited simultaneously is plotted against the total (Au + Pt) thickness, l, in Fig. 1. The adjusted standard error in R_μ is shown for each set of eight films; l is taken to be accurate within the limits of oscillator stability, that is ± 0.5 Å.

The conduction in these films was found to end abruptly for values of l less than 10.8 Å. Combinations of gold and platinum thicknesses to lower this limit by even 1 Å were not successful. It may, however, be noted that the sticking coefficient, α, of the evaporated films decreases rapidly with increasing lattice mismatch between the substrate and evaporant materials[11]. α also depends on thickness and approaches unity above 250 Å; values for α are not known at 10 Å. The actual thickness of the film of 10.8 Å may, therefore, be only three or four monolayers and conducting films of smaller thickness may not be feasible. The colour of the films obtained varied from light grey at 11 Å to greenish-grey as l approaches 50 Å.

We found that using gold as a nucleating layer did not produce ultra-thin, conducting films of platinum in this range.

It is known that the interatomic forces of attraction between the evaporant atoms, of gold or platinum in this case, cause liquid-like coalescence and consequent island formation, resulting in discontinuous films at thicknesses less than 50 Å. We postulate that, before the onset of island formation, a monolayer or so of a suitable nucleating material has the adatoms locked in the potential well sites on an ionic substrate surface. These then form an effective barrier against surface migration of the oncoming gold atoms and thus induce film growth in monolayers rather than discrete three-dimensional nuclei. The method may also be one with which to induce epitaxy. The choice of nucleating material seems critical.

Preliminary annealing studies on gold-on-platinum films 25 Å thick showed a slight decrease in R_μ up to 210° C; above this temperature R_μ increased slightly. The resistance of gold films alone increased irreversibly with temperature. Although detailed *in situ* electron microscopy and diffraction studies[12] will be necessary to obtain direct evidence, the high electrical conductivity and annealing behaviour of films of gold on platinum may be taken as a reasonable indication that these are continuous. Further work on the ageing and annealing properties, together with a determination of the temperature coefficient of resistance of these films, is in progress. Other nucleating materials are also being investigated.

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1 Cornely, R., and Fuschill, N., *Bull. Am. phys. Soc.*, **18(1)**, 16 (1973).
2 Chaurasia, H. K., thesis, Univ. British Columbia, Vancouver, Canada (1964).
3 Tolansky, S., in *Microstructure of Surfaces using Interferometry*, 15 (American Elsevier, New York, 1968).
4 Slater, J. C., in *Microwave Electronics*, 30 (Van Nostrand, New York, 1950).
5 Chaurasia, H. K., and Voss, W. A. G., *IEEE Trans. Microwave Theory Tech.*, MTT-21(1), 51 (1973).
6 Lepselter, M. P., *Bell System tech. J.*, **45**, 233 (1966).
7 Novak, W. B., and Dyre, R. N., *J. Vac. Sci. Technol.*, **9(1)**, 279 (1972).
8 Glang, R., in *Handbook of Thin Film Technology*, 1–34 (edit. by Maissel, L. I., and Glang, R.) (McGraw-Hill, New York, 1970).
9 Niedermeyer, R., and Hillecke, D., in *Ultra Micro Weight Determination in Controlled Environment*, 213 (edit. by Wolsky, S. P., and Zdanuk, E. J.), (Wiley-Interscience, New York, 1969).
10 Warner, A. W., in *Ultra Micro Weight Determination in Controlled Environment* (edit. by Wolsky, S. P., and Zdanuk, E. J.), 154 (Wiley-Interscience, New York, 1969).
11 Yang, L., Birchenall, C. E., Pound, G. M., and Simmond, M. J., *Acta. Metall.*, **2**, 462 (1954).
12 Poppa, H., Moorhead, R. D., and Heinemann, K., *Nucl. Instrum. Meth.*, **102**, 521 (1972).

Epoxy groups responsible for crosslinking in natural rubber

STORAGE hardening in natural rubber, which is of considerable industrial interest, has been ascribed to the crosslinking of carbonyl groups present in the rubber chain[1]. Supporting evidence is that the crosslinking reaction can be prevented or accelerated by the addition of monofunctional or difunctional amines, respectively. These observations may be explained if the monofunctional amine is regarded as condensing with, and hence destroying, the carbonyl group, whereas the difunctional reagent is capable of reaction with carbonyl groups on separate chains, thus effecting a crosslink. The ability of dimedone (5,5-dimethylcyclohexane-1,3-dione) to inhibit the crosslinking reaction led to the conclusion that the groups were aldehydic[1].

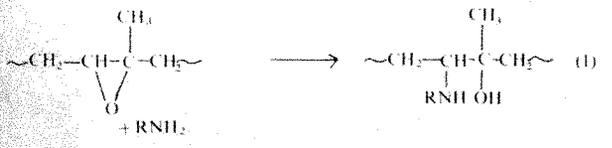
Involvement of aldehyde groups in storage hardening necessitates the presence of complementary 'aldehyde condensing groups' (for example, amino groups) on the macromolecule[2]. It is not easy to account for the presence of aldehyde groups, let alone 'aldehyde condensing groups', on the natural rubber molecule in terms of the known biosynthetic pathways for isoprenoid compounds.

Can other functional groups account for this behaviour? The presence of epoxy groups can provide a mechanism for storage hardening. Epoxidation of the olefinic double bond

has ample precedents among naturally occurring compounds. All classes of terpenoid compounds (with the exception, hitherto, of natural rubber itself) include members that have epoxide groups, and they are found also on the isoprenyl residues of some other compounds of mixed biogenetic origin. Direct evidence for epoxide groups in natural rubber is presented later in this paper.

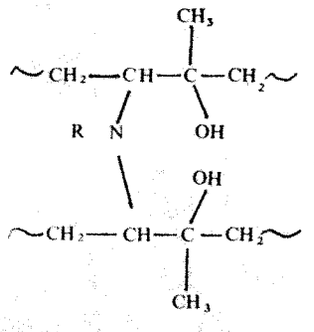
Storage hardening may be envisaged to occur by the following reactions:

(1) Epoxy groups are ring opened by naturally occurring amino-compounds in the latex, for example



(1)

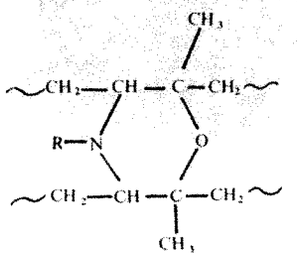
(2) This substituted amine could interact with an epoxy group on an adjacent chain with formation of a crosslink such as



(2)

which may be partially formed at the latex stage.

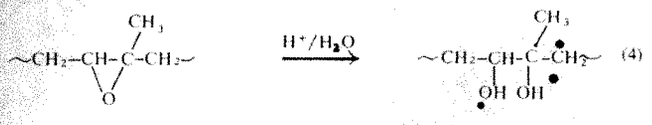
(3) During storage and drying, ring closure of the product of reaction (2) could occur, forming an additional crosslink, for example, the cyclic structure



(3)

The presence of epoxy groups is fully consistent with the experimental results, which apparently favoured aldehydic groups, since ring opening of epoxy groups by amines is well known[3]. Similarly the reactions of epoxides with active hydrogen compounds analogous to dimedone have been reported[4].

Furthermore the present proposals provide insight into the previously unexplained observations that the addition of sodium azide as a preservative to rubber latex leads to a decrease in the number of 'condensing groups'[2], and that conditions of low *p*H during coagulation of the latex reduces the extent of crosslinking on storage[5]. The former is readily explained since the azide ion cleaves epoxide rings to the β-azido alcohol[6]. Similarly the latter observation may be attributed to ring opening of epoxy groups under acidic conditions, for example



(4)

The resulting glycols have no crosslinking ability.

The presence of epoxy groups in natural rubber was demonstrated by making use of reaction (4). A fresh latex sample was treated with acid in order to ring open the epoxide links. The resulting 1,2-diols were cleaved with lead tetraacetate by a method analogous to that used by Flory and Leutner[7]. The chain scission resulting from oxidation of the 1,2-diols was detected by viscometry. The intrinsic viscosity $[\eta]$ of a chloroform solution of the natural rubber sample, at 30° C, before and after treatment with lead tetraacetate is 3.75 and 0.39, respectively.

It is immediately apparent that considerable degradation has occurred. Unforunately, the parameters of the Mark-Houwink viscosity equation for natural rubber in chloroform are not available but, if a reasonable value of $\alpha = 0.7$ is assumed, then the decrease in viscosity corresponds to a decrease in molecular weight by a factor of about 14. This in turn leads to an average number of about 13 epoxy groups per chain. The value is only approximate since not all of the epoxy groups may have ring opened but nevertheless it seems significant that the value is of the same order as that reported previously for 'aldehyde groups' in natural rubber[8].

That the decrease in molecular weight, on degradation with lead tetraacetate, is due to the cleavage of 1,2-diols rather than oxidation of the double bonds is suggested by the rapid reaction, degradation being essentially complete within 30 min. Although oxidation of double bonds by lead tetraacetate has been reported, the reaction is slow and addition products rather than bond cleavage are observed[9].

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[1] Sekhar, B. C., *J. Polymer Sci.*, **48**, 133 (1960).
[2] Sekhar, B. C., *Fourth Rubb. Tech. Conf., London*, 1 (1962).
[3] March, J., *Advanced Organic Chemistry*, 334 (McGraw-Hill, New York, 1968).
[4] Adams, R. M., and Vander Werf, C. A., *J. Am. chem. Soc.*, **72**, 4368 (1950).
[5] Sekhar, B. C., *Malaysia Natural Rubber—New presentation processes*, (Rubber Research Institute of Malaya, 1971).
[6] *Heterocyclic compounds with three and four membered rings* (edit. by Weissberger, A.), Part 1, 628 (Interscience, New York, 1964).
[7] Flory, P. J., and Leutner, F. S., *J. Polymer Sci.*, **3**, 880 (1948).
[8] Sekhar, B. C., *Proc. Nat. Rubb. Res. Conf. Kuala Lumpur*, 512 (1960).
[9] Anderson and Winterstein, *J. org. Chem.*, **28**, 605 (1963).

BIOLOGICAL SCIENCES

Geographical uniformity of selectively neutral polymorphisms

In a recent issue of *Nature*, Bulmer[1] criticised the 'neutral theory'[2] of protein polymorphisms on the ground that the infinite neutral allele hypothesis of Kimura and Crow[3], when combined with Malécot's two-dimensional dispersal model[4,5] leads to results which do not fit to actual data[6–8]. In this note, we intend to show that Bulmer's conclusions are entirely due to the unwarranted assumption of infinite population size inherent in Malécot's formulation, and that when the assumption is corrected, the data in fact are consistent with the neutral theory.

Bulmer claims that there are two contradictions. First, if we use Malécot's formulae for a continuous two-dimensional dispersal model, we get a rather strong geographical localisation of allele frequencies, whereas considerable uniformity is observed in the data. He uses Malécot's two quantities: the inbreeding coefficient (f_0) defined as the probability that

two homologous loci in an individual are identical by descent, and the coefficient of kinship (f_x) defined in a similar way, between two individuals at a distance x apart. Using Malécot's formula for f_0 and f_x which are valid only for a population distributed over a two-dimensional habitat of infinite size, Bulmer calculates the ratio, $\rho_x = f_x/f_0$. The numerical values of ρ_x presented in his examples certainly decreases quite rapidly as x increases. On the other hand, the estimates of this ratio using data from *Drosophila pseudoobscura* are very close to unity even for far distant localities, such as Texas, Colorado and California. He claims then that such a discrepancy cannot be resolved except under the absurd assumption that the root mean square dispersal distance, σ, is of the order of 2,000 km.

Secondly, he points out that the expected degree of homozygosity (f_0) would be much less than that of the data, unless the gene dispersion is very restricted. The average homozygosity found by Prakash *et al.*[6] is 0.877. Bulmer claims, using Malécot's formula for f_0, that to make f_0 to be about this magnitude, the mean square dispersal distance between the breeding places of parent and offspring (σ^2) must be an absurdly small value. For example, if the nutation rate u is 10^{-5}, then the number of flies within a circle of radius σ, that is $\pi\sigma^2\delta$, is about 0.38. This seems to be not only untrue but if the dispersion is restricted to this degree, ρ_x should decrease very rapidly.

It is important to realise that Malécot's formulae used by Bulmer are valid only for cases where the total population number (N) is much greater than the reciprocal of mutation rate (u) and the habitat is large. The size of habitat alone, however, is not sufficient, as long as the population is finite. Of course, if the population is truly infinite, his formulae are valid. On the other hand, if N and $1/u$ are about the same order of magnitude, it is necessary to use formulae valid for a finite population. For such a population, Malécot's formulae cannot be used even as an approximation, because they give entirely different values. Exact analysis for a finite case of torus-like space of size $L_1 \times L_2$ was obtained by Maruyama[9]. For a more realistic case of a rectangular habitat of size $L_1 \times L_2$, although exact solutions were not obtained, it turned out that they can be approximated by solutions of the torus-like space of size $2L_1 \times 2L_2$ (ref. 9). So, we use the torus-like space in the following discussion. If we designate position in the space by Cartesian coordinate system and let $f(x, y)$ be the probability that two homologous genes separated distance x and y along the first and second axes respectively are identical by descent, then

$$f(x, y) = \frac{(1-u)^2(1-f_0)}{2\delta L_1 L_2} \cdot \sum_{m=0}^{\infty}\sum_{n=0}^{\infty} \frac{\Delta_m \Delta_n R_{mn}}{[1-(1-u)^2 R_{mn}]} \cos\frac{2\pi m x}{L_1} \cos\frac{2\pi n y}{L_2} \qquad (1)$$

where $\Delta_0 = 1$ and $\Delta_k = 2$ if $k \neq 0$, δ is the population density and

$$R_{mn} = \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} r(\xi, \eta) \cos\frac{2\pi m\xi}{L_1} \cos\frac{2\pi n\eta}{L_2} \, d\xi \, d\eta$$

in which $r(x, y)$ is the probability that a zygote is formed between gametes of parents born distance $\sqrt{(x^2 + y^2)}$ apart. As a special case, if $r(x, y)$ is a two-dimensional normal density and the migration is isotropic,

$$r(x, y) = \frac{1}{2\pi\sigma^2} \exp\left[-(x^2 + y^2)/(2\sigma^2)\right], \qquad (2)$$

and

$$R_{mn} = \exp\left[-2\pi^2\sigma^2(m^2/L_1^2 + n^2/L_2^2)\right]$$

TABLE 1. Theoretical values of the inbreeding coefficient and genetic correlation

$4Nu$	N	u	$\sigma^2\delta$	f_0	Furthest distance possible/σ	f_{min}^*/f_0
0.08	2×10^6	10^{-8}	2	0.9283	707	0.9624
0.12	3×10^6	10^{-8}	3	0.9862	707	0.9624
0.12	3×10^6	10^{-8}	1.5	0.8998	1000	0.9235
0.2	2.5×10^7	2×10^{-9}	100	0.8335	354	0.9983
0.2	2.5×10^7	2×10^{-9}	50	0.8337	500	0.9966
0.4	10^7	10^{-8}	10	0.7206	707	0.9667
0.4	10^7	10^{-8}	5	0.7268	1000	0.9351
1.0	2.5×10^6	10^{-7}	10	0.5196	354	0.9194
4.0	10^8	10^{-8}	100	0.2051	707	0.9664
10.0	2.5×10^7	10^{-7}	100	0.0976	354	0.9191
40.0	10^8	10^{-7}	50	0.0395	1000	0.8823
40.0	10^7	10^{-6}	5	0.1674	1000	0.6818
100.0	2.5×10^7	10^{-6}	50	0.0263	500	0.3308

* f_{min} is the kinship coefficient between two individuals furthest apart.

In our formula (1), f_0 is involved implicitly, but it can be determined explicitly,

$$f_0 = \frac{(1-u)^2 S}{2\delta L_1 L_2 + (1-u)^2 S} \qquad (3)$$

where

$$S = \sum_{m=0}^{\infty}\sum_{n=0}^{\infty} \frac{\Delta_m \Delta_n R_{mn}}{[1-(1-u)^2 R_{mn}]}$$

Using formulae (1) and (3) with $r(x, y)$ given by (2), and assuming $L_1 = L_2$, we have carried out extensive numerical calculations using our computer TOSBAC 3400. Contrary to Bulmer's claim, we found that theoretical expectations and data are consistent. If Nu is 1 or less and $\sigma^2\delta$ is of order of 100 or 10 or even less, ratio $f(x, y)/f_0$ stays very close to 1 and it is roughly independent of distance and habitat size. Here, a remark on mutation rate is in order. As pointed out by Kimura and Ohta[2], the appropriate mutation rate for neutral alleles is at most 10^{-7} per yr per locus. So, for *Drosophila* whose generation time is some 2 weeks, we should take $u = 10^{-8}$ or less to be consistent with the neutral theory. From these assumptions, it also follows that if we take $4Nu = 0.1 \sim 0.2$, the value of f_0 turns out to be about right order of magnitude consistent with the neutral theory[2]. Note here that N represents the effective population size rather than the actual size. Numerical examples are presented in Table 1, where f_{min} is the kinship coefficient between two individuals furthest distance apart. It should be emphasised that the finiteness of population makes the situation completely different from that of a truly infinite case. Thus, Bulmer is unwarranted in using Malécot's formulae which led him to the erroneous conclusions which seem to be inconsistent with data: Bulmer's dilemma is entirely due to an incorrect assumption of infinite population, but this can be simply resolved by the correct assumption. This may be evident by comparing values in column f_{min}/f_0 of Table 1 in this paper with his tables.

It seems appropriate to discuss here the influence of the dimensionality of space on these properties. Through numerical calculations, we found that a two-dimensional population tends to be nearly panmictic, particularly if $4Nu$ is order of 1 or less, and that panmixity can be attained easily. As a consequence, f_0 is related to N almost directly. On the other hand, in an one-dimensional (or a long but narrow) population, the situation is quite different, and the local differentiation appears, if $\sigma^2\delta$ is small compared with the habitat length. When the habitat size is sufficiently long, the value of f_0 becomes nearly independent of the total popula-

tion size. In such a circumstance, a formula derived assuming an infinite population size gives approximately correct values for f_0 and f_x. But as long as the value of f_0 is influenced by the total size, which is often the case in a two-dimensional population, the finiteness must be taken into account. This is where Bulmer has made an error. We conclude that the pattern of geographical distribution of protein polymorphisms in *Drosophila* as discussed by him is indeed consistent with the neutral theory.

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1 Bulmer, M. G., *Nature*, **241**, 199 (1973).
2 Kimura, M., and Ohta, T., *Nature* **229**, 467 (1971).
3 Kimura, M., and Crow, J. F., *Genetics, Princeton*, **49**, 725 (1964).
4 Malécot, G., *Proc. 5th Berkeley Symp. math. stat. Prob.*, **4**, 317 (1967).
5 Malécot, G., *The Mathematics of Heredity* (Translated by Yermanos, D. M.) (Freeman, San Francisco, 1969).
6 Prakash, S., Lewontin, R. C., and Hubby, J. L., *Genetics, Princeton*, **61**, 841 (1969).
7 Ayala, F. J., Powell, J. R., and Dobzhansky, Th., *Proc. natn. Acad. Sci., U.S.A.*, **68**, 2480 (1971).
8 Lakovaara, S., and Saura, A., *Genetics, Princeton*, **69**, 377 (1971).
9 Maruyama, T., *Genetics, Princeton*, **70**, 639 (1972).

Enzymatic acylation of histidine to mengovirus RNA

PLANT virus RNA from the TYMV family[1–3], TMV[4,5] and BMV[6] can be enzymatically esterfied with valine, histidine and tyrosine, respectively. The aminoacylation of intact or fragmented plant virus RNA[7], may be an important biological function and, therefore, the question as to whether some mammalian viral RNA can be charged with amino acids becomes significant. In this work, we show that mengovirus RNA can, in fact, be specifically acylated with histidine by a mouse liver aminoacyl-tRNA synthetase preparation.

Mengovirus was grown in mouse L-929 cells (clone CCL1) and precipitated by polyethylene glycol. The virus particles were purified by banding in CsCl before the extraction of viral RNA (see legend to Fig. 1). This step was found to be important for the elimination of any contaminating cellular tRNA. To test the homogeneity of the viral RNA, samples were analysed by electrophoresis on polyacrylamide-agarose gels[8,9]. No detectable amounts of RNA of low molecular weight could be found in the viral RNA preparation. The apparent molecular weight of the mengovirus RNA was estimated as 2.3×10^6, by comparing its migration with that of TMV RNA and total *E. coli* RNA (Fig. 1). Our value for the molecular weight differs slightly from previous values for mengovirus, which were based on hydrodynamic measurements (1.74×10^6, ref. 10) or sucrose density gradient sedimentation analyses (2.8×10^6, ref. 11).

Mengovirus RNA can be acylated with a mixture of 16 amino acids by mouse liver aminoacyl-tRNA synthetase (Table 1). On the other hand, MS2 bacteriophage RNA or mouse liver rRNA are not aminoacylated under the same experimental conditions. It is interesting that the heterologous aminoacyl-tRNA synthetase from *E. coli* failed to acylate mengovirus RNA, MS2 bacteriophage RNA or TMV RNA. Mengovirus RNA was found to be esterified solely with histidine by the mouse liver aminoacyl-tRNA synthetase preparation. This was deduced from the finding that the acylation of mengovirus RNA with a mixture of labelled amino acids is hardly suppressed by the addition of excess non-radioactive mixture of amino acids lacking histidine, whereas the addition of excess non-radioactive histidine completely inhibited the reaction.

We have devised an electrophoretic method to determine the size of individually labelled aminoacylated tRNA species.

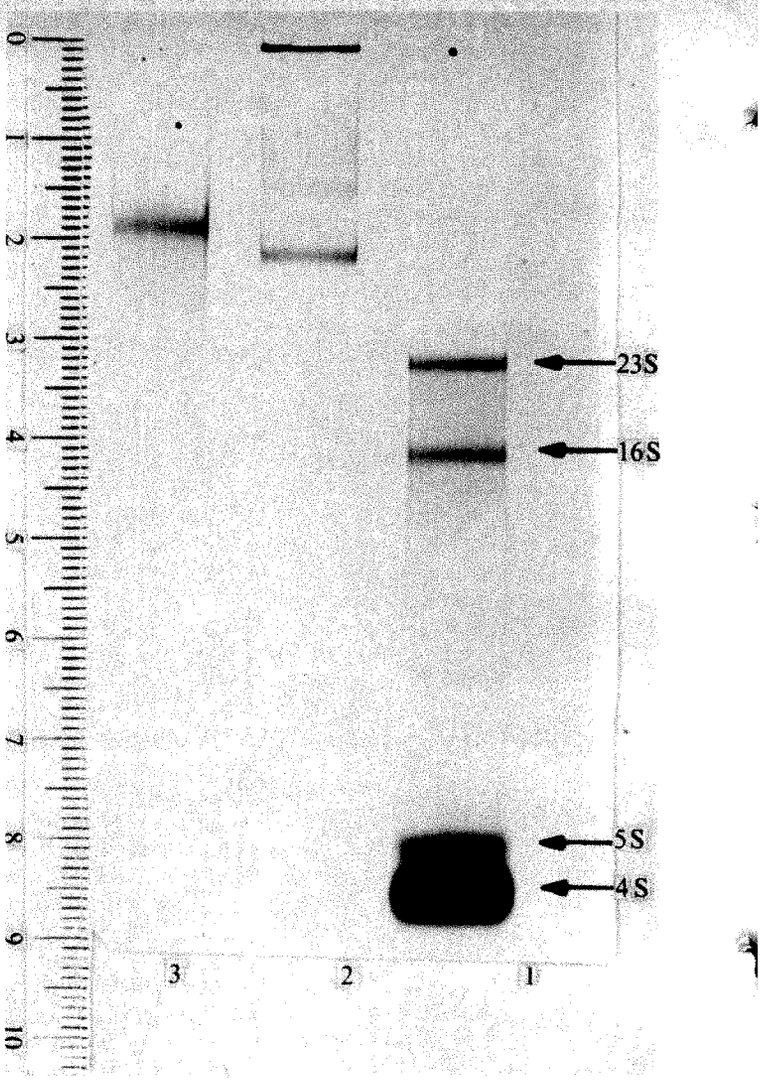


FIG. 1 Polyacrylamide-agarose gel electrophoretic patterns of mengovirus RNA, TMV RNA and total *E. coli* RNA. Confluent monolayers of mouse L-929 cells (clone CCL1) were infected with 2 PFU per cell of mengovirus. After incubation for 1 h at 37° C, 40 ml of Eagle's MEM containing 8% calf serum was added to each 200 cm² bottle. Incubation was continued for 18 h, at which time the cell mass was dislodged by gentle shaking. The suspension was frozen and thawed and the lysate was freed of cell debris by centrifuging at 1000*g* for 5 min at 4° C. The mengovirus suspension was supplemented with 6% polyethylene glycol (6000, Union Carbide) and 0.01% β-mercaptoethanol and stirred at 0° C for 1 h. The precipitate was collected by centrifugation at 12,000*g* for 20 min and suspended with the aid of a glass-teflon homogeniser in 100 mM Tris-HCl, *p*H 7.9 and 10 mM EDTA[11]. The density of the suspension was adjusted to 1.332 g cm^{-3} with CsCl and the virus particles were banded by centrifugation at 40,000 r.p.m. in a type 40 fixed angle rotor in a Beckman Model L ultracentrifuge for 18 h. The band containing the virus particles was collected, diluted three-fold with water and centrifuged for 2 h at 40,000 r.p.m. The RNA was isolated from the virus suspension by phenol–chloroform extraction, followed by ethanol precipitation[15]. Each RNA (1.0–5.0 μg) sample was applied to an 1.7% polyacrylamide–0.5% agarose slab gel. Electrophoresis was carried out for 2.5 h at 0° C at 11.4 V cm^{-1}. The gels were stained with Stains all[9]. Slot 1, Total *E. coli* RNA; slot 2, TMV RNA; slot 3, menogovirus RNA.

To preserve the aminoacyl-RNA ester bond, the aminoacyl-RNA was acylated with N-hydroxysuccinimide acetyl ester to yield N-acetylaminoacyl-RNA. We synthesised the latter, since N-substituted aminoacyl-tRNA derivatives have been shown to be considerably more resistant to hydrolysis than the corresponding unblocked aminoacyl-tRNA derivatives (compare ref. 12). Using N-acetyl-^{3}H-aminoacyl-tRNA molecules, we have been able to estimate their size by monitoring the radioactivity of the sliced gel. Under these conditions, unblocked aminoacyl-tRNA molecules are completely hydrolysed. Figure 2*a* shows the electrophoretic migration of mouse liver N-acetyl-^{3}H-histidyl-tRNA. The electrophoretic pattern of mengovirus RNA bound to N-acetyl-^{3}H-histidine, and run in parallel on the same gel, is shown in Fig. 2*b*. The size of the major RNA peak bearing ^{3}H-histidine, was estimated to be between 0.20×10^6 to 0.35×10^6 daltons. In addition, a small part of the ^{3}H-histidine was attached to RNA of molecular weight 1.25×10^6. When the mengovirus N-acetyl-^{3}H-histidyl-RNA preparation was treated with pancreatic RNase and then analysed by gel electrophoresis, no radioactivity could be detected in the various gel slices.

From these results it is clear that the mengovirus RNA is enzymatically acylated with histidine. It seems that mengovirus RNA is cleaved during the aminoacylation reaction, probably because of contamination of the crude mouse liver aminoacyl-tRNA synthetase preparation with traces of endo-

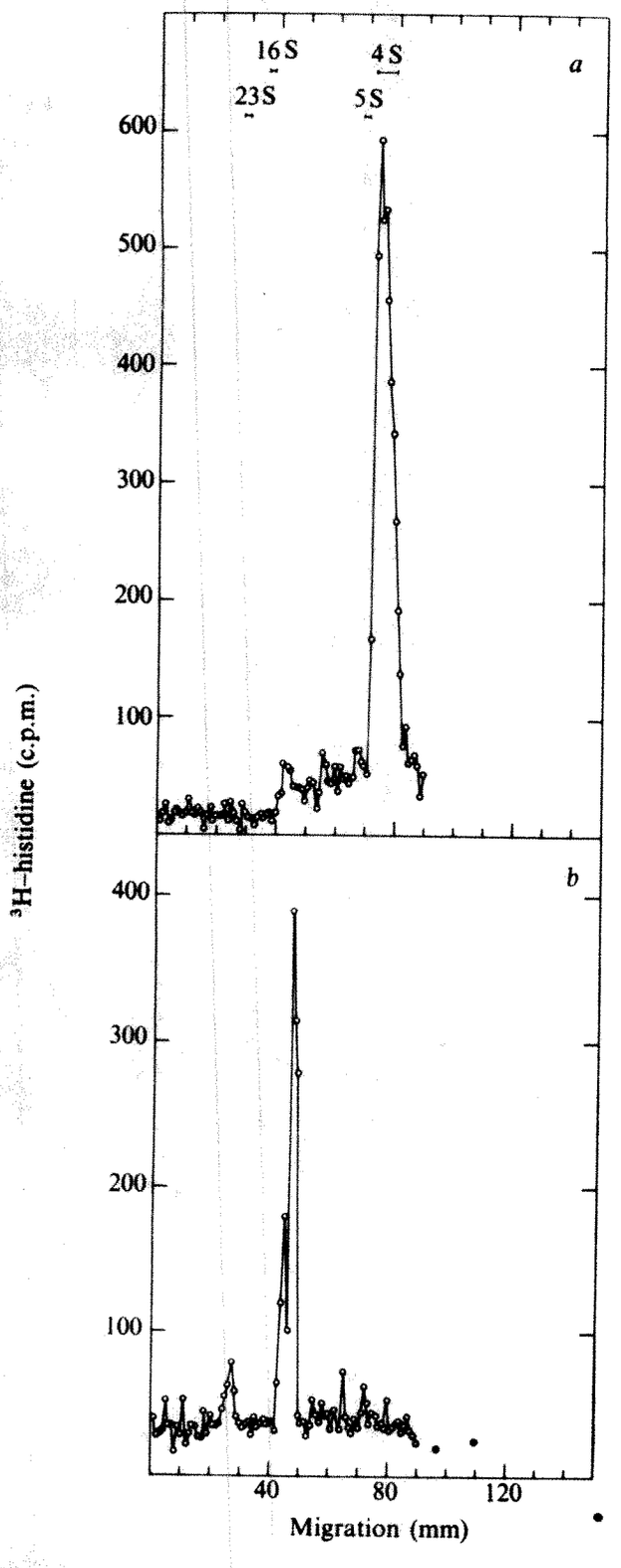


TABLE 1 Aminoacylation of viral RNAs

Source of RNA	Amino acids	Amino acids incorporated (c.p.m. per 10 μg RNA) Mouse liver synthetase	*E. coli* synthetase
Mengovirus	^{3}H-mixture	2,000	170
TMV	^{3}H-mixture	4,800	270
MS2 bacteriophage	^{3}H-mixture	40	190
Mouse liver rRNA	^{3}H-mixture	80	—
Mengovirus	^{3}H-mixture + unlabelled 15 amino acid mixture lacking histidine	1,900	—
Mengovirus	^{3}H-mixture + unlabelled histidine	60	—
Mengovirus	^{3}H-histidine	3,600	—
TMV	^{3}H-histidine	7,800	—

The incubation mixture (0.05 ml) contained: 20 mM Tris-HCl, *p*H 7.5, 1 mM ATP, 10 mM $MgCl_2$, 3 mM dithiothreitol, 0.5 μCi of a mixture of 16 ^{3}H-labelled amino acids (^{3}H-reconstituted protein hydrolysate, Schwartz/Mann, consisting of alanine, arginine, cystine, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine and valine) or ^{3}H-histidine (58 Ci mmol^{-1} Amersham), 10–20 μg RNA and 50 μg mouse liver aminoacyl-tRNA synthetase, or 5 μg of partially purified mouse liver histidyl-tRNA synthetase or 5–10 μg of an *E. coli* enzyme preparation. Where indicated, a mixture of 0.08 mM each of 15 unlabelled amino acids or 0.08 mM histidine was added. The incubations were carried out at 37° C for 30 min. The reaction was stopped by the addition of 0.5 ml of bovine serum albumin solution (0.25 mg) followed by precipitation with cold 5% trichloroacetic acid. The precipitate was filtered through glass filters (Whatman GF/C) washed and counted. The mouse liver aminoacyl-tRNA synthetase was partially purified from a 105,000*g* supernatant by phase partition[13]. The mouse liver histidyl-tRNA synthetase was further purified by DEAE-cellulose chromatography. The *E. coli* aminoacyl-tRNA synthetase was prepared according to Muench and Berg[14].

FIG. 2 Polyacrylamide–agarose gel electrophoresis of mouse liver N-acetyl-(^{3}H-histidyl-tRNA (*a*) and mengovirus N-acetyl-^{3}H-histidyl-RNA (*b*). Mouse liver tRNA and mengovirus RNA were acylated with ^{3}H-histidine (58 Ci mmol^{-1}) with mouse liver histidyl-tRNA synthetase, as described in the legend to Table 1. To 0.05 ml of the labelled aminoacyl-RNA solution, 0.025 ml of 1 M sodium acetate, buffer, *p*H 4.2 and 0.15 ml of ethanol were added. The precipitated RNA was dissolved in 0.025 ml of 0.01 M sodium acetate buffer, *p*H 5.0 and a solution of 0.05 ml of dimethylsulphoxide containing 5 mg of N-hydroxysuccinimide acetyl ester was added. The mixture was then treated as previously described[12]. The mouse liver N-acetyl-^{3}H-histidyl-tRNA (663 c.p.m. μg^{-1}) and the mengovirus N-acetyl-^{3}H-histidyl-RNA (179 c.p.m. μg^{-1}) preparations were applied to two different slots on a 1.7% polyacrylamide–0.5% agarose slab gel. In a third slot 5 μg of total *E. coli* RNA was added. Electrophoresis was carried out for 2 h at 0° C at 11.4 V cm^{-1}. The gel was stained with Stains all and then sliced into 1 mm fractions. Each slice was transferred to a 3.5 ml vial, incubated with 0.3 ml NCS (Amersham/Searle) for 30 min at 20° C and then 3 ml of toluene-based scintillation fluid was added. The vials were inserted into standard 20 ml scintillation vials and counted in a Packard Tricarb scintillation counter.

nucleases. It is still not clear whether specific cleavage of mengovirus RNA is required for its histidine-acceptance activity. The aminoacylated mengovirus RNA fragments have, however, a much larger molecular weight than that of tRNA and, thus, the possibility of tRNA contamination can be excluded. Moreover, the reaction of mengovirus histidyl-RNA with N-hydroxysuccinimide acetyl ester was carried out in 66% dimethylsulphoxide. Under these conditions any contaminating non-covalently linked tRNA would have been dissociated from the mengovirus RNA.

To estimate the extent of mengovirus acylation, we have increased the histidine concentration in the acylation mixture by using ^{14}C-labelled histidine (Schwartz/Mann 342 mCi mmol^{-1}). Under these conditions, 0.18 mol histidine are acylated per mol of mengovirus RNA, as compared with a value of 0.30 mol obtained with TMV RNA. The latter value confirms the results of Litvak *et al.*[5].

In this study we have shown that specific aminoacylation of viral RNA is not confined only to plant viruses. It will be interesting to determine whether other mammalian viral RNA preparations also serve as acceptors for amino acids. In the case of poliovirus RNA, no aminoacylation could be detected by Öberg and Philipson[4]. Whether this is due to the nature of poliovirus or to differences in the aminoacylation system remains to be determined.

The biological function of the amino acid acceptor activity of plant virus and mengovirus RNA is not known. It is possible that the aminoacylation of these viral RNA species may have some regulatory function in protein synthesis or that aminoacyl-RNA may be involved in the recognition site of viral RNA replicase.

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1 Pinck, M., Yot, P., Chapeville, F., and Duranton, H. M., *Nature*, **226**, 954 (1970).
2 Yot, P., Pinck, M., Haenni, A. L., Duranton, H. M., and Chapeville, F., *Proc. natn. Acad. Sci. U.S.A.*, **67**, 1345 (1970).
3 Pinck, M., Chan, S. K., Genevaux, M., Hirth, L., and Duranton, H. M., *Biochimie*, **54**, 1093 (1972).
4 Öberg, B., and Philipson, L., *Biochem. biophys. Res. Commun.*, **48**, 927 (1972).
5 Litvak, S., Tarragó, A., Tarragó-Litvak, L., and Allende, J. E., *Nature new Biol.*, **241**, 88 (1973).
6 Hall, T. C., Shin, D. S., and Kaesberg, P., *Biochem. J.*, **129**, 969 (1972).
7 Sela, I., *Virology*, **49**, 90 (1972).
8 Peacock, A. C., and Dingman, C. W., *Biochemistry*, **7**, 668 (1968).
9 Dahlberg, A. E., Dingman, C. W., and Peacock, A. C., *J. molec. Biol.*, **41**, 139 (1969).
10 Scraba, D. G., Kay, C. M., and Colter, J. S., *J. molec. Biol.*, **26**, 67 (1967).
11 Miller, R. L., and Plagemann, P. G. W., *J. gen. Virol.*, **17**, 349 (1972).
12 Daniel, V., Sarid, S., and Littauer, U. Z., *Science*, **167**, 1682 (1970).
13 Babinet, C., *Biochem. biophys. Res. Commun.*, **26**, 630 (1967).
14 Muench, K. H., and Berg, P., in *Procedures in Nucleic Acid Research* (edit. by Cantoni, G. L., and Davies, D. R.), 375 (Harper and Row, New York, 1966).
15 Littauer, U. Z., Yankofsky, S. A., Novogrodsky, A., Bursztyn, H., Galenter, Y., and Katchalski, E., *Biochim. biophys. Acta*, **195**, 29 (1969).

Structure of inositol hexaphosphate–human deoxyhaemoglobin complex

D-2,3-Diphosphoglycerate (DPG) facilitates the transfer of oxygen from human red blood cells to the tissues by lowering the oxygen affinity of haemoglobin[1]. It does so by combining preferentially with one of the two alternative forms of haemoglobin, namely the deoxy form, in the ratio of 1 mol per mol tetramer. Its binding site was predicted from biochemical and model building experiments and then determined directly by X-ray crystallography[2]. The site lies at the entrance to the central cavity between the N-termini of the β chains and is surrounded by four pairs of basic groups: the α amino group of valine 1 and the side chains of histidines 2 and 143, and of lysine 82. The basic groups are related in pairs by the molecular dyad and arranged so as to complement the acidic groups of DPG by forming seven salt bridges (Fig. 1). On oxygenation the N-termini of the β chains move apart and the cavity closes up so that the stereochemical complementarity is lost[3]. Oxyhaemoglobin does also bind DPG, but much more weakly, and the binding site is still unknown.

Several other multivalent anions, including inositol hexaphosphate (IHP), ATP, GTP and $[Fe(CN)_6]^{4-}$, also lower the oxygen affinity of haemoglobin. The most effective and biologically also the most important of them is IHP, as it is closely related to the inositol pentaphosphate which takes the place of DPG in regulating the oxygen affinity of bird erythrocytes[4,5]. We have determined the binding site of IHP by X-ray analysis and model building. Its six phosphate groups are coordinated to the same basic groups of the β chains as the single carboxyl and the two phosphates of DPG, and the binding of the two compounds also produces similar changes in the tertiary structure of the β subunits.

Crystals of human deoxyhaemoglobin were prepared by the method of Perutz[6], except that the ammonium phosphate normally added to the more than 2 M ammonium sulphate solution was replaced by 20 mM IHP at *p*H 6.5. At this high ionic strength, however, IHP failed to bind. The crystals were therefore soaked for 1 d in an aqueous solution of 70% (v/v) 2-methyl-2,4-pentanediol (MDP) + 1 mM IHP + 20 mM bis Tris adjusted to *p*H 6.5 with HCl. Other crystals were grown in the standard way (that is, without IHP) and soaked in a solution of 70% MPD + 20 mM bis Tris at *p*H 6.5. The structure of the haemoglobin molecules in such crystals is the same as that in crystals suspended in concentrated ammonium sulphate[2]. The intensities of about 14,000 reflections within a limiting sphere of 3.5 Å^{-1} from crystals in MPD with and without IHP were measured on a Crystalign four-cycle diffractometer. A difference Fourier synthesis was calculated using ($|F_{+\text{IHP}}| - |F_{-\text{IHP}}|$) as the coefficients to-

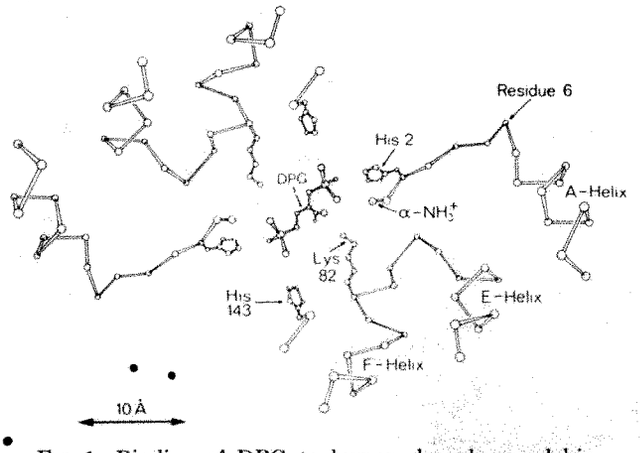


Fig. 1 Binding of DPG to human deoxyhaemoglobin.

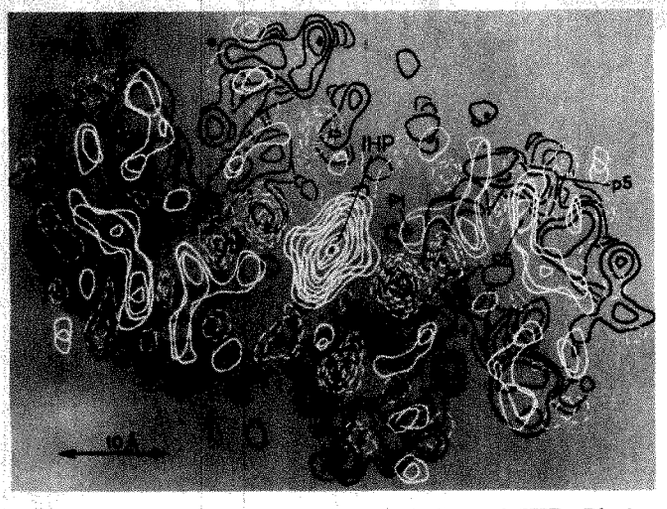


FIG. 2 Fourier map showing the binding of IHP. Black contours: sections −15 to −23 Å of the electron density map of human deoxyhaemoglobin which includes the same parts of the molecule, in the same orientation, as the model sketched in Fig. 1. Difference peaks P1 and p1 straddle the N terminus, peaks P5 and p5 the A helix, peaks P2 and p2 histidine 143. The positions of helices E and F and of the EF corner are obvious by reference to Fig. 1. White contours: difference electron density map ± IHP. Solid contours and upper case letters mark positive difference density; broken contours and lower case letters, negative difference density.

gether with the phase angles determined by Muirhead and Greer[7].

The difference electron density map is dominated by a massive and markedly asymmetrical positive peak at the entrance to the central cavity between the N termini of the β chains. The asymmetry may be an artefact, because the dyad axis passing through the centre of the haemoglobin molecule is not a crystallographic one, so that errors in intensity measurement and in phasing have different effects on points in the electron density which are related by the molecular dyad, but are not crystallographically equivalent. The difference map was therefore averaged about the molecular dyad, as in the previous study of DPG, and then sampled in planes 1 Å apart perpendicular to that axis.

Figure 2 shows a projection of several sections of the symmetry-averaged map. The large difference peak is cross shaped and has its maximum at a level 12 Å below that of the iron atoms in the β subunits. To find the orientation of the IHP molecule, a model built according to the structure of its sodium salt[8] was viewed against the difference map in same Richards box[9] in which a model of human deoxyhaemoglobin had been constructed (A. A., L. F. Ten Eyck, and G. Fermi, unpublished). The model of IHP fitted the difference map only with the cyclohexane ring parallel, but not perpendicular, to the dyad, and it fitted well with the line joining phosphorus atoms 3 and 6 normal to the dyad and inclined at an angle of 32° to x (Fig. 3). An equally good fit can be obtained after rotating the model by 120° so that phosphate 1 occupies the place of 3, and 4 that of 6. The distance between the phosphorus atoms of either of these two phosphates is 7.5 Å as compared to 6.5 Å between those of fully stretched DPG (Fig. 1). In either of these orientations our very limited resolution allows some latitude for rotation of the individual phosphate groups about both the C–O and the O–P bonds and therefore the fit should not be regarded as strong evidence in favour of the conformation of IHP in deoxyhaemoglobin being exactly the same as in the crystals of its sodium salt.

Phosphates 3 and 6 fit in well between the two α amino groups, which are 4 Å from the nearest phosphate oxygen in the undistorted deoxy structure (Fig. 4). This distance is too large for salt bridges, so that IHP (and DPG) pull the two α amino groups closer together. The evidence for this comes from a pair of positive (P1) and negative (p1) peaks which straddle the native electron density peaks of valines 1β, showing that the N termini move towards the phosphates; and peaks P5 and p5 which show that the entire A helix moves towards the centre of the molecule. Peaks P2 and p2 show that histidines 143β also move towards the phosphates. As in the DPG map, a large negative peak (P4) overlaps a peak in the native density, indicating that the atoms represented by that peak are removed by IHP. The peak lies outside the density representing the protein and is probably due to an inorganic anion, either sulphate or phosphate, bound to the α amino groups in the absence of IHP. Unlike the DPG map, the IHP map shows hardly any movement of lysine

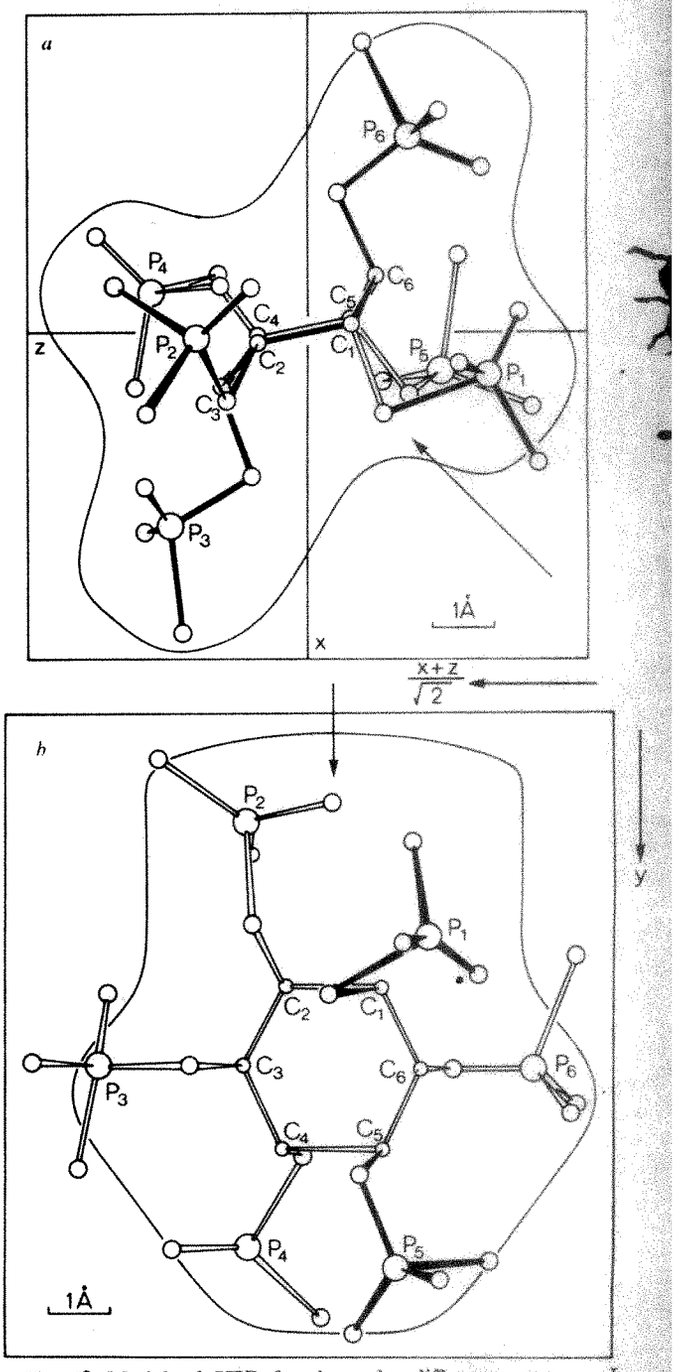


FIG. 3 Model of IHP fitted to the difference map. *a*, In projection on a plane normal to the molecular dyad superimposed on the outline of the central section ($y = -19$) through the difference peak. *b*, In projection along the diagonal between x and z against the outline of the central section through the difference peak. The arrow in (*b*) shows the direction of view in (*a*) and *vice versa*.

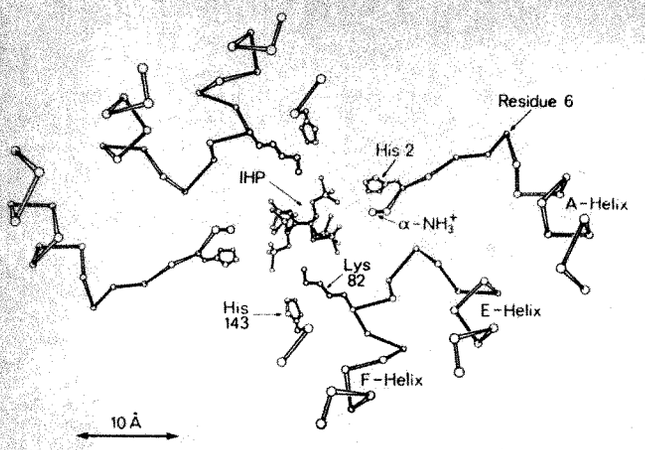


FIG. 4 Binding of IHP to human deoxyhaemoglobin. Note that the conformation of lysine 82 is different from that in Fig. 1. The side chain of asparagine 139 which could form hydrogen bonds with phosphates 1 and 2, lies approximately above that of lysine 82.

82 or of histidine 2. Lack of the former is explained by the fact that the side chain lies in the correct poistion to form salt bridges with phosphates without having to move. The reason for the absence of difference peaks near histidine 2 is not clear because this residue must move with the N-terminus and helix A. The amino groups of asparagines 139β are in positions that allow them to form hydrogen bonds with phosphates 1 and 2.

The DPG difference map showed widespread disturbances to the tertiary structure of the β subunits, and the same are seen in the IHP complex. The movement of the A helix has already been referred to. Weaker difference peaks along helices E and F, and along helix H (not shown in Fig. 2) point to structural disturbances that radiate from the IHP binding site and extend as far as the haem. At our present resolution of 3.5 Å the exact nature and magnitude of these disturbances cannot be determined, but they may be such as to oppose the transition of the tertiary structure from the deoxy to the oxy form, in which case both DPG and IHP should decrease the oxygen affinity of the β subunits in the quaternary deoxy structure.

In summary, the mode of binding of IHP to human deoxyhaemoglobin is similar to that of DPG. There are the same eight basic groups to form salt bridges with the six phosphates of IHP as with the two phosphates and one carboxyl of DPG, but in addition asparagines 139 may form hydrogen bonds with two of the phosphates. Titration of IHP shows that it carries about eight negative charges at *p*H 7.3. Two of the basic groups of the globin, the ϵ amino groups of the lysines, have *p*K's of 10.5 and will therefore be fully charged in the absence of IHP. The four imidazoles and two α amino groups have *p*K's in the neutral range which will rise on combination with IHP, while those of the phosphates will fall. The net effect on the bases, however, clearly dominates, because combination of deoxyhaemoglobin with IHP is accompanied by an uptake of protons[10]. On being bound to crystalline deoxyhaemoglobin grown in concentrated buffers both DPG and IHP displace one inorganic anion from each of the β chains. They both create perturbations to the tertiary structure of the β subunits that extend to the haems and could lower the oxygen affinity by stabilising the quaternary deoxy structure of the tetramer and by constraining the tertiary structure of the β subunits.

In avian erythrocytes inositol 1,3,4,5,6-pentaphosphate (IPP) takes the place of DPG as a regulator of oxygen affinity[5]. The only avian haemoglobin sequence determined so far is that of the chicken[11]. If, as seems likely, IPP takes up the same position in chicken deoxyhaemoglobin as IHP does in human, the number of basic groups facing it would be 12 instead of eight, and would include Val 1, His 2, Lys 82, Arg 135, His 139 and Arg 143. Therefore, the binding energy should be very high indeed.

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1 Benesch, R., and Benesch, R. E., *Nature*, **221**, 618 (1969).
2 Arnone, A., *Nature*, **237**, 146 (1972).
3 Perutz, M. F., *Nature*, **228**, 734 (1970).
4 Rapoport, S., and Guest, G. M., *J. biol. Chem.*, **138**, 269 (1941).
5 Johnson, L. F., and Tate, M. E., *Can. J. Chem.*, **47**, 63 (1969).
6 Perutz, M. F., *J. Cryst. Growth*, **2**, 54 (1968).
7 Muirhead, H., and Greer, J., *Nature*, **228**, 516 (1970).
8 Blank, G. E., Pletcher, J., and Sax, M., *Biochem. biophys. Res. Commun.*, **44**, 319 (1971).
9 Richards, F. M., *J. molec. Biol.*, **37**, 225 (1968).
10 Kilmartin, J. V., *Biochem. J.*, **133**, 725 (1973).
11 Matsuda, G., Maita, T., Mizuno, K., and Ota, H., *Nature new Biol.*, **244**, 244 (1973).

β-2-Microglobulin is part of the HL-A molecule in the lymphocyte membrane

IT has been shown that papain-solubilised HL-A antigens consist of two polypeptide chains, which may be separated under dissociating conditions[1,2]. Peterson *et al.*[3] have shown that β-2-microglobulin (β-2-m) constitutes one of the two polypeptide chains in papain-solubilised HL-A antigens. In these antigens, β-2-m represents a constant small subunit (molecular weight 11,800), whereas a larger subunit (30,000–31,000) carries the allo-antigenic specificity. The studies by Peterson *et al.* do not exclude the possibility that the two subunit structure of HL-A antigens might be a product of the solubilisation process. It has previously been reported[4] that β-2-m, which also occurs in biological fluids[5], is present in high concentrations on the surface of lymphocytes. The

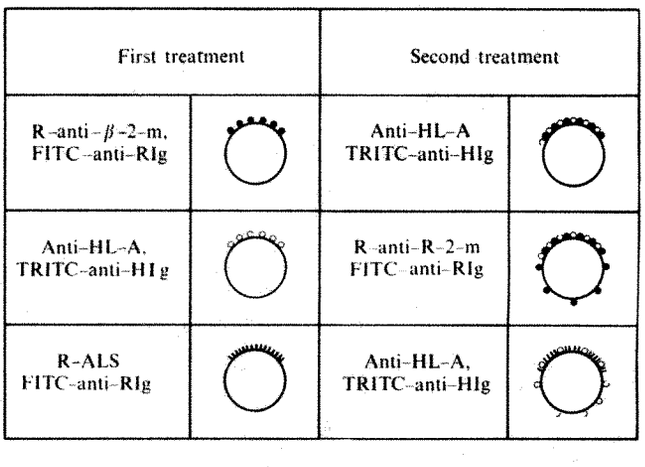


FIG. 1 Diagram of cell membrane antigen aggregation. Lymphocytes were first incubated with one set of antibodies at room temperature followed by incubation for 30 min at 37° C to induce capping of a cell membrane antigen. The cells were then reincubated with other antibodies at 4° C to determine whether the antigenic determinants detected by the second antibody co-capped with the antigen detected by the first antibody. 1, R-anti-β-2-m: Rabbit-anti-human-β-2-microglobulin; 2, R-ALS: Rabbit anti-human lymphocyte serum; 3, Anti-HL-A: Pooled multi-specific anti-HL-A; 4, FITC-anti-RIg: Fluorescein-labelled-swine-anti-rabbit-immunoglobulin; 5, TRITC-anti-HIg: Rhodamine-labelled-goat-anti-human-immunoglobulin".

Table 1 Aggregation/blocking of β-2-m, HL-A or species-specific antigens on human lymphocytes

Pretreatment*	Microcytotoxicity test results: Background (AB-serum)	Anti-HL-A (specific for the cells)	Anti-β-2-m (undiluted)	ALS (Diluted 1:64)
None (native cells)	−†	+++	+++	+++
Anti-β-2-m	(+)	(+)	(+)	+++
Anti-β-2-m + β-2-m	(+)	+++	+++	+++
Anti-HL-A (pool of multispecific sera)	(+)	(+)	+++	+++
Anti-lymphocyte serum (ALS) (1:64)	(+)	+++	+++	(+)

* Sensitised for 2 h at 20° C, washed, incubated with anti-immunoglobulin for 30 min at 4° C, washed, and incubated 30 min at 37° C
† Strength of the reactions: − = < 5% lysis; (+) = 5%–20%; + = 20%–40%; ++ = 20%–60%; +++ = > 60%.

present study was designed to establish whether β-2-m is part of the intact HL-A molecule in the cell membrane. Experiments were performed in which the blocking of HL-A antigens[6,7], β-2-m and other lymphocyte membrane components were induced by selected antisera. Furthermore, to study the relationship between these cell membrane components and the antigen receptors on lymphocytes, the different antisera were also tested for their ability to induce or inhibit lymphocyte activation *in vitro*. Methodological details are published elsewhere[8].

Anti-human-β-2-m (Dacopatts, 2900 Hellerup, Denmark, Catalogue Code T-072 Lot 023) was prepared in rabbits by immunisation with highly purified β-2-m from urine; the antibody preparation was immunochemically monospecific for β-2-m. Aggregation/blocking of cell membrane antigens was carried out by indirect technique. In 0.2 ml Hanks BSS (Flow Laboratories, Irvine, Scotland), 400,000 human lymphocytes prepared by Ficoll/Isopaque flotation, were first incubated with 0.2 ml of either undiluted anti-β-2-m, two rabbit anti-human lymphocyte sera[9] (ALS) diluted 1:64, or with undiluted multispecific cytotoxic anti-HL-A serum. Preliminary experiments showed that these antiserum concentrations gave optimal cap formation. To potentiate aggregation/capping, the cells were then incubated with 0.2 ml anti-immunoglobulin (FITC-labelled or unlabelled swine anti-rabbit immunoglobulin (Dacopatts 21090 or F2190) or rhodamine-labelled goat anti-human immunoglobulin (Highland, Los Angeles) and warmed to 37° C for 30 min[6,7].

By microcytotoxicity testing[10], undiluted anti-β-2-m induced 60–90% lysis of all human lymphocytes tested; the lysis was reduced to 30–40% after dilution 1:2, and lost upon dilution above 1:8. When the lymphocytes were first incubated with anti-β-2-m and anti-rabbit immunoglobulin, immediately followed by microcytotoxicity typing[10], all detectable HL-A antigens on the tested cells were completely blocked; that is, none of the antigens belonging to three segregant SD series[7] could be detected (Table 1). After incubation in a 1:1 mixture of Medium 199 (Flow Laboratories) and AB serum for 24 h at 37° C, blocked cells again showed a normal typing pattern. Preincubation of the cells with pooled anti-HL-A sera blocked the expression of HL-A antigens, but did not prevent the cells from being lysed by anti-β-2-m (Table 1), thus indicating an excess of β-2-m compared with HL-A in the cell membrane.

Aggregation of cell membrane antigens was also studied by immunofluorescence of double-stained cells. Aggregation was induced during the first staining procedure by warming to 37° C for 30 min; and the second staining step was carried out at 4° C to avoid additional aggregation of membrane components. When cells were first incubated with anti-β-2-m and stained with FITC-labelled anti-rabbit immunoglobulin, followed by incubation with anti-HL-A serum and staining with rhodamine-labelled anti-human immunoglobulin, the red fluorescence of the HL-A antigens was superimposed on the green fluorescence of β-2-m, which showed aggregation into patches and caps in virtually all cells. Figure 1 shows these results in diagrammatic form. (The cells were examined immediately with a Leitz Ortholux microscope equipped with a Ploem-type vertical illuminator, and care was taken to keep the preparations cold). In experiments where the cells after incubation with anti-β-2-m and anti-immunoglobulin were incubated with ALS and fluorescein-labelled anti-rabbit immunoglobulin, marked diffuse fluorescence of the whole cell membrane could be observed.

Thus both microcytotoxicity tests and immunofluorescence demonstrate that anti-β-2-m concomitantly aggregated/blocked β-2-m and HL-A molecules in the cell membrane. This strongly indicates that β-2-m is part of the native HL-A antigens in the cell membrane. By contrast, anti-β-2-m did not coaggregate antigens against which our ALS were directed.

When lymphocytes were preincubated with pooled anti-HL-A sera followed by rhodamine-labelled anti-human immunoglobulin, and then reincubated with anti-β-2-m and double-stained with FITC-labelled anti-rabbit immunoglobulin, immunofluorescence microscopy showed green staining superimposed on red patches and caps, but also diffusely distributed over the rest of the cell membrane, confirming the observations by cytotoxicity tests that there is an excess of β-2-m compared with HL-A antigens in the cell membrane.

When the cells were preincubated with ALS followed by anti-rabbit immunoglobulin, no blocking of the expression of the HL-A antigens could be observed in the microcytotoxicity test (Table 1). When stained with FITC-labelled anti-rabbit immunoglobulin after preincubation with ALS, and reincubated with HL-A antisera followed by rhodamine-labelled anti-human immunoglobulin, the green fluorescence was aggregated into patches and caps in virtually all cells while the red fluorescence showed a diffuse distribution in the cell membrane, and was superimposed on the green patches and caps. The ALS tested, therefore, neither blocked the expression of the HL-A antigens as measured by microcytotoxicity technique nor induced aggregation of HL-A antigens as determined by immunofluorescence.

To test the specificity of the reactions, highly purified β-2-m (supplied by Dr Bengt G. Johansson, Lund) was added to anti-β-2-m, pooled anti-HL-A and ALS. β-2-m (50 μg per 0.2 ml antiserum) abolished blocking and aggregation of HL-A and β-2-m determinants by anti-β-2-m as determined by microcytotoxicity test and fluorescence microscopy. The same amount of β-2-m had no influence on the activity of pooled anti-HL-A sera or ALS.

Studies of lymphocyte transformation *in vitro* were carried out by our micromethod[11]. Results from mixed lymphocyte cultures (MLC) cultured for 5 d in the presence of anti-β-2-m or ALS are shown in Fig. 2. As controls we used partly purified immunoglobulin from rabbits prepared in the same way as the anti-β-2-m antibodies and normal rabbit serum. The results show that anti-β-2-m blocks the MLC reactions (ABm), up to a dilution in normal serum of 1:128. In addition, a very slight but definite mitogenic effect is demonstrated by anti-β-2-m diluted 1:2 (AAm). The ALS shows a much more intense mitogenic effect up to a dilution of 1:32. No definite inhibition of the MLC reactions could be demonstrated by any dilution of the ALS. This is in marked contrast to the results with anti-β-2-m.

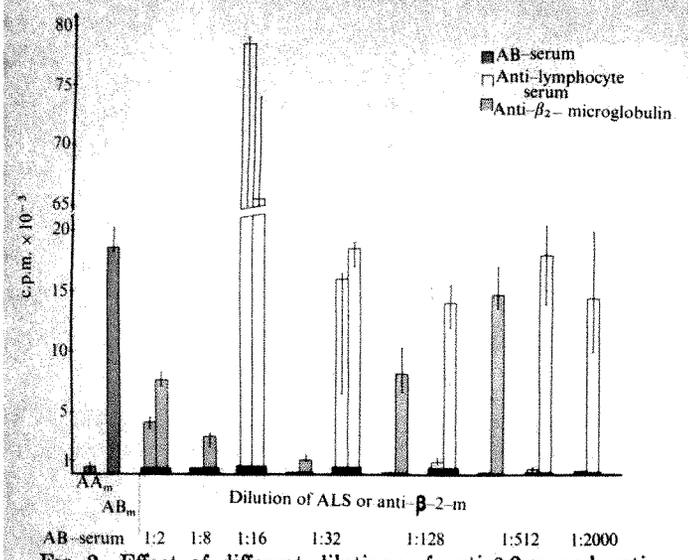


FIG. 2 Effect of different dilutions of anti-β-2-m and anti lymphocyte serum on mixed lymphocyte cultures. 0.15×10^6 responding lymphocytes (from A) were mixed with 0.15×10^6 mitomycin treated stimulating lymphocytes (from either A or B) in the wells of flat bottomed microtitre plates. Each single culture consisted of 0.2 ml Medium 199 supplemented with antibiotics and 20% inactivated normal (AB) or immune serum (diluted in AB-serum). Twenty-four hours before collection, 0.02 ml of 0.05 μCi 2^{-14} C-thymidine was added in physiological saline, the cultures collected on glass fibre filters on day 5, and the degree of proliferation evaluated by liquid scintillation counting. The columns indicate the median of the triplicates, and the brackets show the range. The first column in each pair is the autologous combination (AAm) in test serum, the second is the allogeneic combination (ABm) in test serum. The black areas at the bottom of the columns are the autologous control combinations (AAm) in normal serum. Note the mitogenic affect of both anti-β-2-m and ALS, and the inhibitory effect on MLC exhibited by anti-β-2-m only.

Anti-β-2-m was also found to block completely the stimulation induced by PPD of lymphocytes from a BCG-sensitised individual (10 and 100 μg PPD to 0.15×10^6 lymphocytes in 0.2 ml culture medium; collection on day 4). In parallel experiments, anti-HL-A antibodies directed against the lymphocytes had similar inhibitory activity, while an HL-A antiserum not reactive with the lymphocyte donor and normal AB serum did not affect the response. Addition of 50 μg β-2-m per 0.2 ml antiserum abolished the mitogenic and inhibitory activity of anti-β-2-m, but had no effect on the inhibitory effect of anti-HL-A antisera or the mitogenic effect of ALS.

It has previously been shown[12–14], that antibodies with specificity for HL-A antigens can probably inhibit antigen-induced lymphocyte activation. The results of our present study indicate that anti-β-2-m antibodies also inhibit allogenic lymphocyte stimulation and lymphocyte transformation induced by PPD. Thus, anti-HL-A and anti-β-2-m antibodies which both induce redistribution and removal of HL-A molecules in the cell membrane, inhibit lymphocyte activation *in vitro*. The xenogenic anti-lymphocyte sera tested showed no activity against HL-A antigens or β-2-m, and did not cause detectable inhibition of lymphocyte activation. A slight inhibitory activity in the anti-lymphocyte sera could be overshadowed by the mitogenic activity demonstrated by these sera. There was, however, definitely no inhibition at serum concentrations being 30 to 60 times lower than the optimal concentration for cap formation at the cell surface. By using anti-β-2-m or anti-HL-A sera[13,14], inhibition of lymphocyte activation could be detected at concentrations 30 to 60 times less than those optimal for induction of cap formation. The results with the two anti-lymphocyte sera presented in this study confirm our observations[13,14] that mixed lymphocyte cultures are not necessarily inhibited by all antibodies inducing aggregation of lymphocyte cell membrane components. Inhibitory activity of other anti-lymphocyte sera on lymphocyte transformation has previously been described[15]. This may be due to the presence of anti-β-2-m or other antibodies reacting with the HL-A molecule in these sera.

The efficiency of anti-HL-A and anti-β-2-m sera in inhibiting antigen-induced lymphocyte transformation indicates that both the allo-antigenic subunit and the β-2-m subunit of the HL-A molecule are closely associated with the T lymphocyte receptor. The HL-A molecule may either be part of the T cell receptor, be structurally closely associated with it in the cell membrane, or be functionally associated with lymphocyte activation at a secondary step following antigen binding to the T cell receptor. The convincing homology that has been demonstrated in amino acid sequence between β-2-m and the constant domains of IgG further suggests that β-2-m serves an important effector function in immunological reactions[4].

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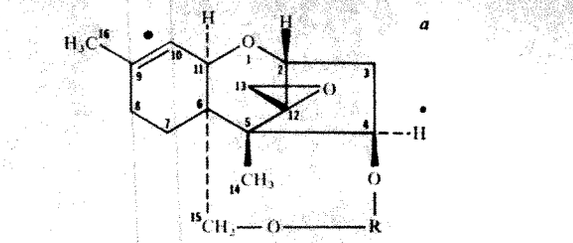
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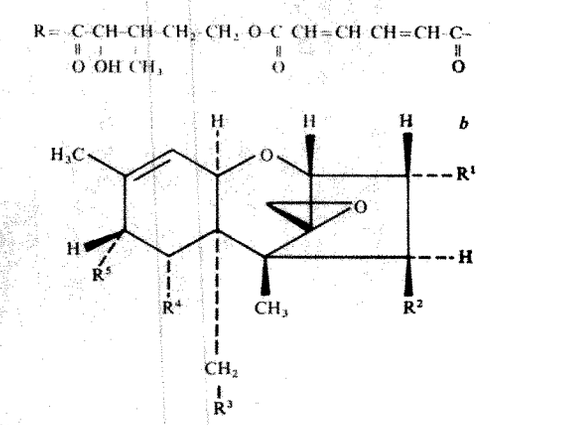
1 Cresswell, P., Turner, M. J., and Strominger, J. L., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1603 (1973).
2 Tanigaki, N., Katagiri, M., Nakamuro, K., Kreiter, V. P., and Pressman, D. *Fedn Proc.* **32**, 1017 (1973).
3 Peterson, P. A., Rask, L., and Lindblom, J. B., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 35 (1974).
4 Peterson, P. A., Cunningham, B. A., Berggård, I., and Edelman, G. E., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1697 (1972).
5 Berggård, I., and Bearn, A. G., *J. biol. Chem.*, **243**, 4095 (1968).
6 Bernoco, D., Cullen, S., Scudeller, G., Trinchieri, G., and Ceppellini, R. *Histocompatibility Testing (1972)*, 527 (Munksgaard, Copenhagen, 1973).
7 Solheim, B. G., Bratlie, A., Sandberg, L., Staub-Nielsen, L., and Thorsby, E., *Tissue Antigens* **3**, 439 (1973).
8 Solheim, B. G., and Thorsby, E., *Tissue Antigens*, **4**, 83 (1974).
9 Thorsby, E., *Vox. Sang.*, **13**, 194 (1967).
10 Kissmeyer-Nielsen, F., and Kjerbye, K. E., *Histocompatibility Testing 1967*, 381 (Munksgaard, Copenhagen, 1967).
11 Helgesen, A., Hirschberg, H., and Thorsby, E., *Manual of Tissue Typing Techniques, Publ.*, No. 74-545, 75 (National Institutes of Health, Bethesda, 1973).
12 Ceppellini, R., *Progress in Immunology*, **1**, 1001 (1971).
13 Thorsby, E., and Solheim, B. G., *Haematologia*, **7**, (1973).
14 Thorsby, E., and Solheim, B. G., *Transpln. Proc.*, **5**, 1859 (1973).
15 Eijsvoogel, V. P., du Bois, M. J. M. J., Schellekens, P. Th. A., and van Loghem, J. J., *Transpln. Proc.*, **1**, 408 (1969).

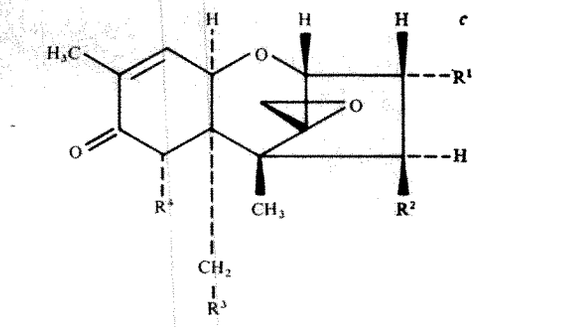
Two classes of inhibitors of peptidyl transferase activity in eukaryotes

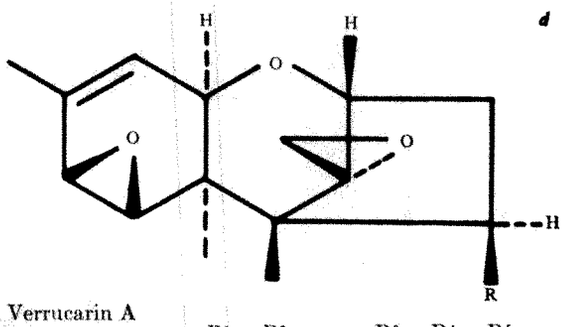
THE 12,13-epoxytrichothecenes (Fig. 1) form a group of fungal toxins with a tricyclic trichothecene ring system. (For review, see ref. 1). They have been shown to inhibit protein synthesis in a variety of eukaryotic systems[2] and in particular they block peptidyl transferase activity as determined by the puromycin-fragment reaction[3]. More recent studies have shown however, that the trichothecenes have differential effects on protein synthesis in whole cells[4]. One subgroup containing verrucarin A and T-2 toxin inhibited initiation (I-type), and the other subgroup inhibited elongation/termination (E-type); trichodermin was in the latter group. Here I show that these two classes can also be distinguished in cell-free systems and suggest analogies with the mode of action of the lincosaminide and macrolide inhibitors of protein synthesis in prokaryotes.

It has been observed that the effect of antibiotics on the reaction of puromycin with nascent peptide chains on polyribosomes *in vitro* accurately reflects their effect on trans-









a	Verrucarin A					
b		R^1	R^2	R^3	R^4	R^5
	Trichodermol	H	OH	H	H	H
	Diacetoxyscirpenol	OH	OAc	OAc	H	H
	Trichodermin	H	OAc	H	H	H
	T-2 toxin	OH	OAc	OAc	H	$OOCCH_2$-$CHMe_2$
	HT-2 toxin	OH	OH	OAc	H	$OOCCH_2$-$CHMe_2$
c		R^1	R^2	R^3	R^4	
	Nivalenol	OH	OH	OH	OH	
	Trichothecin	H	OOCCH=CHMe	H	H	
d						
	Crotocol	R = OH				
	Crotocin	R = OOCCH=CHMe				

FIG. 1 Structure of some 12, 13-epoxytrichothecenes.

peptidation *in vivo*[5]. The various trichothecene inhibitors were tested using polyribosomes prepared from rat liver, and it was found that the E-type inhibitors, such as trichodermin, inhibit the puromycin reaction, whereas the I-type inhibitors have little or no effect (Table 1). This assay allows the expansion of the E-type subgroup to include crotocin and trichothecin, in addition to trichodermol and trichodermin.

TABLE 1 Inhibition of ^{3}H-peptidyl-puromycin reaction and poly (U)-directed polyphenylalanine incorporation

	^{3}H-Peptidyl-puromycin		Phenylalanine incorporation		
	μM	% Activity	μM	% Activity	
No drug	—	100% = 2.8 pmol	—	100% = 55 pmol	
Trichodermin	35	50	100	87	
Trichothecin	35	50	100	70	E-Type
Trichodermol	80	50	100	98	
Crotocin	150	64	100	97	
Diacetoxyscirpenol	54	94	80	77	
Crotocol	190	91	100	89	
Nivalenol	160	106	5.6	50	
T-2 Toxin	104	100	4.0	50	I-Type
Verrucarin A	100	108	1.0	50	
HT-2 Toxin	120	96	0.2	50	

Effect of various trichothecenes on the reaction of puromycin with rat liver polyribosomes and on poly(U)directed phenylalanine incorporation. Polyribosomes from rat liver were prepared as described by Baliga *et al.*[20]. Formation of ^{3}H-peptidyl-puromycin was measured as described previously[6]. Control activity is expressed as pmol of ^{3}H-puromycin incorporated into trichloroacetic acid-precipitable material. ^{14}C-phenylalanine incorporation using yeast extracts was measured as described previously[9]. The average control activity is expressed as total pmol of ^{14}C-phenylalanine incorporated into acid-precipitable, alkali stable material.

The I-type inhibitors inhibit poly(U)-directed phenylalanine incorporation on yeast ribosomes[6]. Various trichothecenes were tested in this system and HT-2 toxin behaved like other I-type inhibitors as it inhibited phenylalanine incorporation at low levels and did not affect peptidyl-puromycin formation even at relatively high levels (Table 1). This suggests that HT-2 toxin will inhibit initiation *in vivo*. It can be seen that the E-type inhibitors do not affect phenylalanine incorporation to the same degree as they inhibit the peptidyl-puromycin reaction. Although crotocol and diacetoxyscirpenol were relatively inactive in both *in vitro* assays, diacetoxyscirpenol inhibits initiation *in vivo*[6].

The fact that the I-type inhibitors (verrucarin A, T-2 toxin, and so on) inhibit the puromycin reaction *in vitro* when using CCA-leu (the fragment reaction)[3] but not the same reaction on polyribosomes is reminiscent of the action of the antibacterial agents lincomycin, certain macrolides such as carbomycin, and the streptogramin A group of antibiotics. These compounds inhibit the puromycin reaction with donors such as CCA-leu in cell-free systems from prokaryotes, but not with nascent peptide chains on polyribosomes; they also seem to inhibit initiation *in vivo*[8]. Further investigation has revealed other similarities between the trichothecenes and the macrolides and lincosaminides.

Surprisingly, although the E-type trichothecenes inhibit elongation *in vivo*, they do not inhibit poly(U)-directed polyphenylalanine synthesis. Similar results have been observed among the macrolides, since erythromycin does not inhibit polyphenylalanine synthesis in a purified *Escherichia coli* system[7], although it inhibits elongation *in vivo*. On the other hand, spiramycin, an I-type macrolide, does inhibit polyphenylalanine synthesis[10].

The macrolide antibiotic erythromycin does not inhibit the fragment reaction although it inhibits the binding of lincomycin, an antibiotic which blocks the fragment reaction on bacterial ribosomes[11]. In addition, erythromycin antagonises the inhibition by lincomycin of the puromycin reaction with $tRNA_f^{met}$ (ref. 12). Lack of inhibition by erythromycin is therefore not the result of an inability to bind to ribosomes under the assay conditions used. I have obtained a similar result with the trichothecenes. Although trichodermin does not inhibit poly(U)-directed phanylalanine incorporation to a large extent, its presence significantly reduces the inhibition produced by I-type inhibitors on yeast ribosomes (Fig. 2*a*). This suggests that trichodermin is bind-

TABLE 2 Summary of effects of protein synthesis inhibitors on various reactions

	Poly(U)-directed polyphenylalanine synthesis	Puromycin-fragment reaction	Peptidyl-puromycin reaction	*In vivo*
Eukaryotes				
Verrucarin A	+	+ (ref. 3)	−	Initiation (ref. 4)
Nivalenol	+	0	−	Initiation (ref. 4)
T-2 toxin	+	0	−	Initiation (ref. 4)
HT-2 toxin	+	0	−	0
Trichodermin	−	+ (ref. 3)	+	Elongation (ref. 4)
Trichodermol	−	+ (ref. 3)	+	0
Crotocin	−	0	+	0
Trichothecin	−	+ (ref. 3)	+	0
Prokaryotes				
Lincomycin	+ (ref. 17)	+ (ref. 18)	− (ref. 19)	Initiation (ref. 8)
Spiramycin	+ (ref. 10)	+ (ref. 18)	− (ref. 19)	Initiation (ref. 8)
Erythromycin	− (ref. 7)	− (ref. 18)	− (ref. 19)	Elongation (ref. 9)

+, Inhibition; −, No inhibition; 0, not done.

ing to the ribosome and preventing the binding of the other inhibitors. This may indicate partially overlapping binding sites on the ribosome, although other possibilities cannot be excluded, such as the binding at one site producing a conformational change at another site. But the similarity in structure between trichodermin and HT-2 toxin, for example, strongly suggests a common site of binding.

The converse experiment was carried out using varying levels of verrucarin A and trichodermin in a puromycin reaction on rat liver polyribosomes (Fig. 2*b*). The highest concentration of verrucarin A (40 μm) did not antagonise inhibition by trichodermin, whereas the same concentration of verrucarin A inhibits poly(N)-directed polyphenylalanine incorporation by 90%. This could be taken as evidence for binding sites which are not mutually exclusive, but this would conflict with the results of the previous experiment which suggested a common binding site. A more likely possibility is that peptidyl-tRNA prevents the binding of verrucarin A to ribosomes as previously suggested by Cundliffe *et al.*[4]. On stripped ribosomes both E- and I-type inhibitors might bind to mutually overlapping sites. This explains why the I-type inhibitors prevent either initiation or steps shortly after initiation of protein synthesis and not elongation, thereby permitting run-off of polysomes. A similar explanation has been used to explain why the lincosaminides do not inhibit elongation of peptide chains on bacterial polyribosomes[13]. Since verrucarin A does not inhibit the puromycin reaction on salt-washed 80S yeast ribsomes, while trichodermin

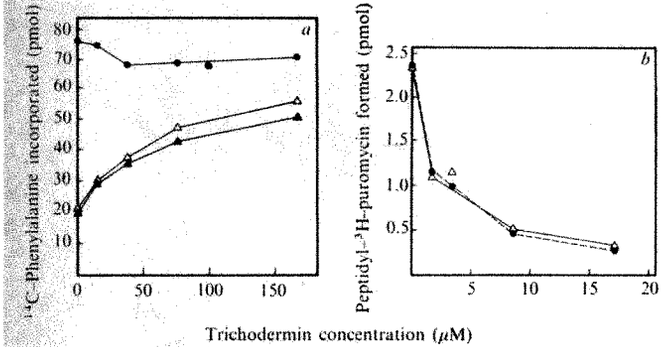


FIG. 2 *a*, Antagonism by trichodermin of the inhibition of poly U-directed phenylalanine incorporation by I-type inhibitors. ^{14}C-phenylalanine incorporation was measured as described previously[6]. Activity is expressed as total pmol of ^{14}C-phenylalanine incorporated into alkali-stable, acid-precipitable material in 30 min. Similar results were obtained using nivalenol (8 μM) and T-2 toxin (2 μM). ▲, Verrucarin A (2.0 μM); △, HT-2 toxin (2.4 μM); ●, control. *b*, Lack of antagonism by verrucarin A of the inhibition of ^{3}H-peptidyl-puromycin formation of trichodermin. ^{3}H-Peptidyl-puromycin was measured as described in Table 1. △, Verrucarin A (40 μM). ●, Control.

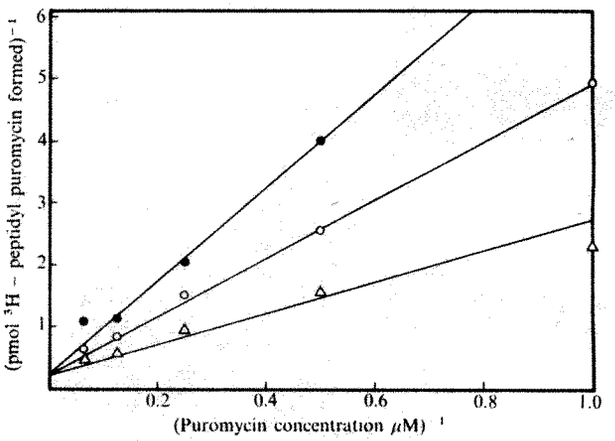


FIG. 3 Competitive inhibition of ^{3}H-peptidyl-puromycin synthesis by trichodermin. Assays were performed as described previously but incubations were carried out for 1 min to provide initial rates. ●, Trichodermin (68 μM); ○, trichodermin (17 μM); △, no trichodermin.

does (unpublished observations), one can probably rule out the involvement of any factor(s) present on unwashed ribosomes which may prevent the binding of verrucarin A.

Further evidence for overlapping sites comes from the fact that a trichodermin resistant strain of *S. cerevisiae*[6] is also resistant to T-2 toxin (100 μg ml^{-1}) and anisomycin (20 μg ml^{-1}). Similarly, bacterial mutants resistant to erythromycin are known to be resistant to lincomycin, spiramycin, and the streptogramin B antibiotics[14].

The nature of the inhibition of the puromycin reaction on rat liver polyribosomes by trichothecenes is of some interest. When plotted on a double reciprocal plot, trichodermin seems to be competitive inhibitor of the puromycin reaction. The K_m for puromycin was 13×10^{-6} M which compares to 8×10^{-6} M previously reported by Pestka[15]. Carbomycin, tylosin, spiramycin, and lincomycin, all I-type macrolides, inhibit binding of CACCA-Phe to prokaryotic ribosomes[16], a model reaction for the interaction of the acceptor aminocyl-tRNA to the peptidyl transferase centre. Trichodermin, trichodermol, and anisomycin inhibit binding of both CACCA-LeuAc and UACCA-Leu to ribosomes, presumably by preventing access of these aminoacyl-tRNA fragments to the donor and acceptor sites of the peptidyl transferase centre[3]. But inhibition of this binding was found to be less than inhibition of the puromycin fragment reaction, which may be due to different experimental conditions in the two assays. The competitive inhibition (elevating the K_m but not changing the V_{max}) which I observe, indicates weaker binding of puromycin and is consistent with blockade of the binding at the acceptor site in the peptidyl transferase centre by tri-

chodermin under conditions more closely resembling protein synthesis (for example, absence of ethanol).

I suggest that the I-type trichothecenes, macrolides, and lincosaminides are similar in their mode of action of protein synthesis inhibition. The basis for the proposed analogy is summarised in Table 2. It fails at one important point for the E-type inhibitors in that the E-type macrolides, such as erythromycin, and the E-type trichothecenes differ in their effects on the peptidyl-puromycin and the puromycin-fragment reactions.

The macrolides which inhibit initiation *in vivo* have been subdivided into two classes based on the size of oligophenylalanine produced in their presence in a poly(U)-directed system[7]; it would be interesting to see if the I-type inhibitors of the trichothecenes can be classified in the same manner. Diacetoxyscirpenol may be a member of a second class of I-type inhibitors because of its anomalous effect on poly(U)-directed polyphenylalanine synthesis.

It has been postulated that the presence of substituents on carbon 15 of the trichothecene ring system may be important in determining whether the toxin will affect elongation or initiation[4]. The assignments of crotocin and trichothecin to the class of E-type inhibitors and HT-2 toxin to the class of I-type inhibitors based on *in vitro* experiments further substantiates this hypothesis. HT-2 and T-2 toxin are substituted at C-15; both crotocin and trichothecin, like other E-type inhibitors, lack a substituent at this position.

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1 Bamburg, J. R., and Strong, F. M., in *Microbial Toxins*, (edit. by Kadis, S., Ciegler, A., and Ajl, S. J.), **7**, (Academic Press, New York, 1971).
2 Ueno, Y., *Proc. U.S.-Jap. Conf. Toxic Microorganisms* (edit. by Herzberg, M.), **76–79** (1970).
3 Carrasco, L., Barbacid, M., and Vazquez, D., *Biochem. biophys. Acta*, **312**, 268–376 (1973).
4 Cundliffe, E., Cannon, M., and Davies, J. E., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 30–34 (1974).
5 Pestka, S., Goorha, R., Rosenfeld, H., Neurath, C., and Hintikka, H., *J. biol. Chem.*, **247**, 4258–4263 (1972).
6 Schindler, D. G., Grant, P., and Davies, J. E., *Nature* (in the press).
7 Pestka, S., *A. Rev. Microbiol.*, **25**, 487–562 (1971).
8 Cundliffe, E., *Antibiotic Inhibitors of Ribosome Functions*, in *Molecular Basis of Antibiotic Action*, (edit. by Gale, E. F., Cundliffe, E., Reynolds, P. E., Richmond, M. H., and Waring, M. J.), **278–379** (Wiley Interscience, Chichester, 1972).
9 Cundliffe, E., and McQuillen, K., *J. molec. Biol.*, **30**, 137–146 (1967).
10 Vazquez, D., *Life Sci.*, **6**, 845–853 (1967).
11 Monro, R. E., Fernandez-Munoz, R., Celma, M. L., and Vazquez, D., *Drug Action and Drug Resistance in Bacteria* (edit. by Mitsuhashi, S.) **1**, 305–336 (University Park Press, Baltimore, 1971).
12 Chang, F. N., thesis, Univ. of Wisconsin (1968).
13 Burns, D. J. W., and Cundliffe, E., *Eur. J. Biochem.*, **37**, 570–574 (1963).
14 Weisblum, B., in *Drug Action and Drug Resistance in Bacteria*, (edit. by Mitsuhashi, S.), **1**, 217–238 (University Park Press, Baltimore, 1971).
15 Pestka, S., Rosenfeld, H., Harris, R., and Hintikka, H., *J. biol. Chem.*, **247**, 6895–6900 (1972).
16 Lessard, J. L., and Pestka, S., *J. biol. Chem.*, **247**, 6909–6912 (1972).
17 Chang, F. N., Sih, C. J., and Weisblum, B., *Proc. natn. Acad. Sci. U.S.A.*, **55**, 431–438 (1966).
18 Monro, R. E., and Vazquez, D., *J. molec. Biol.*, **28**, 161–165 (1967).
19 Pestka, S., *J. biol. Chem.*, **247**, 4669–4678 (1972).
20 Baliga, B. S., Pronczuk, A. W., and Munro, H. W., *J. molec. Biol.*, **34**, 199–218 (1968).

Effect of insulin on (Na^+, K^+)-activated adenosine triphosphatase activity in rat muscle sarcolemma

THE existence of (Na^+, K^+)-activated adenosine triphosphatase (ATPase)[1,2] supports a molecular explanation for the active transport of sodium and potassium ions through cell membranes[3].

Other than results from insulin stimulation of membrane-bound ATPases from human lymphocytes[4], no studies have, so far, given any proof of connecting insulin effects on electrolyte transport through cell membranes with the effect on the ATPases. Our observations show an insulin stimulation of sarcolemma-bound ATPase when muscle tissue is incubated with insulin before measuring the enzyme activity on isolated membrane preparations.

Wistar albino rats weighing 170–250 g were fasted for 20–22 h. The rats were killed with a sharp blow to the head and exsanguinated or decapitated when in ether narcosis. In other experiments the animals were anaesthetised with Nembutal sodium (30 mg kg^{-1}, at 30 min intervals) for 1½ h, then bled by cardiac puncture for sugar analysis[5] and decapitated. The leg muscles were excised, transferred to ice-cold 0.9% NaCl, blotted on filter paper and weighed. In the first series, the sarcolemma was isolated according to the method of Severson *et al.*[6], and the membrane preparations incubated with insulin before enzyme assay. In the other experiments, muscle pieces were incubated with insulin (100 mU ml^{-1}) for 15 min in Tyrode's solution[7] before membrane isolation[6]. The membrane pellets from all experiments were resuspended in 0.01 M Tris HCl buffer (*p*H 8.0) approximately five times the original tissue volume (that is, tissue weight) and subjected to two strokes in a Potter-Elvehjem glass homogeniser with a motor-driven Teflon pestle (5,200

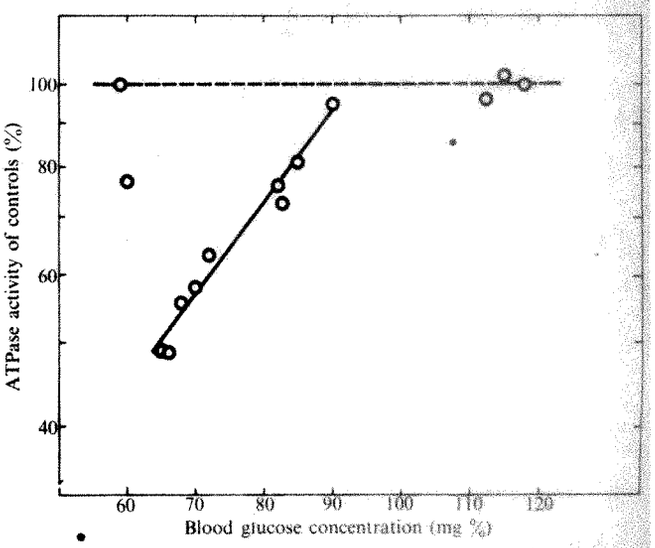


FIG. 1 Semilogarithmic plot of ATPase activity. ●, Variations in specific activities of the controls (%) correlated with blood sugar concentration (mg% whole blood). – – – The specific activities after insulin treatment were taken as 100%; in these experiments only Nembutal-treated rats were used.

TABLE 1 Effect of insulin on isolated muscle sarcolemma ATPase and 5′-nucleotidase activities

Enzyme	No. of experiments	Specific activity ± s.e.m. (μmol P_i per mg protein per min) ×10² Without insulin	With insulin
(Na^+, K^+)-ATPase	15	22.2 ± 1.8	23.3 ± 1.9
5′-nucleotidase	6	2.12 ± 0.15	1.91 ± 0.14

ATPase and 5′-nucleotidase activities at 30° C as liberation of inorganic phosphate from ATP and AMP, respectively. Isolated muscle sarcolemma was preincubated with and without 100 mU ml^{-1} pig insulin at 37° C for 15 min. The membrane preparations were then homogenised in 0.01 M Tris HCl buffer, *p*H 8.0. For the ATPase assay 500 μl of the membranes was mixed with 650 μl Tris HCl buffer, final *p*H 7.6, containing NaCl, KCl, and $MgSO_4$, giving a final concentration of 30, 5, and 0.5 mmol l^{-1}, respectively, after substrate addition. The reaction was started by adding 500 μl 5 mmol l^{-1} ATP and stopped after 2 min by addition of 0.5 ml ice-cold 15% trichloroacetic acid. For the assay of 5′-nucleotidase, 400 μl membrane homogenate and 600 μl Tris HCl buffer, *p*H 8.0, with $MgCl_2$ and AMP to a final concentration of 80 mmol l^{-1}, 0.2 mmol l^{-1}, and 2 mmol l^{-1}, respectively. The reaction was stopped after 10 min. Reaction vessels were centrifuged and the supernatant analysed for phosphate[8]. The precipitate was solubilised in 5 ml 3% deoxycholic acid sodium salt, warmed at 80° C for 5 min and protein content estimated[9] using a bovine serum albumin as standard. Every enzyme sample was run in triplicate.

s.p.m.). The homogenates were used to assay (Na^+, K^+)-activated ATPase and 5′-nucleotidase, as liberation of inorganic phosphate[8] per mg protein[9] per min.

In the third series, non-fasted rats were rendered diabetic by intravenous injection of 55 mg alloxan in a 5% aqueous solution per kg body weight. The severity of diabetes was assessed by measuring the blood glucose[5] 48–72 h after treatment. Blood sugar was sampled at the time the animals were killed. Those having a plasma glucose concentration exceeding 450 mg % were used in the experiments.

Attempts at direct stimulation of the membrane ATPases with insulin did not succeed (Table 1), contrary to the results of Hadden *et al.*[4] Varying the incubation time, temperature, ionic environment, insulin concentration, and preincubation with adrenaline did not alter this. The sample to be examined was compared with a control from the same animal. When muscle cells were preincubated with insulin, ATPase activity was stimulated in the muscle sarcolemma, but the specific activity of the 5′-nucleotidase was unaffected (Table 2).

Although all rats in each series received the same treatment, we obtained great variations in percentage stimulation of ATPase activities. The hormone-stimulated values were quite consistent, but the controls showed great deviations (Table 2). This might indicate that ATPase activity is governed by circulating insulin concentration. If the animals are stressed during handling, there is an increase in blood sugar, followed by an insulin response[10] which, in turn, stimulates ATPase activity. In an attempt to relax the animals, one series of experiments was carried out with the animals anaesthetised with Nembutal. Blood glucose concentration was used as an indication of the circulating insulin level[11].

Figure 1 shows the variations in ATPase activity of the controls, expressed as an exponential function of blood sugar concentration. Insulin stimulation fails when the specific activities approach the maximum level (Fig. 1). The exceptions at the lower sugar concentrations can be explained if one assumes that these low sugar levels result from high circulating insulin concentrations and, consequently, stimulate the ATPase activity, abolishing further stimulation *in vitro*.

The results obtained with muscle tissue from alloxan diabetic rats (Table 2) after *in vitro* insulin treatment show great variation in the stimulation of the ATPase activity, ranging from more than 100% to 0%. When hormone stimulation fails, the specific activity of the control samples is found at the same upper level as those which respond to insulin stimulation. Alloxan in a diabetogenic dose shows a triphasic response, with high insulin secretion in the last phase[12]. Time dependence for reaching nullity of circulating insulin is subjected to great individual variations[13]. Disregarding the irregular data, these results support our hypothesis.

Different types of ATPase activities are recognised within cell membranes. Whether they represent partial activities of the same enzyme system or entirely different enzymes, is still a matter of dispute. The (Na^+, K^+)-activated ATPase has an active site for K^+ outside and an active site for Na^+ inside the cell membrane, and the two ions compete with each other on both sites[3,14].

Omitting K^+ in the enzyme assay resulted in a residual activity of about 8% of controls[15]. This was taken as the criterion for the assumption that the membrane-bound ATPase in question is the (Na^+, K^+)-activated ATPase. Dialysis of sarcolemma preparations against 0.01 M Tris HCl buffer, *p*H 8.0, resulted in a further loss of activities when K^+ was omitted (unpublished data). Omitting both monovalent cations from the enzyme assay resulted in an ATPase activity of 60–70% of that obtained under complete assay conditions, consistent with previous findings[6,16]. We have chosen not to subtract any of these residual or partial activities, because we were able to detect an insulin effect on the ATPase in conditions we consider more 'physiological'.

Our results were obtained with rather high insulin concentrations. The data from a few experiments with physiological hormone concentrations did not show any significant enhancement of the ATPase activity (unpublished data). We interpreted this as a built-in shortage of the *in vitro* system; instead of vascular transport of insulin to the muscle cells, it is distributed within the muscle mass by means of a concentration gradient. When muscle pieces are mechanically divided longitudinally, a leakage of insulin-degrading enzymes from broken cells might occur, which would destroy fractions of the hormone, the degradation being more pronounced, the lower the hormone concentration[17,18]. In addition, insulin might adsorb to glassware. Different species of insulin might also influence the response.

TABLE 2 Effect of insulin on sarcolemma ATPase and 5′-nucleotidase activities after *in vitro* incubation of muscle cells with insulin

Enzyme	Treatment of animals	No. of experiments	Specific activity ± s.e.m. (μmol P_i per mg protein per min) ×10² Without insulin	With insulin
(Na^+, K^+)-ATPase	Ether anaesthesia	15	28.7 ± 2.9	33.5 ± 3.2
(Na^+, K^+)-ATPase	Nembutal	10	21.2 ± 1.5	29.2 ± 0.6
(Na^+, K^+)-ATPase	Alloxan diabetic	5	21.0 ± 3.8	28.0 ± 0.6
5′-nucleotidase	Nembutal	11	2.03 ± 0.13	1.99 ± 0.16

ATPase and 5′-nucleotidase activities assayed at 30° C as liberation of inorganic phosphate from ATP. Except for the alloxan diabetic rats, the animals were fasted for 20–22 h. Small muscle pieces were incubated in cylindrical vessels (1.6 × 10 cm) containing 2.5 ml Tyrode solution (8.0 g NaCl, 0.2 g KCl, 1.0 g $CaCl_2$, 0.05 g $MgCl_2$, 0.05 g NaH_2PO_4, 1.0 g $NaHCO_3$, and 2.0 g glucose diluted to 1 l), flushed with O_2:CO_2 = 95%:5%. Tissue mass per tube was 1.5 g. After temperature equilibrium, 10 μl insulin (1.2 mg ml^{-1}) was added to the incubation medium. Samples and controls were incubated at 37° C for 15 min. The controls and samples were treated equally and isolated simultaneously. The enzyme activities were measured as in Table 1.

It seems reasonable to conclude that insulin stimulates ATPase activity under physiological conditions, as shown indirectly by the variation of the controls as a function of blood sugar concentrations (Fig. 1), and from the fact that the highest control values are not further increased by incubation with high insulin concentrations *in vitro*.

Although this technique is adequate to demonstrate an insulin effect on membrane-bound ATPase activity, a better system must be developed in order to gain an insight into the mechanism of action.

The effect of insulin on stimulation of Na^+ and K^+ transport through muscle cell membranes is long established[19,20] but no attempts to link this effect to an insulin stimulation of the (Na^+, K^+)-activated ATPase have succeeded[2,21]. There might be different reasons for this: the enzyme activity has been assayed with crude cell homogenates in which the insulin effect is masked by gross action of unspecific phosphatases (unpublished results); isolated membrane preparations were exposed to the hormone before the enzyme was assayed without any effects, in agreement with our results, or controls might have been stimulated by insulin release after stress when the animals were killed.

Without any transport data, our experiments led to three conclusions. First, the (Na^+, K^+)-activated ATPase from isolated muscle membranes is not stimulated directly by insulin. Second, incubation of the muscle tissue with insulin before isolation of the membranes resulted in an increased ATPase activity and, third, there seems to be a correlation between blood sugar concentration and the tendency to stimulate ATPase activity *in vitro* by insulin.

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1 Skou, J. C., *Biochim. biophys. Acta*, **23**, 394–401 (1957).
2 Bonting, S. L., Simon, K. A., and Hawkins, N. M., *Archs Biochem. Biophys.*, **95**, 416–423 (1961).
3 Skou, J. C., *Physiol. Rev.*, **45**, 596–617 (1965).
4 Hadden, J. W., Hadden, E. M., Wilson, E. E., Good, R. A., and Coffey, R. G., *Nature new Biol.*, **235**, 174–177 (1972).
5 Hyvärinen, A., and Nikkilä, E. A., *Clin. chim. Acta*, **7**, 140–143 (1962).
6 Severson, D. L., Drummond, G. I., and Sulakhe, P. V., *J. biol. Chem.*, **247**, 2949–2958 (1972).
7 Tyrode, M. V., *Arch. Int.*, **20**, 205–223 (1910).
8 Taussky, H. H., and Shorr, E., *J. biol. Chem.*, **202**, 675–685 (1953).
9 Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265–275 (1951).
10 Mayhew, D. A., Wright, P. H., and Ashmore, J., *Pharmac. Rev.*, **21**, 183–211 (1969).
11 Kraegen, E. W., Young, J. D., George, E. P., and Lazarus, L., *Horm. Metab. Res.*, **4**, 409–413 (1972).
12 Rerup, C. C., *Pharmac. Rev.*, **22**, 485–518 (1970).
13 Lundquist, I., and Rerup, C., *Eur. J. Pharmac.*, **2**, 35–41 (1967).
14 Skou, J. C., *Bioenergetics*, **4**, 203–232 (1972).
15 Wallach, D. F. H., and Kamat, V. B., in *Methods in Enzymology* (edit. by Neufeld, E. F., and Ginsburg, V. G.), **8**, 164 (Academic Press, New York and London, 1966).
16 Peter, J. B., *Biochim. Biophys. Res.*, **40**, 1362–1367 (1970).
17 Mirsky, A., and Broh-Kahn, R. H., *Archs Biochem.*, **20**, 1–9 (1949).
18 Elgee, N. J., Williams, R. H., and Lee, N. D., *J. clin. Invest.*, **33**, 1252–1260 (1954).
19 Creese, R., *Nature*, **201**, 505–506 (1964).
20 Zierler, K. L., Rogus, E., and Hazlewood, C. F., *J. gen. Physiol.*, **49**, 433–456 (1966).
21 Rogus, E., Price, T., and Zierler, K. L., *J. gen. Physiol.*, **54**, 188–202 (1969).

In vitro suppression of cell mediated autoimmunity in NZB mice

The spontaneous autoimmune disease of NZB mice is characterised by the development of a variety of autoantibodies, particularly those reacting with autologous erythrocytes as demonstrated by a positive direct Coombs test. The onset of autoantibody production coincides with thymic hyperplasia and is followed by splenomegaly and glomerulonephritis[1]. All the tissues contain murine leukaemia virus particles (type C) visible on electron microscopy[2] and Gross antigen is detectable immunologically[3]. This virus is vertically transmitted, yet antibodies have been shown to develop to Gross antigen at the time the direct Coomb's test becomes positive[4]. The role of this virus, if any, in the aetiology of the autoimmune features is still unclear but it has been suggested that this progressive loss of tolerance to Gross antigen is of pathogenetic importance[4,5].

Old surviving mice have a high frequency of lymphomata[6,7]. Many of these are transferable to other mouse strains, but autoimmune phenomena are not associated with these[6,8]. It has not been possible to reliably transmit the disease to other strains with a cell-free filtrate nor indeed with whole cells[9–11] unless sufficient immunosuppression is also used to convert the recipient into an effective chimaera[12]. A depression of cell-mediated immunity to certain extrinsic antigens has been demonstrated in these mice before the manifestation of the autoimmune phenomena[13,14], yet the proportion of θ-positive cells in the spleen was shown to remain essentially undiminished[15,16], although one report does describe a progressive decrease with age[17]. Recently, we have provided evidence which suggests loss of the homeostatic control of autoreactivity with respect to cell mediated immunity: lymphocytes from diseased adult NZB mice were cytotoxic *in vitro* to syngeneic or autochthonous foetal fibroblasts. Cells from young NZB mice did not show this reactivity[18,19]. We here provide evidence that this cytotoxicity depends on the presence of θ-positive, presumably thymus-derived, cells. Furthermore, we were able to show inhibition of the cytotoxic effect of lymphocytes from the Coombs-positive, diseased NZB mice (NZB^+) by non-θ-bearing lymphocytes from young, Coombs negative NZB mice (NZB^-), and from young mice of other strains.

A modification of the microcytotoxicity assay of Takasugi and Klein[20] was used as previously described[18]. Test fibroblasts were cultured in Falcon microtest plates at a concentration of 100 cells per well and 24 h later, splenic lymphocytes purified on a Ficoll/Isopaque gradient[21] were added.

In these conditions, lymphocytes from NZB^+ mice were cytotoxic to syngeneic or autochthonous foetal NZB fibro-

Table 1 Effect of treatment of lymphocytes with anti-θ antiserum and fresh complement before incubation of these cells with fibroblast monolayers.

Source of lymphocytes*	Pretreatment	Source of fibroblasts	No. of Fibroblasts Remaining (±s.e.m.)
NZB+	nil	NZB foetal	43 ± 3†
NZB−	nil	NZB foetal	115 ± 8‡
NZB+	Anti-theta serum and C′	NZB foetal	132 ± 19
NZB	Anti-theta serum and C′	NZB foetal	130 ± 8
CBA	nil	NZB foetal	93 ± 7
NZB+	nil	CBA foetal	74 ± 7
NZB−	nil	CBA foetal	87 ± 6
CBA	nil	CBA foetal	97 ± 9

* NZB+, Autoimmune Coombs positive NZB mouse; NZB−, normal Coombs negative NZB mouse (see text); C′, guinea pig complement absorbed with agarose (see text)
† Significantly different from control values ($P < 0.001$)
‡ Control values

TABLE 2 Effect on autoreactivity of NZB+ cells by preincubation with dilutions of anti-θ antiserum with and without complement.

Dilution of anti-θ antiserum	Complement	% fibroblasts remaining* (±s.e.m.)†	
—	−	100	(±7)*
1:1	+	145	(±4)
1:2	+	96	22
1:5	+	106	9
1:10	+	43	5
1:1	−	31	3‡
1:2	−	18	2‡
1:5	−	19	2‡
1:10	−	16	2‡
—	+	10	3
—	−	30	5§
—	−	6	1‖

* Expressed as a % of control value obtained without addition of lymphocytes.
† Converted to a percentage.
‡ Significantly different from results where complement was included.
§ Preincubation with medium alone.
‖ No incubation.

blasts as is seen in Table 1. As our tissue culture system used heat-inactivated foetal calf serum in the culture medium, this phenomenon was most likely the result of cell mediated autoreactivity. In our previous experiments, no significant toxicity was demonstrable against fibroblasts from neonatal or adult BALB/c and CBA mice[18]. The ratios of lymphocytes to fibroblasts were varied from 1:10 to 1:2,000. The dose response curve obtained, showed that near maximal killing was achieved with a cell number ratio of 1:200 (ref. 18). In keeping with the methodology developed by Hellstrom[22], experiments were carried out using a ratio of one fibroblast to 2,000 lymphocytes.

The cellular origin of the NZB-killer activity was assessed by testing for the possible involvement of θ-bearing thymus derived cells. Lymphocytes from NZB$^+$ mice were treated with anti-θ serum and complement as described elsewhere[23]. The potency of this antiserum was verified by its capacity to interfere with the *in vitro* immune response to a T-cell dependent antigen (sheep erythrocytes) but not to a T-cell independent antigen (polymerised flagellin from *Salmonella adelaide* strain 1338). The source of complement was guinea pig serum previously absorbed with agarose for 30 min at 4° C, according to the method of Cohen and Schlesinger[24]. Such serum was free of non-specific cytotoxicity and had no effect on the potency of 'killer' cells from NZB$^+$ mice. Results from a representative experiment shown in Table 1 indicate that the cells involved in the 'self' killing mechanisms bear the θ antigen. The result of diluting the anti-θ antiserum can be seen in Table 2. It is only effective in the presence of complement. Cohen[25] has previously described

TABLE 3 The effect of the addition of lymphocytes from young animals on the cytotoxic ability of cells from diseased NZB mice on their isogeneic fibroblasts.

Source of young	No. Added	No. of NZB+ cells	No. of fibroblasts remaining (±s.e.m.)
—	—	200,000	53 ± 6*
NZB−	20,000	180,000	143 ± 13
NZB−	40,000	160,000	154 ± 21
NZB−	200,000	—	128 ± 20†
BALB/c	20,000	180,000	68 ± 6*
BALB/c	40,000	160,000	109 ± 7
BALB/c	200,000	—	99 ± 9*
CBA/HI	20,000	180,000	123 ± 19
CBA/HI	40,000	160,000	196 ± 31
CBA/HI	200,000	—	156 ± 20†

* Significantly different from control values ($P < 0.01$).
† Control values

the development *in vitro* of cell mediated autoimmunity. The responsible cells here too seem to be of thymic origin.

We have previously reported that the *in vitro* killing of NZB fibroblasts by NZB$^+$ lymphocytes may not be inhibited by serum from young NZB$^-$ mice[26]. It seems that the mechanisms which must prevent *in vivo* autoreactivity in the clinically normal, NZB$^-$ mouse are mediated by either serum factors too weak to be detected *in vitro* or by direct cellular control. To try and re-establish control over the autoimmune response *in vitro*, lymphocytes from NZB$^+$ mice were mixed with cells from 4-week-old mice before being added to the fibroblasts. Table 3 demonstrates that substitution of even 10% of cells from NZB$^-$ mice significantly suppressed the autoreactivity of lymphocytes from diseased NZB mice. This suppression effect was also demonstrable with cells from both BALB/c and CBA/HI strains. In each case, the effect of NZB$^-$ cells alone was taken as the control but each experiment must be considered individually as different lymphocytes have a variable stimulating effect on the growth of the fibroblasts present. It is therefore difficult to compare directly the relative efficacies of different cell types in their capacity as suppressor cells in this system. One may argue that inhibition of fibroblast killing by cells from clinically normal mice could have been due to a dilution effect on the killer cells. It is also possible that NZB$^-$ cells added to

TABLE 4 The effect of pretreating young NZB lymphocytes with anti-θ antiserum and complement on the inhibition of the autoimmune response.

Proportion of young NZB cells in mixture*	Pretreatment	Proportion of old cells	No. of fibroblasts remaining (±s.e.m.)
—	−	100%	43 ± 3†
100%	−	0%	115 ± 8‡
10%	−	90%	93 ± 7
10%	+	90%	111 ± 11
20%	+	80%	128 ± 10
33%	+	66%	132 ± 9
50%	+	50%	111 ± 11
20%§	−	80%	49 ± 4†

* Total number of cells (200,000) taken as 100%
† Significantly different from control value $P < 0.001$
‡ Control value
§ Killed by heating at 56° for 30 min

the autoreactive lymphocytes have acted as alternative targets and may thereby have competed with target fibroblasts for killer cells.

These suggestions seem untenable for the following reasons. First, the reduction in total number of self reactive cells from 200×10^3 to 180×10^3 or even 20×10^3 had little effect on reducing fibroblast killing[18] indicating that there was a gross excess of 'killer' cells when 200×10^3 lymphoid cells from NZB$^+$ mice were added to the 100 fibroblasts in a single well. Second, heat killed lymphocytes no longer inhibited the autoreactivity of NZB$^+$ cells (Table 4).

If the NZB$^-$ cells responsible for the suppression phenomenon were theta-bearing thymus-derived cells, suppression of autoreactivity should no longer be demonstrable after treatment of these cells with anti-theta serum and complement. As seen in Table 4, however, preincubation of young NZB cells with these reagents failed to abolish the *in vitro* suppressive effect. We tentatively suggest, therefore, that the observed suppression of self reactivity is mediated by θ negative bone-marrow derived B cells rather than by thymus-derived T cells. This is in accord with the similar finding of Phillips and Wegmann[27] on tetraparental mice. The possibility is not ruled out, however, that T cells with a low density of the θ surface marker may have escaped the cytotoxic action of anti-θ serum and complement.

Suppression of cell-mediated immunity by humoral factors

is now well known in similar experimental situations to the above. We have suggested earlier on experimental grounds that blocking of immunocompetent cells by antigen–antibody complexes could account for mechanisms of self tolerance[28]. As discussed elsewhere, immunocompetent cells could conceivably become inactivated *in vivo* by antigen–antibody complexes in solution or when attached to the surface of macrophages[29]. Depending on the configuration of a complex (as determined by the relative amounts of complexed antigen and antibody) a matrix of closely spaced free antigenic determinants of identical specificity would provide the optimal conditions for lymphocyte inactivation. In the case of tissue fixed complexes *in vivo*, blocking activity in serum may be minimal or totally absent and this could account for our failure to demonstrate such activity in the serum of NZB$^-$ mice[26]. Furthermore, inhibition *in vitro* of self reactivity by the addition of small numbers of lymphocytes from healthy animals could be due to the release of B cell derived 'auto-antibody' becoming complexed with a fibroblast derived 'self' antigen. These complexes would readily interact directly with immunocompetent effector cells by blocking their reactivity. A key question pertains to the mechanisms responsible for the breakdown of self tolerance in NZB mice as well as to the nature of the relevant antigen in this case. Although our work to date fails to provide information on these points, it opens a new experimental approach to the investigation of self tolerance and its homeostatic control.

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[1] Howie, J. B., and Helyer, R. J., *Adv. Immun.*, **9**, 215 (1968).
[2] East, J., Prosser, P. R., Holborow, E. J., and Jacquet, H., *Lancet*, **i**, 755 (1967).
[3] Oldstone, M. B. A., Aoki, T., and Dixon, F. J., *Science*, **174**, 843 (1971).
[4] Mellors, R. C., Aoki, T., and Huebner, R. J., *J. exp. Med.*, **129**, 1045 (1969).
[5] Mellors, R. C., Shirai, T., Aoki, T., Huebner, R. J., and Krawczynski, K., *J. exp. Med.*, **133**, 113 (1971).
[6] Denman, A. M., Russell, A. S., Loewi, G., and Denman, E. J., *Clin. exp. Immun.*, **20**, 973 (1971).
[7] Mellors, R. C., *Perspectives in Virology*, **6**, (edit. by Pollard, M.) 239 (1968).
[8] East, J., and Branca, M., *Clin. exp. Immun.*, **4**, 621 (1969).
[9] Mellors, R. C., and Chen, Y. H., *J. exp. Med.*, **124**, 1031 (1966).
[10] East, T., de Sousa, M. A. B., and Parrot, D. M. V., *Transplantation*, **3**, 711 (1965).
[11] Russell, P. J., Hicks, J. D., Boston, L. E., and Abbot, A., *Clin. exp. Immun.*, **6**, 227 (1970).
[12] Denman, A. M., Russell, A. S., and Denman, E. J., *Clin. exp. Immun.*, **5**, 567 (1969).
[13] Rodney, G. E., Yunis, E. J., and Good, R. A., *Clin. exp. Immun.*, **9**, 315 (1971).
[14] Ghaffar, A., Krsiakova, M., and Playfair, J. H. L., *Transplantation*, **10**, 432 (1970).
[15] Waksman, B. H., Raff, M. C., and East, J., *Clin. exp. Immun.*, **11**, 1 (1972).
[16] Stobo, J. D., Talal, N., and Paul, W. E., *J. Immun.*, **109**, 692 (1972).
[17] Stutman, O., *J. Immun.*, **109**, 602 (1972).
[18] Liburd, E. M., Russell, A. S., and Dossetor, J. B., *J. Immun.*, **111**, 1288 (1973).
[19] Stiller, C. R., Russell, A. S., McConnachie, P., Dossetor, J. B., and Diener, E., *Clin. exp. Immun.*, (in the press).
[20] Takasugi, M., and Klein, E., *Transplantation*, **9**, 219 (1970).
[21] Kissmeyer-Niehlsen, F., and Kjerbe, K. E., *Histocompatibility testing*. 381–83 (Munksgaard, Copenhagen, 1967).
[22] Hellstrom, I., Warner, G. A., Hellstrom, K. E., and Sjogren, H. O., *Int. J. Cancer*, **11**, 280 (1973).
[23] Diener, E., O'Callaghan, F. B., and Kraft, N., *J. Immun.*, **107**, 1775 (1971).
[24] Cohen, A., and Schlesinger, M., *Transplantation*, **10**, No. 1, 130 (1970).
[25] Cohen, I. R., *Nature new Biol.*, **242**, 60 (1973).
[26] Liburd, E., Russell, A. S., and Dossetor, J. B., *Clin. exp. Immun.*, (in the press).
[27] Phillips, S. M., and Wegman, T. G., *J. exp. Med.*, **137**, 291 (1973).
[28] Diener, E., Feldmann, M., and Armstrong, W. D., *Ann. N.Y. Acad. Sci.*, **181**, 119 (1971).
[29] Diener, E., and Feldmann, M., *Transplantation Rev.*, **8**, 76 (1972).

Intracellular J chain in mouse plasmacytomas secreting IgA, IgM and IgG

THE final step in assembly of IgA and IgM is the polymerisation of intracellular monomeric (H_2L_2) subunits. This event takes place just before, or at the time of secretion[1–7], and involves the incorporation of J chain[8,9] into IgM[10] and polymeric, but not monomeric IgA[10–11]. An unanswered question is whether J chain travels through the membranous elements of the cell, as do the other immunoglobulin subunits[12]. As a prelude to answering this, we have demonstrated intracellular J chain in polymer-secreting mouse myeloma cells. In addition, the postulation of an intraclonal switch from IgM to IgG in B-lymphocytes[13] prompted us to look for, and find, J chain in cells which secrete IgG.

To detect intracellular J chain, mouse myeloma cells were grown *in vitro* in the presence of radioactive leucine[1]. The cells were lysed either with 1% (w/v) Nonidet P40 or 1% (w/v) sodium deoxycholate in the presence or absence of 0.1 M iodoacetamide. Following centrifugation (30,000*g*, 40 min), a rabbit antiserum reactive with free, but not bound, J chain (R.M.E.P., unpublished) was added to the cell lysate, and complexes of radioactive J chain and the rabbit antibody were precipitated with goat anti-rabbit IgG as previously described[1]. The antibody–antigen precipitates were washed, reduced and alkylated and electrophoresed in alkaline urea polyacrylamide gels[10,14], and the amount of radioactivity in J chain was measured as the total found in the J chain peak.

Initial experiments were done with a tissue culture-adapted cell line of MOPC 315 which produces IgA and which was found to secrete 70% of the myeloma protein in dimer form; the remaining myeloma protein consisted of monomer and higher polymers in approximately equal amounts. With this cell line, similar yields of radioactivity in intracellular J chain were obtained whichever of the methods outlined above was used for lysing the cells.

The results of a typical experiment are presented in Fig. 1. When the total radioactive cell lysate was electrophoresed in an alkaline urea gel (Fig. 1*a*) there was no discrete peak of J chain. The presence of intracellular J chain could only be demonstrated in the precipitate formed with the specific anti-J chain reagent (Fig. 1*b*). Electrophoresis of unlabelled cell lysates of MOPC 315 myeloma cells followed by staining of the polyacrylamide gel, however, has been reported to reveal a prominent band in the J chain position[15]. In similar experiments using 6% (w/v) alkaline urea polyacrylamide gels we were unable to demonstrate J chain in MOPC 315 myeloma cell lysates, and we would therefore conclude that the reported prominent J chain band[15] is contaminated by other proteins.

To be certain that the peak of radioactivity in the J chain position (Fig. 1*b*) is authentic, and not the result of another cellular protein with similar electrophoretic properties, the radioactive cell lysate was reacted with a sample of anti-J chain antiserum which had been previously absorbed with a highly purified preparation of mouse J chain[16]. The re-

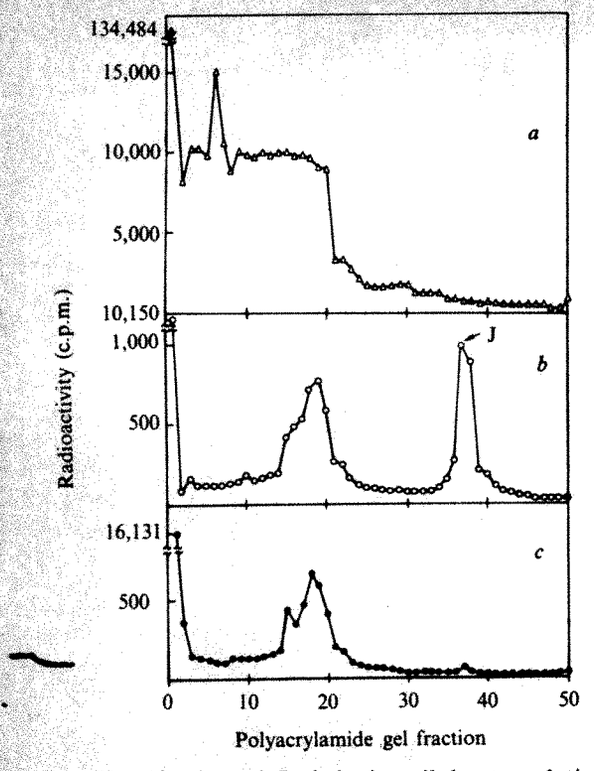


FIG. 1 Identification of J chain in cell lysates of tissue culture-adapted MOPC 315 mouse myeloma cells. Analysis by polyacrylamide gel electrophoresis. Myeloma cell suspensions at 2×10^7 cells ml^{-1} were incubated for 2 h in Eagle's medium containing 0.2 mCi of L- 4,5-^{3}H-leucine (51 Ci $mmole^{-1}$) ml^{-6}. The cells were collected by centrifugation, washed once with tissue culture medium and lysed in 0.075 M NaCl–0.075 M Tris–HCl, *p*H 8.0 containing 1% (w/v) sodium deoxycholate, 0.1 M iodoacetamide and DNAse (50 μg ml^{-1}). After standing at 37° C for 10 min, the cell lysates were centrifuged at 30,000*g* for 40 min and the supernatants were reserved for analysis on alkaline–urea 6% (w/v) polyacrylamide gels[10,14]. Before electrophoresis of the total unfractionated cell lysate, the material was dialysed against 8 mM Tris–25 mM glycine, *p*H 8.7. Radioactive J chain in the cell lysate was isolated by precipitation of soluble complexes formed between rabbit anti-mouse J chain and intracellular J chain with goat anti-rabbit immunoglobulin. As a control precipitation, a sample of the rabbit anti-mouse J chain was first absorbed with an excess of purified J chains[16] and then added to the radioactive cell lysate for precipitation with goat anti-rabbit immunoglobulin. Antibody–antigen precipitates were washed four times with phosphate-buffered saline containing 1% (w/v) Nonidet P-40, once with 8 mM Tris–25 mM glycine, *p*H 8.7, and then dissolved in 9 M urea–10 mM dithiothreitol by heating for 2 h at 56° C. Samples were then applied to alkaline–urea (6% (w/v)) polyacrylamide gels. The gels were sliced, dissolved and counted[10], and fractions are numbered from the negative (top) to the positive electrode. *a*, Total cell lysate; *b*, radioactive protein in the cell lysate precipitated by the rabbit anti-(mouse J chain) antiserum; *c*, radioactive protein in the cell lysate precipitated with the rabbit anti-mouse J chain antiserum which had been previously absorbed with purified mouse J chain.

sulting antibody–antigen precipitate between rabbit IgG and goat anti-rabbit IgG no longer contained radioactive J chain (Fig. 1*c*), thereby unequivocally confirming the original identification (Fig. 1*b*). The problem of non-specific contamination of the antibody–antigen precipitate is overcome by taking advantage of the unique electrophoretic mobility of J chain. The amount of radioactivity coprecipitated by absorbed and non-absorbed anti-mouse J chain is essentially the same, and only when the electrophoretic analysis is done can J chain be identified and quantitated.

The absence of a covalent linkage between intracellular J chain and the intracellular monomer precursor of secreted polymer[7,10] was confirmed with the tissue culture form of MOPC 315 myeloma cells, taking advantage of the specificity of the myeloma protein for 2,4-dinitrophenol. Cells (2×10^7 ml^{-1}) were labelled for 1 h with ^{3}H-leucine (59 Ci $mmol^{-1}$, 0.2 mCi ml^{-1}), and the resulting cell lysate (1% w/v Nonidet P-40, 0.1 M iodoacetamide) was filtered through a column of ε-N-2,4 dinitrophenyl-lysyl-sepharose[17]. The eluate was examined for free J chain by precipitation with the specific antiserum. The intracellular monomeric IgA was eluted from the column with 0.1 M DNP-glycine, reduced (10 mM dithiothreitol) and then electrophoresed in alkaline urea polyacrylamide gels to determine the presence or absence of associated J chains. Free J chain was found in the filtrate from the column and accounted for 1.5×10^{-2}% of the total radioactivity precipitated by 10% (w/v) trichloracetic acid; there was no detectable J chain associated with intracellular myeloma protein, although 10^5 c.p.m. were applied to the polyacrylamide gel.

Having identified J chain in the tissue culture-adapted MOPC 315 myeloma cell line, we then examined a variety of other tumour lines using similar methodology. The results are summarised in Table 1. Not unexpectedly, J chain was detected in cells secreting polymeric IgM (MOPC 104E) and IgA (MOPC 315 and 5647). More interesting was the finding of comparable amounts of J chain within myeloma cells secreting IgG_1 (MOPC 21 and MOPC 70E) and IgG_{2a} (Adj PC5 and 5563). In all cell lysates where a positive identification of J chain was made, there was no characteristic peak on polyacrylamide gel when the rabbit antiserum was absorbed with purified mouse J chain before the formation of the antibody–antigen precipitates with goat anti-rabbit immunoglobulin. We are therefore confident of our identification of J chain in IgG-secreting plasma cells. Relatively less J chain was present in a myeloma which secretes half molecules (HL) of an IgA (MOPC 47A) containing heavy chains of shorter than normal length[18,19]. No J chain could be detected in a tumour line which secretes only monomer (H_2L_2) species of IgA (MPC1). This is further evidence that the peak identified on alkaline-urea polyacrylamide gels is authentic J chain, because if this component were a polypeptide which fortuitously cross-reacted with J chain, its presence would be predicted in all myelomas.

The finding of J chain in IgG-secreting myeloma cells raises the question of whether J chain is restricted to cells of lymphoid origin. We therefore labelled a permanent cell line of mouse L cells and looked for J chain, as detailed in the

TABLE 1 Presence of J chain in mouse myeloma cells.

Tumour	Product	J chain (% total radioactivity $\times 10^{-2}$)
MOPC 104E	IgM, free L	3.29
MOPC 315	IgA (monomer and polymer)	2.81
5647	IgA (monomer and polymer)	1.03
MPC 1	IgA (monomer only)	Not detected
MOPC 47A	IgA (HL half molecule)	0.26
MOPC 21	IgG_1	1.35
MOPC 70E	Free L (K), traces of IgG_1	3.17
Adj PC5	IgG_{2a}	2.45
5563	IgG_{2a}	1.32

Myeloma cell suspensions were prepared from solid tumours, incubated for 2 h with radioactive leucine and cell lysates were prepared as indicated in the legend to Fig. 1. Total radioactivity in protein was measured by precipitation with 10% (w/v) trichloroacetic acid. Samples containing 2×10^7–6×10^7 c.p.m. were reacted with the rabbit antimouse J chain antiserum and goat anti-rabbit immunoglobulin, and the precipitates were analysed on alkaline–urea–polyacrylamide gels as described in the legend to Fig. 1. The radioactivity in J chain was obtained by summing the counts found in the position on the gel characteristic for J chain. This amount is expressed as % of the total trichloroacetic acid-precipitable radioactivity in the cell lysate. Verification of identity was obtained in all cases by electrophoresis of precipitates made using a sample of rabbit anti-mouse J chain previously absorbed with purified mouse J chain (see text and legend to Fig. 1).

legend to Fig. 1. J chain could not be detected in radioactive lysate samples containing up to 6×10^7 c.p.m. Although more non-lymphoid cells need to be examined, nonetheless the tentative conclusion is that J chain is not a ubiquitous cellular constituent and may be confined to cells of lymphoid origin.

What is the fate of J chain synthesised by IgG-secreting cells? One possibility is intracellular degradation and another secretion. The latter is more easily tested. Myeloma cells secreting IgM (MOPC 104E), IgA (MOPC 315), IgG_1 (MOPC 21) and IgG_{2a} (5563 and Adj PC5) were incubated with radioactive leucine and allowed to secrete for a period of 5 h. Culture supernatants containing 2×10^7 c.p.m. in trichloroacetic acid-precipitable radioactivity were tested for the presence of free J chain using the specific rabbit antiserum as already described. In all cases tested free J chain was absent. This makes it more likely that it is degraded following synthesis by IgG-secreting cells. This would be compatible with the absence of covalent and non-covalent interaction between intracellular immunoglobulin and J chain[7,10], and the fact that only at the subcellular site where polymerisation takes place is J chain incorporated into polymer molecules[10,11]. As polymerisation takes place just before, or at the time of, secretion[1-7], it is reasonable to suggest that newly synthesised J chain follows the same intracellular route irrespective of the class of immunoglobulin secreted. If J chain is degraded in IgG-secreting cells, this probably occurs at the same subcellular site as polymerisation.

The failure of the IgA-secreting tumour line MPC1 to secrete polymeric molecules is not entirely attributable to the absence of J chain. The reason for this assertion is that secreted IgA monomers of MOPC 315 can be polymerised *in vitro* when J chain and a disulphide interchange enzyme are supplied[16]. In identical conditions, the secreted monomeric IgA of MPC1 is not polymerised (R.M.E.P., and E. Della Corte, unpublished). A likely explanation would be a structural alteration in the heavy chain, perhaps at the site involved in linkage to the J chain. The secreted HL subunits of MOPC 47A were also unable to form polymers *in vitro* when supplied with J chain and the disulphide interchange enzyme (R.M.E.P., and E. Della Corte, unpublished). Extensive deletions are known to be present in the heavy chain of this tumour line[18,19]. The presence or absence of intracellular J chain, together with the potential for secreted immunoglobulin to be polymerised *in vitro*, should form the basis of a valuable approach for analysis of myeloma variants secreting IgA and IgM.

The MOPC 70E tumour line secretes traces of IgG_1, but the major secretory product of the cell is light chain. In this situation of suppressed heavy chain synthesis, however, intracellular J chain was demonstrated, indicating that the genes for heavy chains and J chains are not coordinately controlled.

What is the significance of J chain present in myeloma cells secreting IgG? While other interpretations must be considered, for example that the cells studied are neoplastic, it is possible to speculate that intracellular J chain in IgG-secreting cells represents a relic from previous commitment to IgM synthesis, which would also allow the same progenitor cell line to switch from IgG to IgA synthesis. Such a developmental succession of immunoglobulin class expression has already been postulated[13].

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1 Parkhouse, R. M. E., and Askonas, B. A., *Biochem. J.*, **115**, 163 (1969).
2 Parkhouse, R. M. E., *Biochem. J.*, **123**, 635 (1971).
3 Parkhouse, R. M. E., *FEBS. Lett.*, **16**, 71 (1971).
4 Parkhouse, R. M. E., *Transplantn Rev.*, **14**, 131 (1973).
5 Bevan, M. J., *Eur. J. Immun.*, **1**, 133 (1971).
6 Bargellesi, A., Periman, P., and Scharff, M. D., *J. Immun.*, **108**, 126 (1972).
7 Della Corte, E., and Parkhouse, R. M. E., *Biochem. J.*, **136**, 589 (1973a).
8 Halpern, M. S., and Koshland, M. E., *Nature*, **228**, 1276 (1970).
9 Mestecky, J., Zikan, J., and Butler, W. T., *Science*, **171**, 1163 (1971).
10 Parkhouse, R. M. E., *Nature new Biol.*, **236**, 9 (1972).
11 Halpern, M. S., and Coffman, R. L., *J. Immun.*, **109**, 674 (1972).
12 Bevan, M. J., Parkhouse, R. M. E., Williamson, A. R., and Askonas, B. A., *Progr. Biophys. molec. Biol.*, **25**, 131 (1972).
13 Cooper, M. D., Lawton, A. R., and Kincade, P. W., *Clin. exp. Immun.*, **11**, 143 (1972).
14 Reisfeld, R. A., and Small, P. A., *Science*, **152**, 1253 (1966).
15 Raam, S. V., and Inman, F. P., *J. Immun.*, **110**, 1044 (1973).
16 Della Corte, E., and Parkhouse, R. M. E., *Biochem. J.*, **136**, 597 (1973b).
17 Goetzl, E. J., and Metzger, H., *Biochemistry*, **9**, 1267 (1970).
18 Seki, T., Appella, E., and Itano, H. A., *Proc. natn. Acad. Sci. U.S.A.* **61**, 1071 (1968).
19 Mushinski, J. F., *J. Immun.*, **106**, 41 (1971).

Expression of polyoma-induced antigens in low malignant hybrids derived from fusion of a polyoma-induced tumour with a fibroblast line

HARRIS *et al.*[1] have shown that hybrid cells (A9/SEWA) derived from fusion of cells of polyoma-induced sarcoma (SEWA) and an aneuploid long-established fibroblast line (of L origin), A9, had low tumorigenicity (Table 1)[2]. They have also shown[3] that the polyoma-induced transplantation antigen (PTA) was present in low tumorigenic hybrids of SEWA/T6 T6. To investigate the relationship between malignancy and cell surface modifications, we analysed, in this system, the polyoma-specific surface antigen revealed by immunofluorescence[4] concurrently with other characteristics of these cells, such as karyotype, transplantability and the presence of T antigen.

Antigen T was demonstrated by immunofluorescence on fixed cells as previously described[5,6]. Two positive antisera were used. The counts were done on 10^3 cells for three independent experiments. The parental SEWA cells and the A9/SEWA hybrid were both strongly positive. The parental A9 strain was negative (Table 2).

Surface antigen was studied on living cells using the technique we have previously described[4]. Five hundred cells were counted and two repeat counts performed for each experiment. The antisera were produced in A.SW mice injected with 10^7 PFU polyoma virus once a week for 3 weeks. After an interval of 1 week, the mice were challenged with 10^5 SEWA cells injected intraperitoneally. The sera collected from the tumour-free mice, 2 weeks later, were tested by immunofluorescence using SEWA cells as the positive control and A9 and A.SW mouse kidney cells as negative controls. Positive results were also obtained when these sera were tested against cells of a cell line (C3H Py Broc.) that had been

TABLE 1 Growth of cells *in vivo**

Cell type	Genotype of host	Unirradiated animals: No. takes (Total no. animals with progressive tumours/total no. inoculated)	Unirradiated animals: % takes	Irradiated animals: No. takes (Total no. animals with progressive tumours/total no. inoculated)	Irradiated animals: % takes
A9	C3H			3/25	12
SEWA/A9	C3HxA.SW F_1			8/25	32
SEWA†	A.SW	4/4	(100)	17/17	100
SEWA†	A.SW	45/61	73	93/98	93

* 5×10^4 to 2×10^6 cells were injected subcutaneously in syngeneic newborn animals
† Tumour cells adapted to growth *in vitro*

transformed *in vitro* by polyoma virus. The results, summarised in Table 2, show that the hybrid does carry the polyoma-specific membrane antigen in very similar proportions (about 80%) whatever the passage level.

TABLE 2 Polyoma antigens

Cells	A9	SEWA	A9/SEWA 8th passage*	A9/SEWA about 10th passage*	A9/SEWA about 40th passage*	A9/SEWA 43rd passage*
T antigen % of positive cells	0	60 ± 10		30 ± 10	20 ± 10	
Polyoma-specific surface antigen 11% of positive cells	0.4	88 ± 5	82 ± 5			88 ± 5

* Average results of four experiments done on subcultures about tenth or fortieth serial passage after the receipt of the hybrid at Marseilles.

Experiments with antiserum absorption gave results which did not differ significantly between the parent lines and the hybrids. The following technique was used in these antiserum absorption experiments. The limiting dilution (1/320) of the anti S-polyoma serum was first determined using a

TABLE 3 Absorption of antiserum by ascitic SEWA and A9/SEWA cells

Cell line	Number of cells ($\times 10^7$) used for absorption	% positive cells
	0.5	80
	1	80
SEWA	2.5	30
	5	5
	10	5
	0.5	80
	1	20
A9/SEWA	2.5	5
	5	5
	10	5

Anti S-Polyoma serum at 1/320 dilution was absorbed by contact with the cells for 1h at 37°C and overnight at 4°C. The serum was then retested on the target cells (SEWA and A9/SEWA).

1/10 dilution of fluorescein-labelled anti-mouse immunoglobulin. The absorption was then carried out by contact of the serum at 1/320 with the cells for 1 h at 37° C and overnight

TABLE 4 Surface histocompatibility antigens measured by immunofluorescence

Cell antigens	A9	SEWA	A9/SEWA
H-2^k*	45%	2%	35%
H-2^s*	4%	30%	23%

* Once a week A.SW mice were injected intraperitoneally with 10^6 C3H mouse spleen cells for 3 consecutive weeks. These spleen cells were prepared for injection as follows: the spleen was removed and the cells scraped off with a file. The resulting cell suspension, which contained many red blood cells was first washed in PBS free of Ca^{++} and Mg^{++} by centrifuging at 200*g* for 5 min. After treatment by hypotonic shock, the cells were collected by a second centrifugation. The same operation was performed with C3H mice injected with spleen cells of A.SW mice; sera were used at 1/30 dilution.

at 4° C. The serum was then retested with the target cells (SEWA cells in ascitic form). Absorption with 5×10^7 SEWA cells in ascitic form ml^{-1} and 2.5×10^7 A9/SEWA cells ml^{-1} was necessary to obtain identical extinctions (Table 3). When the cell size and the fact that about 30% of the cells in the ascitic form of the SEWA tumour are not tumour cells (for example the macrophages and endothelial cells) are considered, this difference cannot be regarded as significant.

TABLE 5 Immunogenicity

A.SW pretreated with	Total N° of inoculated cells	Takes in A.SW mice 20 d after challenge
10^6 PFU polyoma	—	2/11
A9/SEWA*	3×10^6	3/22
UV irradiated SEWA (3,000 erg mm^{-2})	3×10^6	2/6
A9	3×10^6	5/5
Control	—	14/15

* Average results of three experiments done on subcultures about thirtieth and sixtieth serial passage after receipt of hybrid at Marseilles.
Growth in 2 month old A.SW mice of SEWA in ascitic form after inoculation of various agents once a week for 3 week interval of 2 week and then a challenge I/P injection of 1×10^3 SEWA cells.

The results of testing for H-2 antigens are shown in Table 4 and the ability of various test cells to induce a rejection reaction of TATA-positive SEWA cells in syngeneic A.SW mice are shown in Table 5.

The karyotype study, which mainly took into account the evolution of the mode and the presence of markers with respect to the passage level of the cell lines, was carried out both for the hybrid and parental strains, and is summarised by the histogram in Fig. 1.

The presence of these antigens indicates that the viral genome is expressed in the hybrid cells. We have demonstrated previously that at least half of the polyoma genome necessary for replication is involved in the coding for the expression of T and S antigen[7,8] and one fifth for the expression of TATA. It is also clear that the introduction of a fibroblast genome represses malignant behaviour, as shown previously[9] but does not interfere with the polyoma-specific antigen expression of the transformed cells. In view of the fact that only certain cells can be transformed by polyoma virus, the genetic or the epigenetic constitution of the target cell is obviously decisive for its competence to behave as

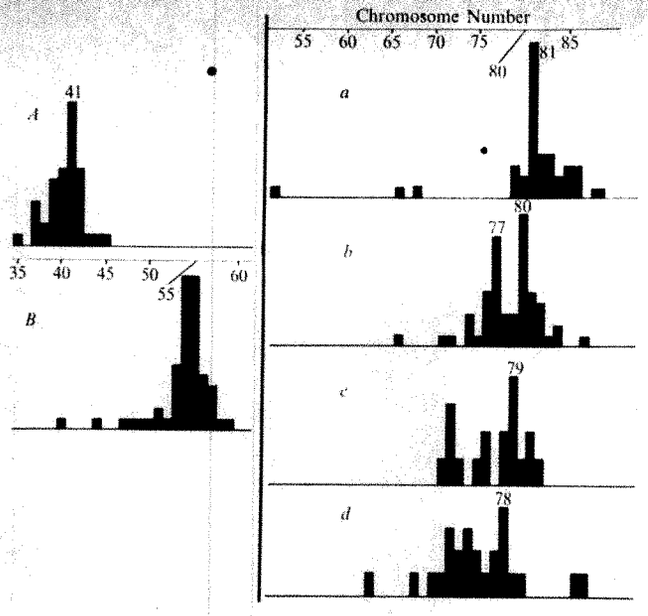


FIG. 1 Karotype analysis of hybrid and parental strains. *A*, SEWA; *B*, A19; *a* A9/SEWA hybrid, third passage; *b*, twenty-eighth passage; *c*, seventieth passage; *d*, 118th passage.

a malignant cell after the covalent integration of the viral genome. The role of the cellular genome is also suggested by the experiments on reversion[10], showing that rearrangement of the genetic material may suppress the expression of transformed and/or neoplastic properties, in spite of the continued presence of the viral genome. The same conclusion is reinforced by our findings on hybrid cells. Obviously the genome of at least certain fibroblasts will inhibit tumour growth, in spite of the continued presence of the virally determined antigens. Although these antigens may be necessary for the malignant behaviour of the polyoma transformed cell, with the appropriate genetic or epigenetic constitution, it will follow from this reasoning that this is not necessarily sufficient and other modifying factors may play a decisive role for the realisation of the virally determined neoplastic potential.

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1 Harris, H., Miller, O. J., Klein, G., Worst, P., and Tachibana, T., *Nature*, **223**, 363 (1969).
2 Klein, G., Bregula, U., Wiener, F., and Harris, H., *J. Cell. Sci.*, **8**, 659 (1971).
3 Klein, G., and Harris, H., *Nature new Biol.*, **237**, 163 (1972).
4 Lherisson, A. M., Meyer, G., and Bonneau, H., *Bull. Cancer*, **54**, 419 (1967).
5 Meyer, G., Lherisson, A. M., and Bonneau, H., *Bull. Cancer*, **54**, 79 (1967).
6 Fogel, M., Gilden, R. V., and Defendi, V., *Proc. Soc. Exp. Biol. Med.*, **124**, 1047 (1967).
7 Meyer, G., and Birg, F., *J. gen. Virol.*, **9**, 127 (1970).
8 Meyer, G., Lherisson, A. M., and Bonneau, H., *Int. J. Cancer*, **4**, 520 (1969).
9 Wiener, F., Klein, G., and Harris, H., *J. cell. Sci.*, **8**, 681 (1971).
10 Rabinowitz, Z., and Sachs, L., *Nature*, **225**, 136 (1970).

Lipopolysaccharides inhibit lymphosarcoma cells of bone marrow origin

BACTERIAL lipopolysaccharides (LPS) stimulate DNA synthesis specifically in B (thymus-independent) lymphocytes[1–3], increase immunoglobulin synthesis and secretion in B cells[5], and stimulate the immune response of antibody production to various antigens in the apparent absence of T (thymus-dependent) lymphocytes[4,6–8]. Recently a mouse B lymphosarcoma, PU-5-1, has been described which has surface immunoglobulin[9], and produces a small amount of immunoglobulin (R. Asofsky, unpublished). Cells of this tumour were adapted to culture and tested for the LPS stimulation of immunoglobulin secretion. Surprisingly, growth of the tumour cells was inhibited by LPS, although there was no effect on mouse myelomas or T lymphomas. Inhibition also occurred with an Abelson leukaemia virus induced tumour believed to represent a B lymphoma[10] or immature myelomonocytic neoplasm[11]. No effect was observed on the growth rate of a number of human B cell lines tested. LPS is not cytotoxic for the tumour cells: growth resumes when it is removed. Incorporation of precursors into DNA is inhibited before RNA and protein synthesis is affected. The active com-

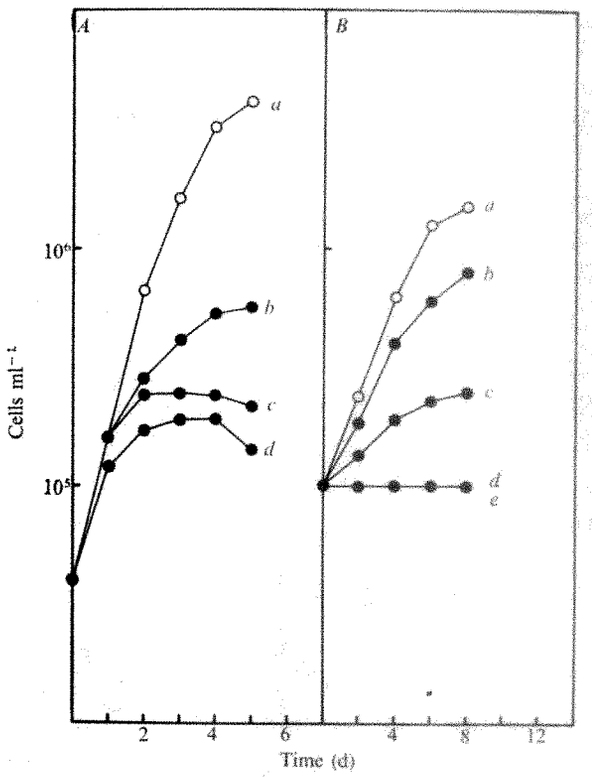


FIG. 1 Growth inhibition by LPS. *A*, RAW 8 cell line was adapted to culture from the second transplanted generation of a BALB/c tumour originating from infection of spleen cells *in vitro* with Abelson leukaemia virus[30] (in preparation). The cells grow as round floating cells in Dulbecco modified Eagle's medium containing 10% foetal calf serum in Petri dishes[16]. LPS (*S. typhosa* W 0901, Difco Laboratories, Detroit) was dissolved in phosphate buffer, *p*H 8.0, at 10 mg ml^{-1}, boiled for 60 min, and stored frozen until use. Cultures were innoculated at 0.4×10^5 ml^{-1} with *a*, 0; *b*, 1; *c*, 5 or, *d*, 50 μg ml^{-1} as shown. Viable cell counts were made daily. *B*, PU-5-1 is a spontaneous lymphosarcoma from a germ-free BALB/c mouse with immunoglobulin μ and κ determinants on its surface[9] (obtained from R. Asofsky, N.I.H., Bethesda, Maryland). The tumour was adapted to culture at generation 35 and grown in RPMI-1640 medium with 10% horse serum as above. Cultures were initiated at 1×10^5 cells ml^{-1} with, *a*, 0; *b*, 1; *c*, 5; *d*, 20 or *e*, 50 μg ml^{-1} LPS as shown.

TABLE 1 Cell lines tested for LPS inhibition

Murine		Primate	
Sensitive		Sensitive	
PU-5-1.8	B lymphoma	None tested	
RAW 8.3	Lymphosarcoma		
Resistant		Resistant	
S49.1	T lymphoma		
WEHI 22.1	"	MLC	Marmoset T cell line
EL4	"		
R1	"		
C1	Myeloma	Human B cell lines	
P3	"		
J558	"		
MOPC 315	"	RAJI	Burkitt's lymphoma
S117	"	DAUDI	"
GM-86	Erythroleukaemia	WIL2	Normal
P815	Mastocytoma	8866	
C1498	Myeloid leukaemia	UM-37	

Cell lines were tested for growth inhibition by LPS as in Fig. 1. Cultures of cell lines listed as Resistant contained more than 50% of the number of cells in control cultures when incubated 4–8 d with 100 μg ml^{-1} LPS. P815-X2 was obtained in culture from D. Grausz, University of California at San Diego; WEHI-22.1 from A. Harris, Walter and Eliza Hall Institute, Melbourne; GM-86 from Institute for Medical Research, Camden, New Jersey. The other mouse cell lines are listed in ref. 16. Marmoset and human cell lines were obtained through the courtesy of R. Lerner[28], Scripps Clinic and Research Foundation, La Jolla, California; G. Moore[17], Denver General Hospital, Denver; A. Bloom[29], University of Michigan, Ann Arbor; and G. Klein, Institute of Tumor Biology, Stockholm. UM-37 was derived from a patient with myeloma. The primate lines were grown in RPMI-1640 medium containing 10% foetal calf serum in Petri dishes.

ponent of LPS seems to be the lipid A, as shown for its pyrogenic[12], mitogenic[8,13–15], and adjuvant[8,13] properties.

At 1–50 μg ml^{-1}, LPS from *Salmonella typhosa* inhibited the growth of RAW 8 cells (Fig. 1), a lymphosarcoma of a BALB/c mouse derived from *in vitro* infection of spleen cells with Abelson leukaemia virus. Cell growth continued normally for one or two doublings, and then was completely inhibited in the presence of 5 μg ml^{-1} LPS. Growth of PU-5-1, a spontaneous BALB/c B-type lymphoma[9] was inhibited by LPS at 5–50 μg ml^{-1} without a delay (Fig. 1). LPS from *E. coli* (W 055:B5 and 0111:B4, Difco) gave similar results. Lipid A derived from LPS by mild acid hydrolysis also inhibited RAW 8 and PU-5-1 cell growth at 5–20 μg ml^{-1} (not shown). These concentrations of LPS and lipid A which are inhibitory to the tumour cells were previously shown to be optimally mitogenic for normal spleen B lymphocytes[4,8].

Cell lines which are not sensitive to LPS inhibition of growth are listed in Table 1. Among murine tumours these include. θ^+ T lymphomas, myelomas, a myeloid leukaemia, an erythroid leukaemia, and a mastocytoma. Some cell lines show a partial inhibition of DNA synthesis 24 h after LPS addition, such as the T lymphoma S49[16], but DNA synthesis resumes and overall growth does not seem to be affected. A number of human lymphoid cell lines with surface immunoglobulin, considered to be B cells, and a marmoset T cell line are also unaffected by LPS (Table 1). A human myeloma line[17], RPMI 8226, is partially inhibited by LPS.

Experiments were performed to determine the sequence of events during the LPS inhibition of synthesis of macromolecules. Figure 2 shows that 20 μg ml^{-1} LPS causes a rapid inhibition of incorporation of ^{3}H-thymidine into DNA in RAW 8 cells, followed by a decrease in apparent RNA synthesis, both approaching 30–40% of control levels by 24 h. As two doublings occur before growth stops, one possibility is that an early event involves the LPS inhibition of transport of nucleic acid precursors rather than direct inhibition of DNA synthesis. Protein synthesis, as measured by the uptake of an essential amino acid, continues unaffected for more than 24 h before showing impairment. PU-5-1 cells also exhibit a rapid decline of incorporation into DNA, but not into RNA or protein (Fig. 2).

As cells remained viable for up to 5 d during LPS inhibition of growth, reversal of the LPS effect was tested. Both RAW 8 and PU-5-1 cells, cultured in the presence of 20 μg ml^{-1} LPS for 2 d, resumed DNA synthesis and multiplication when washed and recultured without LPS (not shown).

A screening was made of drugs with biological effects similar to LPS. BCG is an immunological adjuvant stimulating the reticuloendothelial system[18], and synthetic polyribonucleotides have toxic and adjuvant properties like LPS[19], but BCG (at 4 × 10^5 viable bacteria ml^{-1}; Eli Lilly), poly(I)·poly(C) (100 μg ml^{-1}; P L Biochemicals) and poly(A)·poly(U) (100 μg ml^{-1}; Miles Laboratories) did not inhibit growth of RAW 8 cells. Some thymus-independent antigens such as dextran (molecular weight 500,000, Pharmacia) and polyvinyl pyrrolidone (molecular weight 160,000, Baker; molecular weight 360,000, Sigma) are reported to be mitogenic specifically for B lymphocytes[20], as is LPS, but do not affect RAW 8 growth at 1 mg ml^{-1}. Dextran and zymosan, like LPS, activate complement components[21], which bind to B lymphocytes. Zymosan caused only moderate inhibition of RAW 8 growth at 1 mg ml^{-1} (Sigma, 50% of control cell number at day 4 as in Fig. 1) and the LPS inhibition occurred equally well in fresh or heat-inactivated foetal calf serum, indicating that complement is not involved.

A variety of microbiological products and synthetic polynucleotides are under investigation for nonspecific stimulation of host immunological resistance to tumours[18]. LPS and endotoxins are reported to have inhibitory effects on tumours *in vivo*[22], and this has been correlated with the local Schwartzman reaction and haemorrhagic necrosis[23]. With some tumours, the endotoxin effect may be mediated by factors released by the host rather than a direct action on the tumour[23]. The experiments described here suggest that certain lymphosarcomas may be susceptible also to direct inhibition by LPS. In preliminary experiments, mice given RAW 8 and PU-5-1 tumour cells, preincubated 1 h with LPS, or mice treated with LPS injections 2 d after innoculation, survived significantly longer than controls. LPS treatment did not increase survival of mice with other types of tumours.

Not all tumours derived from B lymphocytes are inhibited by LPS. Mouse myelomas and human B lymphoid lines are

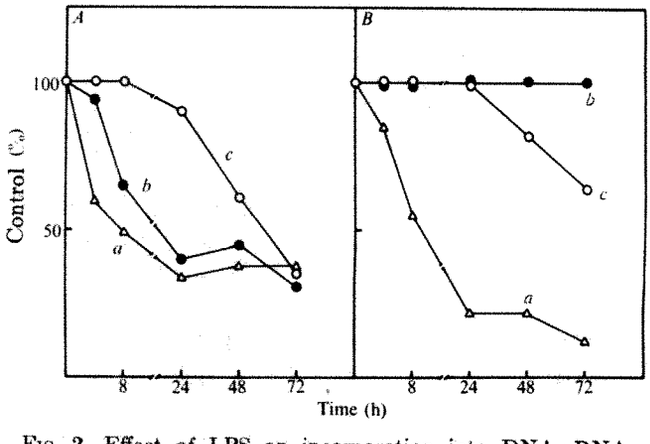


FIG. 2 Effect of LPS on incorporation into DNA, RNA and protein. Two ml cultures of *A*, RAW 8 or *B*, PU-5-1 were innoculated with or without 20 μg ml^{-1} LPS as in Fig. 1. At the times shown, duplicate dishes were incubated 1 h with 1 μCi ^{3}H-thymidine (50 Ci mmole^{-1}), 1 μCi ^{3}H-uridine (25 ci mmole)$^{-1}$, or 10 μCi ^{3}H-leucine (medium contains 0.8 mM leucine) to measure incorporation into, *a*, DNA; *b*, RNA or, *c*, protein, respectively. At the end of 1 h, the cells were collected and TCA-precipitable radioactivity determined. Each point is the mean of measurements from duplicate cultures which agreed within 10%. Results are shown as incorporation in the presence of LPS as a % of control cultures.

resistant (Table 1). There may be some types of human lymphosarcomas, however, which resemble RAW 8 and PU-5-1 in LPS sensitivity. Human peripheral blood lymphocytes[24] and spleen cells (P.R., and I.N., unpublished) do not respond to the mitogenic effect of LPS. LPS is not mitogenic or toxic to the mouse strain C3H/HeJ (ref. 25). Experiments are in progress to induce Abelson leukaemia virus tumours in these mice to see which biological activities are correlated with lymphosarcoma inhibition.

The inhibition of B lymphomas by LPS which is a mitogen for normal B lymphocytes is curious. A similar paradox was seen in the toxicity of PHA, a T lymphocyte mitogen, for T lymphomas but not other tumours[16,26]. The stimulating signals from the mitogens on the already dividing tumour cells may result in a push to a differentiated end cell type incapable of further division. Further proof is needed that the LPS-sensitive cells described here are tumours of B lymphocytes rather than of a form of monocyte as LPS is reported to have inhibitory effects on macrophages or phagocytic mononuclear cells[27].

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1 Janossy, G., and Greaves, M. F., *Clin. exp. Immun.*, **9**, 483 (1971).
2 Andersson, J., Möller, G., and Sjöberg, O., *Cell. Immun.*, **4**, 381 (1972).
3 Kruger, G., and Spiesel, S. Z., *J. Immun.*, **108**, 1088 (1972).
4 Watson, J., Epstein, R., Nakoinz, I., and Ralph, P., *Immunology*, **110**, 43 (1973).
5 Melchers, F., and Andersson, J., *Transpln. Rev.*, **14**, 76 (1973).
6 Jones, J. M., and Kind, P. D., *J. Immun.*, **108**, 1453 (1972).
7 Schmitdke, J. R., and Dixon, F., *J. exp. Med.*, **136**, 392 (1972).
8 Watson, J., Trenkner, E., and Cohn, M., *J. exp. Med.*, **138**, 699 (1973).
9 Green, I., Shevach, E. M., Stobo, J., Frank, M., and Herberman, R., *Adv. exp. Med. Biol.*, **29**, 491 (1972).
10 Abelson, H. T., and Rabstein, L. S., *Cancer Res.*, **30**, 2213 (1970).
11 Siegler, R., Zajdel, S., and Lane, I., *J. natn. Cancer Inst.*, **48**, 189 (1972).
12 Galanos, C., Rietschel, E. Th., and Lüderitz, O., *Eur. J. Biochem.*, **19**, 143 (1971).
13 Chiller, J. M., Skidmore, B. J., Morrison, D. C., and Weigle, W. O., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2129 (1973).
14 Peary, D. L., Shands, Jun., J. W., Adler, W. H., and Smith, R. T. *J. Immun.*, **111**, 352 (1973).
15 Janossy, G., Humphrey, J. H., Pepys, M. B., and Greaves, M. F., *Nature new Biol.*, **245**, 108 (1973).
16 Ralph, P., and Nakoinz, I., *J. natn. Cancer Inst.*, **51**, 883 (1973).
17 Matsuoka, Y., Yagi, Y., Moore, G. E., and Pressman, D., *J. Immun.*, **102**, 1136 (1969).
18 Yashphe, D. J., in *Immunological Parameters of Host-Tumor Relationships* (edit. by Weiss, D. W.) 90 (Academic Press, New York, 1972).
19 Han, I. H., Johnson, A. G., Cook, J., and Han, S. S., *J. infect. Dis.*, **128**, S232 (1973).
20 Coutinho, A., and Möller, G., *Nature new Biol.*, **245**, 12 (1973).
21 Janossy, G., Humphrey, J. H., Pepys, M. B., and Greaves, M. F., *Nature new Biol.*, **245**, 108 (1973).
22 Nauts, H. C., Fowler, G. A., and Bogatko, F. H., *Acta med., scand.*, **145**, S276 (1953).
23 Old, L. J., and Boyse, E. A., in *The Harvey Lectures*, **67**, 273, (Academic Press, New York, 1973).
24 Peavy, D. L., Adler, W. H., and Smith, R. J., *J. Immun.*, **105**, 1453 (1970).
25 Sultzer, B. M., and Nilsson, B. S., *Nature new Biol.*, **240**, 198 (1972).
26 Ralph, P., *J. Immun.*, **110**, 1470 (1973).
27 Neter, E., in *Current Topics in Microbiology and Immunology*, **47**, 82, (Springer-Verlag, Berlin, 1969).
28 Kennel, S. J., and Lerner, R. A., *J. molec. Biol.*, **76**, 485 (1973).
29 Poulik, M. D., and Bloom, A. D., *J. Immun.*, **110**, 1430 (1973).
30 Potter, M., Sklar, M. D., and Rowe, W. P., *Science*, **182**, 592 (1973).

Complement-mediated mixed aggregation of murine spleen cells

RECEPTORS on the surface at B cells for the fixed C3 component of complement have previously been studied by investigating the adherence to lymphocytes of complement-coated immune complexes and their function interpreted in the light of this reaction[1–10]. Other nucleated cells, however, notably macrophages, in the secondary lymphoid organs, also have receptors for fixed C3 (refs 3 and 4). It is therefore possible that alexinated complexes might mediate mixed adherence of different cell types which could have more significant functional consequences for complement receptor lymphocytes than just direct binding to their C3 receptors.

In vivo and *in vitro* studies indicate that C3 plays a part in the induction of thymus-dependent humoral antibody formation, but not in the response of B cells either to mitogens or thymus-independent antigens (refs 11, 12, 12a and unpublished results of M.B.P.). I have suggested on the basis of this evidence that C3 fixed on antigen-containing complexes might, as a consequence of its interaction with cell surface C3 receptors, enhance or facilitate T-dependent presentation of antigen to B cells at the macrophage surface (refs 11 and 13 and M.B.P., unpublished). Here I confirm the feasibility of this model and demonstrate the complement-mediated mixed adherence of spleen cells which results from binding of fixed C3 to cell surface C3 receptors.

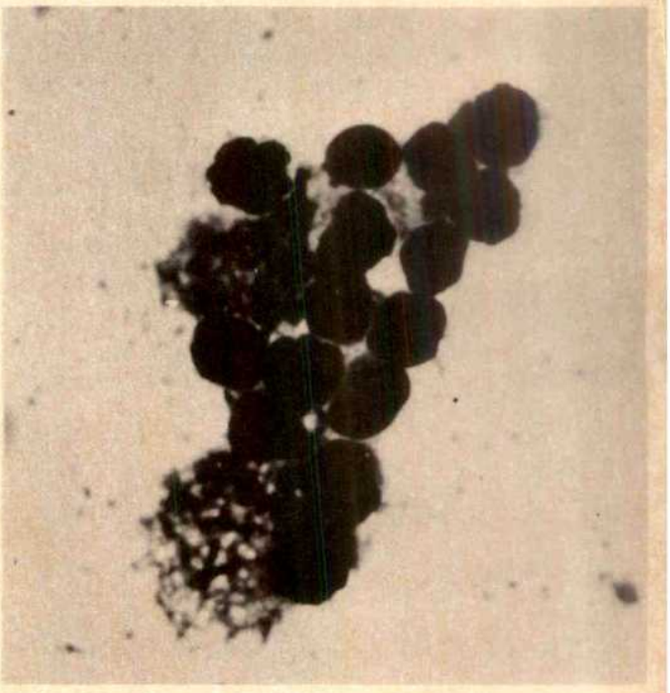


FIG. 1 Complement-mediated spleen cell aggregation. A dissociated spleen cell suspension from a mouse which had received 0.2 ml of Freund's complete adjuvant by intraperitoneal injection 3 weeks previously was rotated at 37°C for 45 min with fresh autologous serum (1/5 in CFT). The appearance of a typical cell clump in a fixed preparation obtained at the end of incubation, stained with Giemsa stain is shown. Cells with morphology characteristic of small lymphocytes, macrophages and blast cells were present. (Magnification × 1,550).

TABLE 1 Complement-mediated spleen cell aggregation*

Lymphocyte suspension†	Complement‡	Other treatment	% Clumps‖¶	Size of clumps**
Normal spleen	NMS	—	15	15–50
Normal spleen	NMS/inact	—	2	3–5
Normal spleen	NMS/EDTA	—	0.2	3–5
Adjuvant spleen	NMS	—	16	>50
Adjuvant spleen	NMS/inact	—	3	5–15
Adjuvant spleen	NMS/EDTA	—	1	3–5
Adjuvant spleen	NMS	CFT§	14	15–50
Adjuvant spleen	NMS	EDTA§	0.4	3–5
Adjuvant spleen	NMS	IgG_1 ‖	14	15–50
Adjuvant spleen	NMS	anti-C3 ‖	1	3–5
Normal thymus	NMS	—	3	3–5
Normal thymus	NMS/inact	—	3	3–5

* Lymphocyte suspensions were rotated for 45 min at 37°C with active or inactive complement.
† Cell suspensions of spleens from normal mice, mice which had received an intraperitoneal injection of 0.1 ml of complete Freund's adjuvant during the preceding month, or of normal adult thymus cells.
‡ NMS, Fresh autologous normal mouse serum; NMS/inact, same serum heated to 56°C for 30 min; NMS/EDTA, same serum containing 0.01 M EDTA.
§ CFT or EDTA to 0.01 M added to cell suspensions in complement after 45 min incubation and rotation continued for further 15 min.
‖ Cell suspensions incubated for 45 min with complement were washed in CFT and then incubated at room temperature with either eluted anti-C3 antibodies or the IgG_1 fraction of anti-mouse C3.
¶ Number of clumps of >3 cells among 500 free nucleated cells expressed as a %.
** Approximate mean of the number of cells in the clumps.

Lymphoid cells were prepared by crushing the freshly removed spleens or thymuses of adult BALB/c mice with a silicone rubber bung and suspending the cells in complement fixation test diluent (CFT) (veronal buffered physiological saline containing Ca^{2+}, Mg^{2+}, gelatin 0.1 % w/v and 0.015 M sodium azide). Debris and clumps were removed by rapid filtration through separate 2.0 ml cotton wool columns for individual organs; the resulting suspensions after two washes in CFT at 4° C consisted exclusively of single cells. Each mouse was exsanguinated, the serum separated within 12 h at room temperature and kept at 4° C until use on the same day. For the production of complement-mediated cell clumps, lymphoid cell suspensions (5×10^6–15×10^6 cells ml^{-1}) in CFT were rotated (20 r.p.m.) in sealed plastic tubes at 37° C for 45 min with autologous complement at a final concentration of 1:5. Control tubes contained either inactivated (56° C, 30 min), autologous complement or fresh complement containing 0.01 M EDTA. After rotation, the mixture was mounted on siliconised slides and the number of nucleated cell clumps, consisting of three or more cells, amongst 500 free nucleated cells was counted under phase contrast. Dried smears of preparations were fixed with formol-saline and stained with May–Grunwald or Giemsa stain.

For treatment of formed clumps with EDTA, 0.2 M EDTA was added to a final concentration of 0.01 M and the suspensions were then reincubated on the rotator for a further 15 min. For treatment with anti-C3 antibodies, complement-treated lymphoid cell preparations were first washed twice in CFT, then incubated at room temperature with 100 μg ml^{-1} of either isolated sheep anti-mouse C3 antibodies or the IgG_1 fraction of the same antiserum from which all anti-C3 activity had been absorbed (M.B.P., A. J. Bell and I. F. Rowe, unpublished). The anti-C3 preparation used had 170 μg ml^{-1} of protein and a titre of 1:256 for agglutination of 1% EAC.

For the detection of cells with complement receptors, preparations were first washed twice with CFT, centrifuging at 90g for 5 min at room temperature. Indicator cells bearing fixed murine C3 were prepared by sensitising sheep erythrocytes (E) with 6 MHD of IgM rabbit anti-sheep erythrocyte antibody (A) and then alexinating them by incubation (15 min, 37° C) of 1% EA with 1:10 fresh normal mouse complement (C). The EAC were washed twice in CFT before use. Lymphoid cells (20 μl at approximately 2×10^6 cells ml^{-1}) and 0.5% EAC were mixed in siliconised glass Rh rubes and centrifuged at 90g for 5 min at 4° C. The cell pellet was then gently but thoroughly resuspended with a Pasteur pipette and mounted in 0.5% toluidine blue on siliconised slides. Nucleated cells with three or more adherent red cells were scored as rosettes and the proportion of these among at least 200 nucleated cells was counted for each tube. In some experiments, parallel assays in which EDTA to a final concentration of 0.02 M was present were included. Four replicates of each assay were performed, and the % of rosette-forming cells was corrected for the number of rosettes formed by EA instead of EAC.

Incubation of a suspension of normal mouse spleen cells with fresh autologous serum induced the formation of clumps of cells (Table 1). The size and number of clumps were greater when spleen cells were used from mice which had received an intraperitoneal injection of complete Freund's adjuvant in the preceding 2–4 weeks. If the serum was inactivated by heating at 56° C for 30 min, or if it was made to 0.01 M EDTA, no cell clumps formed, nor did they if cell suspensions were incubated in the absence of serum. The formation of clumps was optimal after about 45 min of incubation. Continued rotation at 37° C was associated with progressive dissolution of the aggregates; they were, however, more stable on standing at room temperature and persisted unchanged for up to 48 h at 4° C. The clumps consisted of a heterogeneous population of cells which included small lymphocytes, macrophages, occasional polymorphs and, when stimulated spleens were used, occasional blast cells (Fig. 1). Thymus cell suspensions did not aggregate when incubated with complement under the same conditions.

Incubation of the source of complement with the C3-cleaving protein (CoF) of cobra venom (refs 14 and 15 and M.B.P., unpublished) so as to induce complete conversion of C3 on antigen–antibody crossed electrophoresis rendered it inactive in the production of cell clumps. Confirmation of specific dependence of the phenomenon on C3 was obtained by incubating washed clumps at room temperature with specific eluted anti-mouse C3 antibodies; this resulted in almost complete dissolution of the aggregates (Table 1). Addition of EDTA to 0.01 M to spleen cell suspensions clumped by complement resulted in rapid and complete dissociation of clumps (Table 1), suggesting that macrophages, (to which, in the mouse, the adherence of fixed C3 requires magnesium ions[3]), are involved.

If complement-clumped spleen cell preparations were washed and then rosetted with EAC in the presence of divalent cations, numerous red cells adhered to the clumps and the overall proportion of rosettes on mononuclear cells was diminished by comparison with the initial spleen cell suspension untreated with complement (Table 1). When the

TABLE 2 Effect of preincubation of spleen cells with complement on complement rosettes

Treatment of spleen cells*	% Complement rosettes† CFT	EDTA	Clumps‡ CFT	EDTA
Nil	36.3 ± 5.5	20.7 ± 2.1	−	−
NMS	23.7 ± 3.5	12.7 ± 3.1	+	−
NMS then EDTA	23.7 ± 2.5	12.3 ± 2.1	+	−
NMS/inact	45.0 ± 4.4	23.0 ± 2.6	−	−

* Spleen cell suspensions were rotated at 37°C for 45 min with CFT, autologous fresh serum or heat inactivated autologous fresh serum, before washing in CFT. Some cells incubated with complement were also washed with EDTA.

† Mean ± s.d. of four replicates of cells rosetting with EAC in the presence or absence of Mg^{2+}.

‡ The presence or absence of appreciable clumping of spleen cells. When clumps were present many indicator cells adhered to them.

clumped preparations were rosetted in the presence of 0.01 M EDTA the clumps dissociated, but there was an even greater reduction in the proportion of rosettes formed. The numbers of rosettes formed by clumped preparations which had been dissociated by EDTA, before rosetting in the presence and absence of divalent cations, showed a similar degree of reduction in comparison with the values obtained with the starting preparation. In contrast, treatment of spleen cells with inactivated mouse serum had no effect on the subsequent capacity of the cells to form rosettes with EAC (Table 2). These results suggest first, that after incubation of spleen cells with complement, cells with C3 receptors are included in the clumps; second, that as most of the rosettes which do form among the complement-treated cells are inhibitable by EDTA, and are therefore presumably on macrophages, the lymphocyte C3 receptors are apparently 'blocked' following incubation with complement and do not bind EAC[16].

Spontaneous dissociation of the complement-mediated clumps in the continued presence of the complement source at 37° C may have resulted, at least in part, from alternative pathway-mediated dissociation of fixed C3 from B cells[17]. This is probably due to generation of fluid-phase C3 fragments which have been shown in both a homologous human system (ref. 11 and M.B.P., and A. E. Butterworth, unpublished) and a mixed mouse–guinea pig system[18] to compete with fixed C3 for the C3 receptors. The fact that C3-dependent mixed cell adherence described here does reverse spontanously provides further support for the model which has been proposed for a possible role of this process in cell cooperation (ref. 11 and M.B.P., unpublished).

Complement-mediated C3-dependent aggregation of the different cell types in spleen cell suspensions which bear receptors for fixed C3 is of interest for several reasons. First, it illustrates the feasibility of an *in vivo* role for C3 in mediating the approximation of different cell types, particularly B cells and macrophages, which may be relevant to the mechanisms of cell cooperation in the induction of antibody production. Second, it provides evidence for the existence in normal spleens, and possibly to a greater extent in the spleens of mice stimulated by antigen, of a cell-associated mechanisms or mechanisms capable of fixing C3.

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1 Uhr, J. W., *Proc. natn. Acad. Sci., U.S.A.*, **54**, 1599 (1965).
2 Uhr, J. W., and Phillips, J. M., *Ann. N.Y. Acad. Sci.*, **129**, 792 (1966).
3 Lay, W. H. and Nussenzweig, V., *J. exp. Med.*, **128**, 991 (1968).
4 Henson, P. M., *Immunology*, **16**, 107 (1969).
5 Bianco, C., Patrick, R., and Nussenzweig, V., *J. exp. Med.*, **132**, 702 (1970).
6 Bianco, C., and Nussenzweig, V., *Science*, **173**, 154 (1971).
7 Dukor, P., Bianco, C., and Nussenzweig, V., *Proc. natn. Acad. Sci., U.S.A.*, **67**, 991 (1970).
8 Dukor, P., Bianco, C., and Nussenzweig, V., *Eur. J. Immun.*, **1**, 491 (1971).
9 Eden, A., Bianco, C., and Nussenzweig, V., *Cell. Immun.*, **2**, 658 (1971).
10 Nussenzweig, V., Bianco, C., Dukor, P., and Eden, A., *Prog. Immun.*, **1**, 73 (1971).
11 Pepys, M. B., *Nature new Biol.*, **237**, 157 (1972).
12 Janossy, G., Humphrey, J. H., Pepys, M. B., and Greaves, M. F., *Nature new Biol.*, **245**, 108 (1973).
12a Feldman, M., and Pepys, M. B., *Nature* (in the press).
13 Feldmann, M., and Nossal, G. J. V., *Transplantn Rev.*, **13**, 3 (1972).
14 Ballow, M., and Cochrane, C. G., *J. Immun.*, **103**, 944 (1969).
15 Muller-Eberhard, H. J., and Fjellstrom, K.-E., *J. Immun.*, **107**, 1666 (1971).
16 Dukor, P., Suter, E. R., Dietrich, F. M., and Probst, H., *Proc. 4th Int. Conf. Lymphatic Tissue Germinal Centres Immune Reactions*, (Plenum Press, 1972).
17 Miller G., and Nussenzweig, V., *J. Immun.*, **111**, 299 (1973).
18 Eden, A., Bianco, C., Nussenzweig, V. and Mayer, M. M., *J. Immun.*, **110**, 1452 (1973).

Role of heterochromatin in the control of cell cycle duration

RECENTLY Bennett has demonstrated that most annual plants have less nuclear DNA than perennial plants[1]. Since there is a positive correlation between DNA content and minimum duration of the mitotic cell cycle[2], Bennett suggested that low DNA content and short cycles might be factors determining the generation time of short-lived plants. These assumptions also fit the general findings that DNA contents have increased strikingly during general evolution[3] but have decreased during specialisation[4–6].

Although the progression from DNA-rich conservative species to DNA-poor advanced species could also be demonstrated in some perennial Anthemideae (Asteraceae, Dicotyledones)[7], several annuals have been found in this subfamily which have significantly more DNA than perennials of the same genera[8]. In spite of this, the annual species develop much more rapidly than the perennials. Therefore, a factor unconnected with nuclear DNA content might be expected to play a role in the control of cell cycle and generation times in those species.

I have studied the relationship between nuclear DNA content, percentage of heterochromatin, and cell cycle duration in the genera *Anacyclus*, *Anthemis*, and *Artemisia*. I have analysed three perennial species, three annual species with a higher DNA content, and two annual species with a lower DNA content than the related perennials. I found evidence that the amount of heterochromatin per genome is related to the reduction of cell cycle times in annual plants with large DNA contents.

Seedlings were grown in controlled environment chambers at 22° C and 15 h light; root tips were fixed in ethanol:acetic acid (3:1) and Feulgen-stained for DNA measurement by cytophotometry. The roots were immersed in a solution of 10 μCi ml^{-1} 6-^{3}H-thymidine specific activty 26 C $mmol^{-1}$) at 22° C for 1 h and fixed every other hour for 2 d. Labelled metaphases were counted after an exposure time of 3 d.

The duration of the mitotic cell cycle can be estimated for every species for which the DNA content is known,

TABLE 1 Nuclear DNA contents, heterochromatin content and duration of cell cycle in root tips of annual and perennial *Anthemideae*

Species	Habit	DNA (10^{-12} g per 2C nucleus)	Heterochromatin-DNA (% of total DNA)	Cell cycle time (h) Expected	Actual	Difference
Anacyclus depressus Bail.	Perennial	12.42	1.62	14.3	15.9	+1.6
—*clavatus* (Desf.) Pers.	Annual	10.48	8.55	13.0	11.0	−2.0
—*radiatus* Lois.	Annual	16.92	6.84	16.2	13.6	−2.6
Anthemis tinctoria L.	Perennial	7.46	2.43	12.3	12.3	0.0
—*cota* L.	Annual	15.78	9.09	16.0	6.5	−9.5
—*austriaca* Jacq.	Annual	9.63	34.11	13.0	7.0	−6.0
Artemisia absinthium L.	Perennial	7.28	45.45	11.5	9.5	−2.0
—*annua* L.	Annual	4.05	14.13	10.5	7.7	−2.8

DNA content was measured in Feulgen-stained nuclei by the two-wavelength method of cytophotometry[14]; *Allium cepa* nuclei have been used for the conversion into absolute values. The percentage of DNA within the heterochromatic portion of the genome was calculated from measurements, after the extinction values for euchromatin had been set to zero[15]. The expected cell cycle times were calculated from the diagram given by Evans *et al.*[2]. The actual duration was established after pulse labelling, by the method of labelled mitoses curves[16]. Striking negative differences between the expected and the actual cycle times can be seen in species with high amounts of heterochromatin-DNA, especially in some annuals.

because there is a linear relationship between these two parameters[2]. In some of the species studied, however, the measured cycle duration differed significantly from the expected value (Table 1). In all annuals tested the cell cycle time was shorter than in the related perennials, in spite of the greater DNA content in three of the annual species. These three species have a proportionately greater amount of DNA as heterochromatin than their perennial relatives.

My results indicate that the nuclear DNA content can increase by the addition of heterochromatin without lengthening of the cell cycle. The cycle may even become shorter.

The percentage of heterochromatin within a given genome might, therefore, represent a correcting factor in the control of the duration of the cell cycle, in addition to the nuclear DNA content (the basic determinant). Heterochromatin is rich in repetitive DNA[9] and replicates faster than euchromatin[10]. Thus annual species can become able to grow and develop rapidly, either by decreasing the nuclear DNA content or by increasing the DNA content through an increase in the percentage of heterochromatin. An increase in DNA, when found in specialised and progressive groups, is possibly always accompanied by a proportionally greater increase in repetitive sequences. Fox[11] reported, for example, that the heterochromatic portion of the genome in several beetles increased faster than did the euchromatic portion; increasing amounts of repetitive DNA were also found to be correlated with increasing DNA content in conifers[12]. In *Vicia*, differences between species-specific DNA contents were reported to be caused by different numbers of rRNA genes, which appear in the form of heterochromatic chromosome segments[13]. Thus, the amount of heterochromatin is evidently an important factor in determining both nuclear DNA content and growth rate parameters. Details will be published in a forthcoming paper.

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1 Bennett, M. D., *Proc. R. Soc.*, **B181**, 109 (1972).
2 Evans, G. M., Rees, H., Snell, C. L., and Sun, S., *Chromosomes Today*, **3**, 24 (1972).
3 Rees, H., and Jones, R. N., *Int. Rev. Cytol.*, **32**, 53 (1972).
4 Bachmann, K., and Rheinsmith, E. L., *Chromosoma*, **43**, 225 (1973).
5 El-Lakany, M. H., and Dugle, J. R., *Evolution*, **26**, 427 (1972).
6 Rees, H., and Hazarika, M. H., *Chromosomes Today*, **2**, 158 (1969).
7 Nagl, W., and Ehrendorfer, F., *Oesterr. Bot. Z.*, **121**, 165 (1973).
8 Nagl, W., and Ehrendorfer, F., *Plant Syst. Evol.*, **123** (in the press).
9 Yunis, J. J., and Yasmineh, W. G., *Science*, **174**, 1200 (1971).
10 Bostock, C. J., Prescott, D. M., and Hatch, F. T., *Expl Cell Res.*, **74**, 487 (1972).
11 Fox, D. P., *Chromosomes Today*, **3**, 32 (1972).
12 Miksche, J. P., and Hotta, Y., *Chromosoma*, **41**, 29 (1973).
13 Maher, E. P., and Fox, D. P., *Nature new Biol.*, **245**, 171 (1973).
14 Patau, K., *Cromosoma*, **5**, 341 (1952).
15 Van't Hof, J., *Expl Cell Res.*, **39**, 48 (1965).
16 Wimber, D. E., *Am. J. Bot.*, **47**, 828 (1960).

Mode of action of soybean trypsin inhibitor (Kunitz) as a model for specific protein–protein interactions

THE protein trypsin inhibitors are proteins which bind very strongly to trypsin, blocking its active site (K_i = 10^{-9} to 10^{-14} M). We have carried out a crystal structure analysis of the complex of soybean trypsin inhibitor (Kunitz) (STI), one of the largest inhibitors, with porcine trypsin. Huber and his colleagues have determined the structure of a complex of a small inhibitor, bovine pancreatic trypsin inhibitor (Kunitz) (PTI), with bovine trypsin[1]. These studies improve our understanding of the catalytic mechanism of trypsin, demonstrate that various trypsin inhibitors act in a similar way and provide insight into the development of strong, specific binding between protein molecules.

From preliminary results on the crystal structure of isolated PTI[2], using available knowledge about chymotrypsin[3] and trypsin[4], it was possible to predict a unique mode of binding in which the inhibitor is oriented similarly to a true trypsin substrate[4,5]. This prediction was confirmed by the crystal structure analysis of the complex of PTI with trypsin[1].

Native PTI is not detectably modified by trypsin, but cleavage of a specific peptide bond of STI (Arg 63′–Ile 64′) is catalysed by trypsin. An equilibrium between STI and a modified form, in which this bond is hydrolysed, has been demonstrated[6]. This provides strong evidence that binding of STI to the catalytic site of trypsin mimics the productive mode of substrate binding.

The STI:porcine trypsin complex was crystallised and the first isomorphous derivative was discovered by H. T. Wright in this laboratory. The structure analysis has been taken to a resolution of 2.6 Å. The STI molecule, omitting certain uninterpretable regions, is shown in Fig. 1. As in the case of PTI, we observe that only a very small part of the molecule

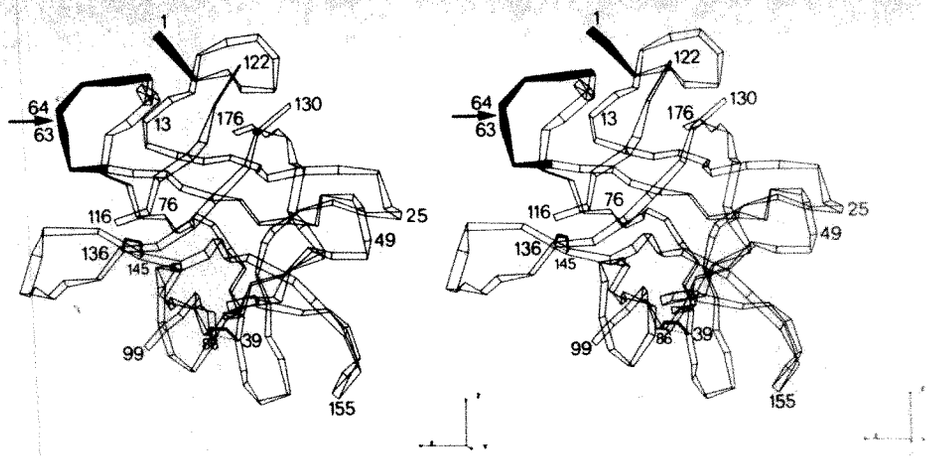


FIG. 1 Conformation of the polypeptide chain of soybean trypsin inhibitor as determined from the crystal structure of the complex with trypsin, and the amino acid sequence[7]. Residues 107′–115′, 123′–129′, 177′–181′ could not be located in the electron density map; as a consequence, the identification of residues 116′–122′ is tentative. The two disulphide bridges are indicated. Residues which make contact with trypsin are blackened : the arrow indicates the scissile bond 63′–64′.

[*Note added in proof.* More accurate co-ordinates show that residues 13′, 60′, 71′, 72′ also make a few contacts with trypsin.]

is involved in the contact region. If the inhibition of trypsin is the function of these molecules, most of the protein does nothing more than stabilise the structure.

In the PTI complex a disulphide bridge adjacent to the active site effectively excludes access of water to the bond (Lys 15′–Arg 16′) which lies at the active site of trypsin[5]. In the STI complex water is also excluded from the catalytic site, mainly by the chain (11′–14′), but this is held in place only by a few hydrogen bonds and van der Waals' contacts. The looser attachment may account for the measurable rate of hydrolysis of the STI complex of trypsin.

Both PTI and STI have a positively charged amino acid at P_1 to occupy the primary specificity pocket of the enzyme (Arg 63′ in STI). The main chain NH of this amino acid is hydrogen-bonded to CO(214), as found in a chymotrypsin: substrate complex[8]. Interactions of adjacent residues in both the STI and PTI complexes (Fig. 2) agree with the interactions proposed for polypeptide substrates of chymotrypsin[9–12]: (a) from a study of models Segal *et al.*[9] pointed out that a bulky group at P_2 can interact hydrophobically with Ile 99. This is observed in both PTI and in STI, where P_2 is Tyr 62′. (b) At P_3 which is Ser 61′ in STI, the NH group is hydrogen bonded to CO(216) in both complexes. Segal *et al.*[9] found a similar interaction for substrate analogues of γ-chymotrypsin. A second hydrogen bond which they found, involving NH(216), cannot occur in trypsin because it is differently oriented, as a consequence of the deletion of residue 218 (ref. 13). (c) In both complexes a residue in P_1' (Leu 64′ in STI) makes hydrophobic contacts with the disulphide bridge 42–58. It was recently shown that interactions at P_1' accelerate deacylation of acylchymotrypsins by 50–100 times[11]. There was no evidence of enhanced binding of the deacylating agent.

Structural models[5] showed that the favourable interactions at P_1' could not occur in the Michaelis complex with a polypeptide substrate, because of unfavourable interactions of the substrate with the enzyme's active serine, Ser-195 (ref. 11). Similarly when the peptide bond P_1–P_1' is hydrolysed, the residue at P_1' is forced away from P_1 by about 1 Å and the favourable interactions are again disrupted. In an intermediate state, when the carbonyl group of the scissile bond is bonded to both Ser-195 and its amide nitrogen, the substrate could make its best fit to the surface of the enzyme. This explained how the energy of this interaction could strain the enzyme:substrate complex toward the transition state, reducing the activation energy of acylation, rather than improving the binding constant[11].

In addition to the primary binding at P_1, all three interactions (a)–(c) are observed in both inhibitor complexes, which therefore both exhibit the mode of binding already inferred as the productive mode for trypsin substrates. The similarity of the interactions at P_3–P_2–P_1–P_1' is confirmed by the close agreement of the recorded dihedral angles in the three intervening peptide bonds (Table 1), which must be close to experimental error. Although the agreement of chain conformation extends no further, one additional similarity exists: (d) In both PTI[1] and STI complexes, arginine is found at P_2'. In each case its guanidinium group lies parallel to Tyr-151 probably forming a charge-transfer complex. The guanidinium group is hydrogen bonded to CO-40. Despite this similarity, the main chain conformations at P_2' are

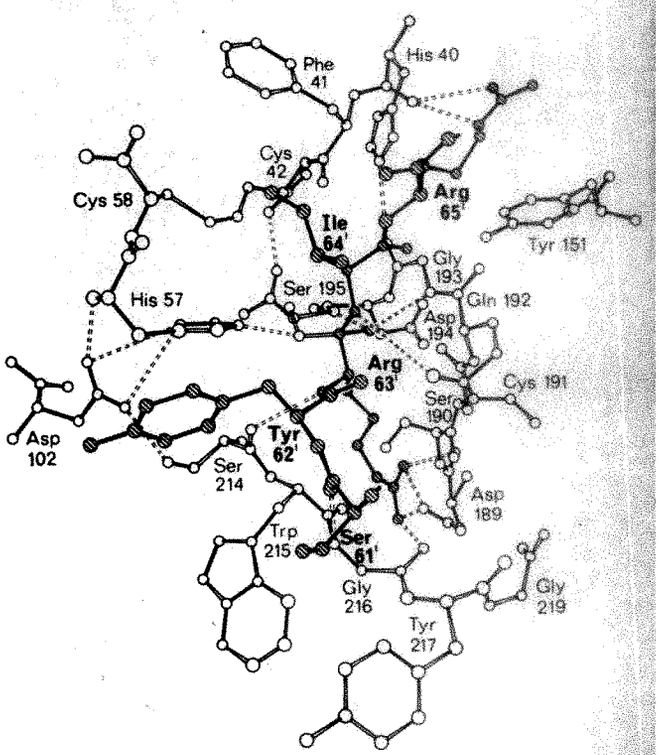


FIG. 2 Residues 61′–65′ of soybean trypsin inhibitor and the parts of the trypsin molecule involved in binding them. Atoms of the inhibitor molecule are shaded and many of the interactions which they make are mentioned in the text. The carbonyl carbon of the scissle peptide bond 63′–64′ is shown tetrahedrally coordinated. Trypsin residues Asn-97 and Leu-99 which lie in front of Asp-102 and touch the distal part of Tyr-62′ have been omitted for clarity.

TABLE 1 Main chain conformations at the active site of bovine pancreatic trypsin inhibitor (Kunitz) and soybean trypsin (Kunitz)

Pancreatic Trypsin Inhibitor		Dihedral angles*	Trypsin site	Soybean Trypsin Inhibitor		
Pro-13′	ϕ	− 86	P_3	ϕ	− 46	Ser-61′
	ψ	− 28		ψ	− 21	
Cys-14′	ϕ	− 64	P_2	ϕ	− 77	Tyr-62′
	ψ	152		ψ	136	
Lys-15′	ϕ	−116	P_1	ϕ	− 89	Arg-63′
	ψ	87		ψ	85	
Ala-16′	ϕ	−135	P_1'	ϕ	−135	Ile-64′
	ψ	166		ψ	−115	
Arg-17′	ϕ	−116	P_2'	ϕ	−175	Arg-65′
	ψ	87		ψ	−179	

* R. Huber *et al.*, private communication

completely different in the two inhibitor complexes. Other trypsin inhibitors do not have arginine at P_2'.

Rühlmann *et al.*[1] were obliged to interpret the electron density at the active site of the PTI complex in the form of a tetrahedral intermediate, in which there is a covalent bond between the enzyme and inhibitor, whereas the peptide Lys 15′–Ala 16′ at the active site is still linked. This tetrahedral form had been generally assumed to exist in the chemical pathway[14,15] but in a normal hydrolysis reaction its lifetime is so short that it had never been convincingly demonstrated.

In Table 2 we give the distances observed in STI between C^{α} (Arg 63′), C^{β} (Ser 195) and C^{α} (Ile 64′), together with the distances expected on stereochemical grounds for the various possible intermediate forms. The Michaelis and acyl forms both cause impossible crowding at the active site. We feel that this provides conclusive evidence that the STI complex is also in the tetrahedral form.

Structural data have indicated two aspects of the interaction between trypsin-like enzymes and their substrates which may favour the tetrahedral intermediate. One of these concerns the binding of the leaving group at P_1' and has been described above. The other concerns hydrogen bonds from the NH groups of Gly-193 and Ser-195 to the carbonyl group of the scissile bond, which are better oriented when the carbonyl carbon becomes tetrahedral[16] and will be strengthened by any negative charge which exists on the oxygen. In the tetrahedral intermediate the buried negative charge, which may be thought of as originating from COO^- (Asp-102), is maximally delocalised across His-57 and the tetrahedral ester group.

The tetrahedral intermediate is close to the transition state of the reaction[17,18]. The essential requirement for a catalyst is to stabilise the transition state[19] and an effective enzyme must provide a means of doing this[20]. The two trypsin inhibitors do this so effectively that the tetrahedral intermediate constitutes a minimum of free energy in the reaction pathway, and at least in crystals it becomes the most abundant state. Formation of a covalent bond between the enzyme and the inhibitor does not in itself explain the strong binding, as is shown by the high affinity of PTI, STI and other inhibitors for anhydrotrypsin, in which the oxygen of Ser-195 has been removed[21,22]. In anhydrotrypsin the overcrowding at the active site is relieved, and the intact inhibitor can bind favourably to the active site. Calorimetric measurements show that the binding of STI to trypsin is endothermic[23], reflecting the relatively high enthalpy of the tetrahedral form, but a large entropy term stabilises the complex.

The entropy of association between protein molecules derives largely from the shells of immobilised, or partly ordered, water molecules which surround the two separate molecules. These are reduced in volume on association[24]. An external polar group is normally hydrogen-bonded to a water molecule, which becomes immobilised. On association, provided that such hydrogen bonds are exchanged for an intermolecular hydrogen bond, there is little change of enthalpy; but the release of immobilised water molecules may be an important contribution to the entropy of association.

There is an important difference between the association of an enzyme with a folded protein molecule which makes a perfect fit, and the binding of a loosely coiled molecule. When a small polypeptide substrate binds to the enzyme, its loss of internal degrees of freedom reduces the free energy of binding. Binding of a correctly folded protein molecule may be achieved without change of internal freedom. These entropic factors, even over the small interaction areas observed in PTI and STI, are evidently sufficient to yield the high association constants which are observed.

Enzyme–substrate interactions have evolved to lower the activation energy of a chemical reaction step. These studies show in detail how the energetics of these interactions may provide the driving force for very strong binding, with a highly specific recognition system. To evolve a receptor, which recognises a specific effector and signals its presence to a linked biochemical system, one would require in addition that substrate binding triggered a structural change.

TABLE 2 Distances at the active site compared with expected distances for different intermediate forms of the complex

	$C^{\alpha}(63')$–$C^{\beta}(195)$ (Å)	$C^{\alpha}(63')$–$C^{\alpha}(64')$ (Å)
Observed distance	3.7	3.9
Expected distances:		
Michaelis complex	5.2	3.8
Tetrahedral intermediate	3.7	3.8
Acyl enzyme	3.7	5.0

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1 Rühlmann, A., Kukla, D., Schwager, P., Bartels, K., and Huber, R., *J. molec. Biol.*, **77**, 417–436 (1973).
2 Huber, R., Kukla, D., Rühlmann, A., and Steigemann, W., *Cold Spring Harb. Symp. quant. Biol.*, **36**, 141–150 (1971).
3 Birktoft, J., and Blow, D. M., *J. molec. Biol.* **68**, 187–240 (1972).
4 Stroud, R. M., Kay, L. M., and Dickerson, R. E., *Cold Spring Harb. Symp. quant. Biol.*, **36**, 125–140 (1971).
5 Blow, D. M., Wright, C. A., Kukla, D., Rühlmann, A., Steigemann, W., and Huber, R., *J. molec. Biol.*, **69**, 137–144 (1972).
6 Niekamp, C. W., Hixson, H. F., jun., and Laskowski, M., jun., *Biochemistry, N.Y.*, **8**, 16–22 (1969).
7 Koide, T., and Ikenaka, T., *Eur. J. Biochem.*, **32**, 417–431 (1973).
8 Steitz, T. A., Henderson, R., and Blow, D. M., *J. molec. Biol.*, **46**, 337–348 (1969).
9 Segal, D. M., Powers, J. C., Cohen, G. H., Davies, D. R., and Wilcox, P. E., *Biochemistry, N.Y.*, **10**, 3728–3738 (1972).
10 Segal, D. M., *Biochemistry, N.Y.*, **11**, 349–356 (1972).
11 Fersht, A. R., Blow, D. M., and Fastrez, J., *Biochemistry, N.Y.*, **12**, 2025–2034 (1973).
12 Blow, D. M., *Israel J. Chem.*, **12**, 1974 (in the press).
13 Hermodson, M. A., Ericsson, L. H., Neurath, H. and Walsh, K. A., *Biochemistry, N.Y.*, **12**, 3146–3153 (1973).
14 Bruice, T. C., *Proc. natn. Acad. Sci. U.S.A.*, **47**, 1924–1928 (1961).
15 Bender, M. L., Kezdy, F. J., *J. Am. chem. Soc.*, **86**, 3704–3714 (1964).
16 Henderson, R., *J. molec. Biol.*, **54**, 341–354 (1970).
17 Caplow, M., *J. Am. chem. Soc.*, **91**, 3639–3645 (1969).
18 Fastrez, J., and Fersht, A. R., *Biochemistry, N.Y.*, **12**, 1067–1074 (1972).
19 Wolfenden, R., *Nature*, **223**, 704–705 (1969).
20 Pauling, L., *Chem. Engng. News*, **24**, 1375 (1946).
21 Ako, H., Foster, R. J., and Ryan, C. A., *Biochemistry, N.Y.*, **13**, 132–139 (1974).
22 Lazdunski, M., Vincent, J.-P., Schweitz, H., Peron-Renner, M., and Pudles, J., *Second int. Conf. on Proteinase Inhibitors* (in the press).
23 Baugh, R. J., and Trowbridge, C. G., *J. biol. Chem.*, **247**, 7498–7501 (1972).
24 Kauzmann, W., *Adv. Prot. Chem.*, **14**, 1–63 (1959).

Prostaglandins and the regulation of uterine blood flow in pregnancy

In late pregnancy, uterine blood flow (UBF) has increased by as much as thirty- to forty-fold[1] and uterine vascular reactivity to vasoconstrictor hormones and adrenergic nerve stimulation has decreased[2,3]. For example, angiotensin II increases blood flow to the gravid uterus[3,4], an uncommon response to this agent which is the most potent vasoconstrictor hormone[5]. Prostaglandins of the E series (PGE) have been reported to maintain some regional blood flows[6,7] and to modulate the vasoconstrictor activity of angiotensin II and noradrenaline[8,9]. In view of the findings that the uterus synthesises prostaglandins[10,11], we have investigated the possible participation of locally synthesised prostaglandins in the regulation of blood flow and vascular reactivity of the gravid uterus.

Mongrel dogs in late pregnancy weighing 20–30 kg were anaesthetised with morphine sulphate (2 mg kg^{-1}, subcutaneously) and chloralose (100 mg kg^{-1}, intravenously). The trachea was cannulated and the lungs were ventilated mechanically. The abdominal cavity was opened by a midline incision. A short segment of the main uterine artery of one horn was isolated from the surrounding tissue. Blood flow to this horn was measured by an electromagnetic flowmeter. In three experiments, we measured blood flows to both uterine horns which were similar. Mean aortic blood pressure (MABP) was measured by a Statham transducer via a catheter inserted into a brachial artery. A direct writer (Hewlett-Packard) was used to record UBF and MABP. The assay of PGE-like material ('PGE') in blood which has been reported[12] requires chromatographing acidic lipid extracts of the blood on thin-layer plates, after which eluates from the PGE zones of the plates were then assayed *in vitro* by bracket assay using the method of Vane[13] in which three tissues, rat stomach strip, rat colon and chick rectum are superfused in series with Krebs solution. Samples of blood (50 ml) were obtained from a cannula in the uterine vein. Angiotensin II was given intravenously for 10–15 min, by a Braun continuous-infusion pump through a cannula inserted into the right forepaw vein. Indomethacin was administered intravenously in 10% alcohol in Krebs solution at doses which decreased UBF by more than 20%. This dose of indomethacin proved highly variable (2–120 mg kg^{-1}) in contrast to the dose required to effect similar reductions in renal blood flow in male dogs[6]. Student's *t* test for paired data and Wilcoxon's signed ranks test were used to determine statistical significance.

The mean basal concentration of 'PGE' in uterine venous blood in nine experiments was 0.39 ± 0.08 (range 0.11 to 0.92) ng ml^{-1}. In eight of these experiments, angiotensin II increased UBF from a mean control of 84 to 111 ml min^{-1} ($P < 0.05$), and MABP from a mean control of 100 to 118 mm Hg ($P < 0.01$) (Table 1). 'PGE' concentrations in uterine venous blood, measured at the time of maximal increase in UBF produced by angiotensin II infusion, increased in every instance; the mean concentrations of 'PGE' increased about two-fold, that is from 0.26 to 0.51 ng ml^{-1} ($P < 0.05$). Arterial levels of 'PGE' measured in one experiment were one-twentieth those in uterine venous blood. In six experiments, within 30 min of administration of indomethacin, MABP increased from a mean of 102 ± 6 to 113 ± 5 mm Hg ($P < 0.02$), UBF decreased from a mean of 89 ± 32 to 21 ± 5 ml min^{-1} ($P < 0.05$), and the concentration of 'PGE' in uterine venous blood decreased from a mean of 0.37 ± 0.12 to 0.03 ± 0.02 ng ml^{-1} ($P < 0.05$). After indomethacin, infusion of PGE_2 into the abdominal aorta at rates as high as 3,000 ng min^{-1} did not increase the depressed UBF. This finding which seems to contradict the importance of a PGE compound in regulating blood flow to the gravid uterus is understandable, if we assume that exogenous PGE_2 does not reach the active site within the uteroplacental circulation because of either degradation, binding or relative inaccessibility of injected PGE_2 to this site. To test this possibility, we used bradykinin which has been shown to release prostaglandins[14,15]. Indomethacin attenuated the uterine vasodilator action of bradykinin (100 ng kg^{-1} min^{-1}, intraaortic): in two experiments before indomethacin, bradykinin increased UBF by 56 and 64 ml min^{-1} (control 60 and 21 ml min^{-1}, respectively), whereas after indomethacin, bradykinin increased UBF by 4 and 16 ml min^{-1} (control 26 and 13 ml min^{-1}; respectively). Further, in the non-pregnant dog 'PGE' is not found in uterine venous blood (three experiments) and BK does not affect UBF. These results suggest that 'PGE' synthesised locally dilates the uterine vascular bed, although exogenous PGE_2 cannot. Similar results were ob-

TABLE 1 Angiotensin II-induced changes in mean aortic blood pressure, uterine blood flow and concentrations of 'PGE' in uterine venous blood

Dog	Dose Ang. II* (ng kg^{-1} min^{-1})	Mean aortic blood pressure Control (mm Hg)	Mean aortic blood pressure Ang. II (mm Hg)	Uterine blood flow† Control (ml min^{-1})	Uterine blood flow† Ang. II (ml min^{-1})	'PGE' Control (ng ml^{-1})	'PGE' Ang. II (ng ml^{-1})
1	10	85	95	69	72	—	—
2	20	80	95	157	186	0.49	0.56
3	10	105	135	100	181	0.21	0.55
4	37.5	110	125	33	44	0.11	0.22
5	37.5	95	105	115	155	0.18	0.92
6	37.5	100	125	52	63	0.23	0.33
7	20	90	115	62	76	—	—
8	37.5	135	145	—	—	0.37	0.46
$\bar{x}$		100	118	84	111	0.26	0.51
s.e.		±6	±6	±16	±23	±0.06	±0.10

* Angiotensin (Ang.) II was infused intravenously.
† Represents blood flow to one horn of the uterus.

tained with arachidonic acid, the precursor of PGE_2[16], which has also been shown to release PGE_2[14]. Thus, arachidonic acid increased UBF when given before, but not after indomethacin.

The levels of 'PGE' that we found in uterine venous blood are more than ten times those reported in the venous effluent of the hind limb[6] and are equal to or greater than those in renal venous blood[17]. The capacity of the kidney to synthesise prostaglandins is surpassed only by the seminal vesicle[18] and in view of our findings, perhaps by the gravid uterus as well. Since prostaglandins of the E series have been shown to oppose the vasoconstrictor activity of pressor systems[8], our findings of high uterine venous levels of 'PGE' which are increased further by angiotensin II may account in part for the increased UBF produced by angiotensin II and the altered reactivity of the uterine vasculature to other vasoconstrictor stimuli. Renin, which is found in abundance in the pregnant uterus[19] has been suggested to participate in the control of uterine vascular resistance[3], possibly through the generation of an angiotensin which in turn stimulates uterine prostaglandin synthesis. In this way, angiotensin-prostaglandin interactions may participate in the regulation of uterine blood flow. The importance for the uterine circulation of uninterrupted synthesis of prostaglandins by the gravid uterus was revealed after inhibition of prostaglandin synthetase with indomethacin[20]. Uterine blood flow fell to levels less than 30% of control. Simultaneously, the concentration of "PGE" in uterine venous effluent declined to a mean value less than 10% of control.

This work recalls our previous studies on the renal circulation: (1) continued production of PGE_2 was shown to maintain resting renal blood flow[6], and (2) angiotensin II and noradrenaline were shown to increase renal synthesis of PGE_2 above basal levels[8]. The importance of this function of the kidney was revealed after inhibition of prostaglandin synthetase by indomethacin. Thus, resting renal blood flow decreased by more than 40%[6] and the renal vasoconstrictor action of angiotensin II and noradrenaline increased several-fold[21]. The previously reported ameliorating effects of pregnancy on both experimental hypertension[22] and essential hypertension[23] may reside partially in the capacity of the gravid uterus to synthesise one or more substances which have antihypertensive properties, such as PGE_2[24]. Finally, we have not distinguished the site of origin within the uteroplacental circulation of the 'PGE' which appears in uterine venous blood. One or more sites including the foetal as well as the maternal components of the placenta, nor excluding the myometrium, should be considered. Thus, we were not able to relate changes in uterine blood flow after indomethacin to a primary effect on one or more of the several elements of the uteroplacental circulation.

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1 Metcalfe, J., Romney, S. L., Swartwout, J. R., Pitcairn, D. M., Lethin, jr., A. N., and Barron, D. H., *Am. J. Physiol.*, **197**, 929 (1959).
2 Ladner, C., Brinkman III, C. R., Weston, P., and Assali, N. S., *Am. J. Physiol.*, **218**, 257 (1970).
3 Ferris, T. F., Stein, J. H., and Kauffman, J., *J. clin. Invest.*, **51**, 2827 (1972).
4 Assali, N. S., Allan Westersten, E. E., *Circulation Res.*, **9**, 189 (1961).
5 Barer, G. R., *J. Physiol.*, **156**, 49 (1961).
6 Lonigro, A. J., Itskovitz, H. D., Crowshaw, K., and McGiff, J. C., *Circulation Res.*, **32**, 712 (1973).
7 Bowery, N. G., Lewis, G. P., and Matthews, J., *Br. J. Pharmac.*, **40**, 437 (1970).
8 McGiff, J. C., Crowshaw, K., Terragno, N. A., and Lonigro, A. J., *Nature*, **227**, 1255 (1970).
9 Ferreira, S. H., Moncada, S., and Vane, J. R., *Br. J. Pharmac.*, **47**, 48 (1973).
10 Ryan, M. J., Clark, K. E., Van Orden, D. E., Farley, D., Edvinsson, L., Sjoberg, N. O., Van Orden III, L. S., and Brody, M. J., *Prostaglandins*, **4**, 629 (1973).
11 Blatchley, F. R., Donovan, B. T., Poyser, N. L., Horton, E. W., Thompson, C. J., and Los, M., *Nature*, **230**, 243 (1971).
12 McGiff, J. C., Crowshaw, K., Terragno, N. A., Malik, K. U., and Lonigro, A. J., *Clin. Sci.*, **42**, 223 (1972).
13 Vane, J. R., *Br. J. Pharmac.*, **35**, 209 (1969).
14 Palmer, M. A., Piper, P. J., and Vane, J. R., *Br. J. Pharmac.*, **49**, 226 (1973).
15 Terragno, N. A., Lonigro, A. J., Malik, K. U., and McGiff, J. C., *Experientia*, **28**, 437 (1972).
16 Frölich, J. C., Wilson, T. W., Carr, K., Nies, A. S., and Oates, J. A., *Clin. Res.*, **21**, 687 (1973).
17 McGiff, J. C., Terragno, N. A., Malik, K. U., and Lonigro, A. J., *Circulation Res.*, **31**, 36 (1972).
18 Sameulsson, B., *J. biol. Chem.*, **238**, 3229 (1963).
19 Ferris, T. F., Gorden, P., and Mulrow, P. J., *Am. J. Physiol.*, **212**, 698 (1967).
20 Vane, J. R., *Nature new Biol.*, **231**, 232 (1971).
21 Aiken, J. W., and Vane, J. R., *J. Pharm. exp. Ther.*, **184**, 678 (1973).
22 Page, E. W., *Am. J. Obst. Gynec.*, **53**, 275 (1947).
23 Chesley, L. C., and Annitto, J. E., *Am. J. Obst. Gynec.*, **53**, 672 (1947).
24 McGiff, J. C., and Itskovitz, H. D., *Circulation Res.*, **33**, 479 (1973).

Role of *rel* gene in translation during amino acid starvation in *Escherichia coli*

It has been thought that the *rel* gene in *Escherichia coli* is responsible for the stringent control of stable RNA synthesis dependent on a supply of amino acids, since the gene was identified as the site of mutation to the relaxed phenotype[1]. But in our previous reports[2,3], we suggested that mutational defects in *rel*⁻ cells are probably in the translational mechinery, the integrity of which may be required for stringent control. This supposition is based on the following observations: (1) When *rel*⁻ cells are shifted down in carbon or nitrogen source, the stringent control of RNA synthesis is observed in spite of their *rel*⁻ genotype[2,4]. Only when deprived of amino acids do *rel*⁻ cells fail to restrict RNA synthesis, in contrast to cells having the *rel*⁺ allele. (2) Ribosomal inhibitors like chloramphenicol abolish the stringent control in both *rel*⁺ and *rel*⁻ cells[2,5,6]. Furthermore, a cold-sensitive mutant defective in protein synthesis at low temperature carring a mutation in *spcA* locus, also shows the relaxed phenotype under non-permissive conditions[3]. It has been reported that several other mutants of *rel*⁺ strains having temperature-sensitive phenylalanyl-tRNA synthetase[7], peptidyl-tRNA hydrolase[8], or elongation factor G (ref. 9) all failed to control the synthesis of RNA at the higher temperature. This suggests that normal synthesis of protein is required for stringent control. (3) During shift-down of a carbon or nitrogen source there is no difference between the growth of *rel*⁺ and *rel*⁻ cells. Only after shift-down of amino acids do *rel*⁻ cells require a much longer lag period before they resume growth[2,10]. This is due to the inability of *rel*⁻ cells to carry out the normal synthesis of protein when the supply of amino-acids is limited.

The work described here was carried out to extend our previous reports[2,3] that the *rel*⁻ mutant is defective in translation under amino acid deprivation. We found that polysomes in the amino acid-starved *rel*⁺ cells function actively in protein synthesis while in *rel*⁻ cells they are inert.

When the cells growing in an amino acid-rich medium are shifted down to one poor in amino acids, *rel*⁺ cells are able to resume growth much more rapidly than *rel*⁻ cells[2]. Enzymes responsible for the synthesis of amino acids are produced immediately after shift-down in *rel*⁺ cells, but not in *rel*⁻ cells[2]. Formation of several other enzymes including β-galactosidase[11], alkaline phosphatase[12], and ornithine transcarbamylase[13] was also impaired in *rel*⁻ cells during amino acid starvation.

When the cells were shifted down to an amino acid-poor medium, the overall rate of protein synthesis was reduced to less than 10% of that in pre-shift conditions in both *rel*⁺ and *rel*⁻ strains (see zero time in Fig. 1*a* and *b*). But as shown in Fig. 1*a* the rate of protein synthesis in *rel*⁺ cells recovered rapidly during incubation in minimal medium and reached a plateau which was sufficient to maintain the growth of cells in shift-down conditions. On the other hand, the rate of protein synthesis in *rel*⁻ cells gradually declined (Fig. 1*b*). The potential protein synthetic activity as measured by incorporation of ^{14}C-phenylalanine in the presence of a mixture of twenty amino acids was preserved in *rel*⁺ cells during amino acid starvation, while in *rel*⁻ cells it decreased.

The change of the rate of RNA synthesis following the amino acid shift-down is also shown in Fig. 1. After deprivation of amino acids, RNA synthesis in *rel*⁺ cells was extremely

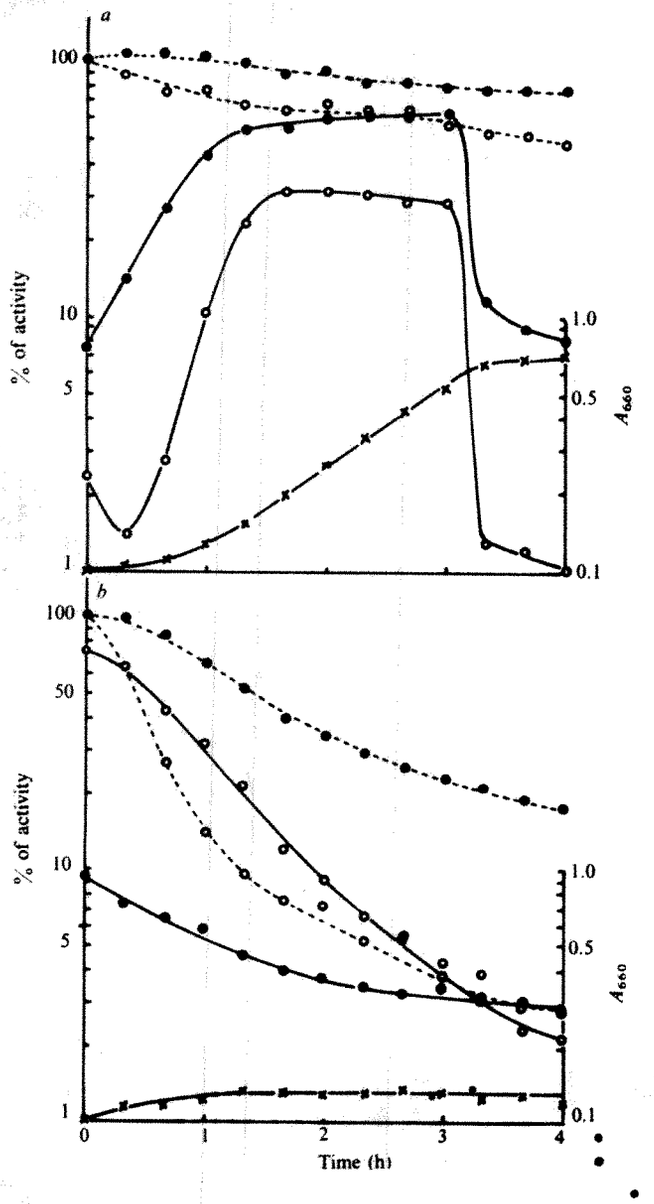


Fig. 1 Change in protein and RNA synthesis in *rel*⁺ and *rel*⁻ cells following the shift-down of amino acids. CP78(*rel*⁺) and CP79(*rel*⁻), isogenic except for the *rel* locus[14], were used. They are descendants of K12 strain and require leucine, threonine, histidine, arginine, and thiamine. Cells were grown at 37°C with shaking in glucose (0.4%) required amino acids (20 μg ml^{-1} each), thiamine (2 μg ml^{-1}), M9 medium; further supplemented with all twenty L-amino acids (0.1 mM each). The cultures grown to log phase were centrifuged, washed and resuspended in a fresh glucose-M9 medium containing only required amino acids and thiamine. During incubation in the shift-down medium, at the time indicated aliquots were withdrawn and the activities of protein and RNA syntheses, and the turbidity at 660 nm (1 cm light path) were estimated. For the measurement of protein synthesis, two samples (0.5 ml each) were taken and pipetted into two different test tubes; one contained 15 μl of ^{14}C-phenylalanine (5 μCi ml^{-1}; 0.25 μmol ml^{-1}) and 50 μl of distilled water, and the other contained 15 μl of ^{14}C-phenylalanine and 50 μl of nineteen L-amino acids mixture except phenylalanine (1 mM each). For RNA synthesis, 0.5 ml samples were added to the test tubes containing 15 μl of ^{3}H-uracil (5 μCi ml^{-1}; 0.25 μmol ml^{-1}) with or without 50 μl of a mixture of twenty amino acids (1 mM each) as above. After incubation for 10 min at 37°C, 3 ml of ice-cold 5% trichloroacetic acid was added. The precipitates were collected by filtration on Whatman GF83 glass fibre papers, washed three times with 3 ml of 5% trichloroacetic acid and then once with acetone. Radioactivity was determined in a liquid scintillation spectrometer using toulene-based counting mixture. Activities of protein and RNA syntheses were represented by the radioactivities incorporated per A_{660} nm of the culture. Solid lines indicate the activities which were measured by the incorporation of ^{14}C-phenylalanine or ^{3}H-uracil without addition of amino acids mixture. Dashed lines show the activities measured with addition of amino acids mixture (potential activity). *a*, CP78 (*rel*⁺); *b*, CP79 (*rel*⁻). ●, protein synthesis; ○, RNA synthesis; ×, cell growth.

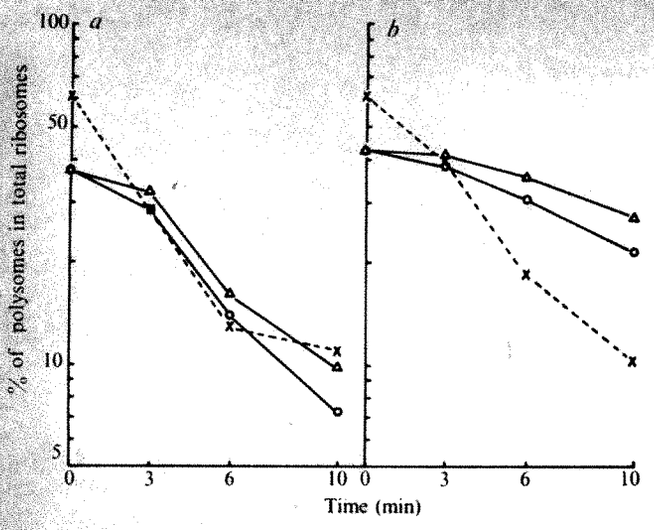


FIG. 2 Decay of polysomes in CP78 (*rel*$^+$) and CP79 (*rel*$^-$) in the presence of rifampicin. Cells, grown to log phase in a glucose-M9 medium supplemented with twenty amino acids, were collected, washed and resuspended in M9-buffer to approximately 7×10^8 cells ml. The cell suspension was poured into the flasks containing 0.6 ml of glucose (0.2 g ml^{-1}), 0.6 ml of thiamine (10 μg ml^{-1}), 0.6 ml of mixture of required amino acids (0.1 mg ml^{-1} each), and 3 ml of mixture of twenty amino acids (1 mM each) when specified. The total volume was adjusted to 30 ml. After incubation for 10 or 30 min 1 ml of rifampicin solution (6 mg ml^{-1}) was added to each flasks. The incubation was stopped by pouring over crushed ice at indicated times. Cell lysates were prepared according to the method of Godson[17]. The cells were collected by centrifugation and resuspended in 0.4 ml of 25% (w/v) sucrose solution containing 10 mM Tris-HCl (pH 8.1). Then to the cell suspension were added 50 μl of lysozyme (1 mg ml^{-1}) and 50 μl of EDTA (10 mM). After mixing by gentle swirling for 3 min in the cold, 0.5 ml of lytic mixture was added, which contained 40 mM Tris-HCl (*p*H 8.1), 1% (w/v) Brij-58, 20 mM $MgCl_2$, 0.2% (w/v) deoxycholate and 1 μg ml^{-1} DNase. Lysed solutions were centrifuged for 10 min at 10,000 r.p.m. to remove the cell membranes and debris. The lysates (3.4 A_{260} unit) were analysed in 4.6 ml of 0.4 M to 1.3 M linear sucrose density gradients containing 10 mM Tris-HCl (*p*H 7.5), 10 mM $MgCl_2$, and 60 mM KCl. Gradients were centrifuged in Spinco SW 50.1 rotor for 50 min at 45,000 r.p.m., then unloaded from the top with ISCO gradient fractionator, which monitored the optical density at 254 nm. The polysome content was determined by cutting and weighing the tracings after correction of the base lines. *a*, CP78 (*rel*$^+$); *b*, CP79 (rel$^-$). ×, Cells were incubated with twenty amino acids for 10 min before addition of rifampicin; ○, incubated in the medium containing only required amino acids for 10 min; △, incubated in the medium containing only required amino acids for 30 min.

suppressed, but it started to rise rapidly before the recovery of growth (Fig. 1*a*). The initial suppression of RNA synthesis in *rel*$^+$ cells is the consequence of stringent control. In the case of *rel*$^-$ cells, the rate of RNA synthesis was not restricted by deprivation of amino acids but during incubation in the minimal medium it declined gradually (Fig. 1*b*). Potential RNA synthesis, like protein synthesis was preserved in *rel*$^+$ cells, but *rel*$^-$ cells were not able to maintain the activity at pre-shift level.

We have suggested that the regulation of RNA synthesis observed in *rel*$^+$ bacteria is dependent on the function of protein synthesising machinery under shift-down conditions[2,3]. It seems that the cells having the *rel*$^+$ allele have the ability to carry out protein synthesis under a limiting supply of amino acids. This teleological significance of the function of the *rel* gene was not considered until the shift-down experiment was carried out, since in the experiments in which required amino acids are withheld, the amino acid auxotrophs are not able to grow (see refs 2 and 15).

When proteins are being synthesised actively in the cells, polysomes are in a dynamic state, being rapidly formed and degraded. Rapid degradation of the active polysomes can be observed when the synthesis of messenger RNA is blocked by addition of actinomycin or rifampicin. On the other hand, polysomes are stabilised and accumulated when ribosome movement on messenger RNA is arrested by ribosomal inhibitors such as chloramphenicol or fusidic acid (see ref. 16). The translational activity on polysomes can therefore be estimated by measuring the stability of polysomes after inhibition of messenger RNA synthesis by rifampicin.

We have measured the stability of polysomes of both *rel*$^+$ and *rel*$^-$ cells in either growth or amino acid shift-down conditions in the presence of rifampicin (Fig. 2). Polysomes in the actively growing cells of both *rel*$^+$ and *rel*$^-$ strains are degraded rapidly with halflife of about 3 to 4 min but the stability of polysomes in amino acid-deprived cells is distinctly different in *rel*$^+$ and *rel*$^-$ strains. Polysomes in amino acid-deprived *rel*$^+$ cells decay almost at the same rate as these in the growing cells although there is a lag before degradation starts (Fig. 2*a*). In a *rel*$^-$ strain, polysomes of cells incubated in an amino acid-deprived medium are much more stable than those of growing cells (Fig. 2*b*). The stability of polysomes is increased by prolonged incubation in the minimal medium.

These findings clearly indicate that the translation on polysomes in *rel*$^+$ cells is occurring actively even under the limiting supply of amino acids, whereas in amino acid-deprived *rel*$^-$ cells polysomes are inert, as when the ribosome movement is arrested by addition of chloramphenicol.

Ron[18] and Silengo[19] have reported essentially the same results as ours on polysome turnover in *rel*$^+$ and *rel*$^-$ bacteria. But Cozzone and Donini[20] have reported a conflicting result: that when *rel*$^+$ and *rel*$^-$ cells carrying temperature-sensitive valyl-tRNA synthetase are treated with rifampicin at a higher temperature, polysomes in both *rel*$^+$ and *rel*$^-$ cells decay as rapidly as those in growing cells under permissive conditions.

From the results of polysome turnover, it was expected that, under the amino acid-starved condition, proteins are synthesised normally on polysomes and released to the soluble fraction in *rel*$^+$ strains, but not in *rel*$^-$ strains. To prove this, we have carried out the following chase experiment. The amino acid-starved cells of both *rel*$^+$ and *rel*$^-$ strains were labelled for 1 min with ^{3}H-amino acids of high specific activity. After labelling, an excess of unlabelled amino-acids was added. Figure 3A shows the kinetics of incorporation of radioactive amino acids into the cell lysate and its acid-insoluble fraction with *rel*$^+$ and *rel*$^-$ cells. Although the specific activities of amino acids were reduced about a thousand-fold by addition of unlabelled amino-acids, the incorporation of radioactive amino acids into the acid-insoluble fraction was not suppressed immediately. In this experiment the amino acid pool of the cells contained radioactivity more than ten times higher than the protein fraction, since the protein synthesis was greatly reduced by amino acid starvation. Therefore, even after the addition of excess unlabelled amino acids there is a time lag before the labelled nascent polypeptides on polysomes are replaced by the unlabelled polypeptides (see Fig. 3C).

Figure 3B shows the pattern of sucrose density gradient centrifugation of lysates of *rel*$^+$ and *rel*$^-$ cells which were chased for 30 and 90 s by the addition of an excess of unlabelled amino-acids. Fig. 3C shows the time course of the incorporation of radioactive amino acids into the acid-insoluble fraction of polysomes, ribosomes, and supernatants calculated from the results of Fig. 3B. As expected, after the addition of unlabelled amino acids the labelled nascent polypeptides on polysomes in amino acid-starved *rel*$^+$ cells were displaced by unlabelled polypeptides and released to the supernatant fraction, while in amino acid-starved *rel*$^-$ cells the radioactive polypeptides were retained on polysomes.

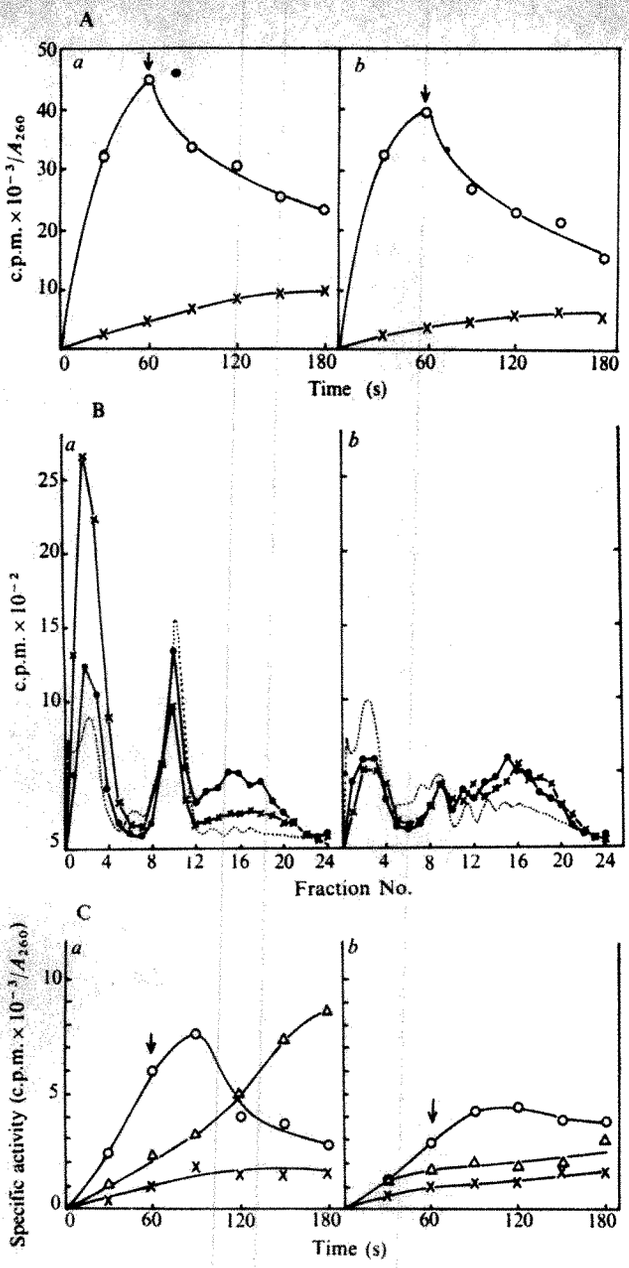


FIG. 3 Pulse labelling of proteins in amino acid-starved cells of both CP78 (*rel*⁺) and CP79 (*rel*⁻). A 60 ml culture, grown to log phase under the supply of twenty amino acids, was washed and resuspended in a glucose (0.4%)-M9 minimal medium without addition of any amino acid. After starvation for 15 min at 28°C, 10 μCi each of radioactive amino acids mixture (8.7 Ci mmol⁻¹ ³H-L-phenylalanine (G), 28.7 Ci nmol⁻¹ ³H-L-lysine-4,5 and 42 Ci mmol⁻¹ ³H-L-tyrosine-3,5 was added. After 60 s of labelling, unlabelled phenylalanine, lysine and tyrosine were added to the final concentration of 25 μM each. At 30 s intervals 10 ml portions of the culture were poured over ice containing 1 mg of chloramphenicol. The cell lysates were prepared and analysed by sucrose density gradients as in Fig. 2. For the determination of radio-activity, 0.2 ml fractions of the gradient were collected and precipitated with 2.5 ml of cold 4% perchloric acid. They were then filtered on Whatman GF83 glass filter papers, washed with 0.4% perchloric acid, 50% ethanol and dried. Radioactivity was determined by scintillation counting in a toulene-based mixture. *a*, CP78 (*rel*⁺); *b*, CP79 (*rel*⁻). *A*, Incorporation of radioactive amino-acids into cell lysate and its acid-insoluble fraction. The arrow indicates the time when unlabelled amino-acids were added. ○, Lysates were applied directly to glass filter papers, dried and counted; ×, lysates were precipitated with cold 4% perchloric acid and counted as described above. *B*, Sucrose density gradient pattern of incorporation. Dotted line shows optical density. ●, The radioactivities were chased for 30 s by addition of unlabelled amino acids after labelling with ³H-amino-acids for 60 s; ×, chased for 90 s. *C*, Release of radioactive polypeptides from polysomes to supernatant fraction. Specific activities of each fraction of polysome, ribosome and supernatant were calculated from the patterns of sucrose density gradients, which denote total counts of corresponding region per total A_{260} unit of that region. The arrow indicates the time when unlabelled amino acids were added. ○, Radioactivities in polysome region; ×, ribosome region; △, supernatant region.

The above results are consistent with those obtained from polysome turnover experiments. Thus, the translational defect in *rel*⁻ cells under the restriction of amino acid supply may be located in some processes involving ribosomal functions.

Many studies have been reported on the mechanism of induction or derepression of enzymes in bacteria. In most cases, the induction or derepression of enzymes is achieved by the regulation at the level of transcription, and the mechanism involved in this process is largely understood. The mechanism of translational control in enzyme formation during adaptation has not yet however been clarified.

From the study on the formation of β-galactosidase in amino acid-starved cells of *Salmonella typhimurium*, Engbæk *et al.*[21] have pointed out that a mechanism for successful use of a limiting supply of aminoacyl-tRNA must exist in the cells. We consider that the *rel* gene is responsible for this mechanism. As we have shown before[2], the mutation in the *rel* gene causes the impairment of formation of enzymes when the supply of amino acid is limited. We have observed that polysome turnover is suppressed in *rel*⁻ cells under amino acid starvation, whereas in *rel*⁺ cells polysomes actively turn over and the polypeptides synthesised on polysomes are transferred normally to the supernatant fraction. These results lend additional support to the view that *rel* gene product functions at the translational level during amino acid starvation. Hall and Gallant[22] have also found that an abnormal β-galactosidase with reduced specific enzymic activity and increased thermolability is formed in amino acid-starved *rel*⁻ cells.

Pedersen *et al.*[23] and Haseltine and Block[24] have reported that ppGpp and pppGpp, the compounds which accumulate in response to amino acid starvation in *rel*⁺ cells, are synthesised on ribosomes in a cell-free system of *E. coli*. The synthesis requires the presence of messenger RNA, deacylated tRNA, and a protein factor isolated from the ribosomal washing of *rel*⁺ cells[25]. Their results suggest that ppGpp and pppGpp are formed on polysomes, when deacylated tRNA is bound to the A site of ribosomes due to the unavailability of the appropriate aminoacyl-tRNA species.

As is shown here, polysomes in *rel*⁺ cells are able to continue to synthesise proteins after amino acid deprivation whereas in *rel*⁻ cells the movement of polysomes is inhibited. It would be of interest to examine whether there is any close correlation between the formation of the unusual guanine nucleotides and the successful use of limiting amount of aminoacyl-tRNA during deprivation of amino acids.

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1 Stent, G. S., and Brenner, S., *Proc. natn. Acad. Sci. U.S.A.* **47**, 2005 (1961).
2 Sokawa, Y., Sokawa, J., and Kaziro, Y., *Nature new Biol.* **234**, 7 (1971).
3 Sokawa, J., Sokawa, Y., and Kaziro, Y., *Nature new Biol.* **240**, 242 (1972).
4 Neidhardt, F. C., *Biochim. biophys. Acta*, **68**, 365 (1963).
5 Pardee, A. B., and Prestidge, L. S., *J. Bact.*, **71**, 677 (1958).

[6] Aronson, A. I., and Spiegelman, S., *Biochim. biophys. Acta*, **53**, 70 (1961).
[7] Atherly, A. G., and Suchanek, M. C., *J. Bact.*, **108**, 627 (1971).
[8] Atherly, A. G., and Menninger, J. R., *Nature new Biol.*, **240**, 245 (1972).
[9] Rabbani, E., and Srinivasan, P. R., *J. Bact.*, **113**, 1177 (1973).
[10] Alföldi, L., Stent, G. S., Hoogs, M., and Hill, B., *Z. Vererbungslehre*, **94**, 285 (1963).
[11] Fiil, N., *J. molec. Biol.*, **45**, 195 (1969).
[12] Hall, B., and Gallant, J., *J. molec. Biol.*, **61**, 271 (1971).
[13] Gallant, J., Erlich, H., Hall, B., and Laffler, T., *Cold Spring Harb. Symp. quant. Biol.*, **35**, 397 (1970).
[14] Fiil, N., and Friesen, J. D., *J. Bact.*, **95**, 729 (1968).
[15] Edlin, G., and Broda, P., *Bact. Rev.*, **32**, 206 (1968).
[16] Schlessinger, D., *The Mechanism of Protein Synthesis and its Regulation* (edit. by Bosch, L.), 441 (North-Holland, Amsterdam and London, 1972).
[17] Godson, G. N., *Methods in Enzymology*, **12** A, 503 (1967).
[18] Ron, E. Z., *J. Bact.*, **108**, 263 (1971).
[19] Silengo, L., *J. Bact.*, **115**, 447 (1973).
[20] Cozzone, A., and Donini, P., *J. molec. Biol.*, **76**, 149 (1973).
[21] Engbæk, F., Kjeldgaard, N. O., and Maaløe, O., *J. molec. Biol.*, **75**, 109 (1973).
[22] Hall, B., and Gallant, J., *Nature new Biol.*, **237**, 131 (1972).
[23] Pedersen, F. S., Lung, E., and Kjeldgaard, N. O., *Nature new Biol.*, **243**, 13 (1973).
[24] Haseltine, W. A., and Block, R., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1564 (1973).
[25] Haseltine, W. A., Block, R., Gilbert, W., and Weber, K., *Nature*, **238**, 381 (1972).

Selective pressure on HL-A polymorphism

THE advantages of heterozygotes is one of the most frequent explanations of the maintenance of a polymorphism. This advantage may have the form of a lower fitness of all homozygotes, whatever the allele they possess. According to observations on a small Tuareg isolate, such is apparently the case for the polymorphism of the histocompatibility system HL-A.

The HL-A system (human leukocyte antigen A) is immunologically detected by means of specific sera. It depends on two loci which lie close together on the same chromosome. At the present time (including the blanks), 15 alleles are well defined at the first locus (SD1) and 20 alleles at the second (SD2). Each combination of one allele at the first locus and one at the second is called a 'haplotype'. The number of these combinations is therefore 300. Because of the low recombination fraction between the loci (about 0.008) (ref. 1), the system can, for certain purposes, be considered as a single locus system with 300 alleles.

Homozygotes (having two identical haplotypes) are very rare since all the haplotypes have a lower frequency than 10% in Caucasoid populations. It is pointless to try to show a discrepancy between the observed frequency of homozygotes and that expected in a Hardy-Weinberg equilibrium. The only exception is in very small, isolated populations in which, because of founder effect or genetic drift, the number of haplotypes present in the genetic makeup of the group is greatly reduced.

This is the case with the Kel Kummer Tuareg tribe, of the South Sahara. This tribe was founded in the seventeenth century, since when it has lived entirely apart from surrounding populations and has rigorously respected the very strict rules of mating which require marriage with the mother's brother's daughter. At present it numbers about 300 people.

The genealogies, collected by A. Chaventré[2] give an exhaustive description of the descent of the tribe, and some of the process of transmission of the genetic heritage through three centuries. They cover a total of 2,500 individuals, and an example of such a genealogy is shown in Fig. 1.

These genealogies gathered from oral tradition, have been confirmed by many cross checks with written records (military registers and accounts of travellers), systematic research on paternity exclusion and research on common ancestors of individuals with rare genes. These verifications have shown the genealogical information to be reliable. The 2,500 individuals, dead or living, of the tribe all descend from a few ancestors, with extremely intricate lines of descent.

This inbreeding may be measured by the classical F coefficient[3]. It is possible to calculate the F values by computer taking into account all the possible paths between living individuals and their common ancestors (in some cases the number of paths is about 10^6). For the set of 106 individuals whose HL-A genotype is known, the mean of the computed F coefficients is 0.1108, which is extremely high. In two sibships it is even greater than 0.24 ($F = 0.25$ for offspring of sib-mating).

The homogeneity that results from this inbreeding is better shown by taking the 'probabilities of a gene's origin', that is, the set of probabilities that, for the various founders, a gene taken at random in the population is derived from him, where a 'founder' is an ancestor whose parents are unknown[3].

Classifying the 97 founders in order of decreasing importance, we obtain Table 1. This shows that 90% of the genetic makeup of the tribe comes from only 22 individuals. These individuals represent 44 haploid sets, some of which are probably identical since the founders certainly were related (but in a way we do not know). It is therefore evident that the Kel Kummer Tribe exhibits an uncommon genetic homogeneity.

To discover the HL-A genetic structure of the Kel Kummer tribe we tested for antigens: HLA1, 2, 3, 9, 10, 11, W29, W30+31 and W28 for the first locus (SD1), and HLA5, 7, 8, 12, 13, W5, W10, W14, W15, W17, W18, W21, W22, W27 and DA(6) for the second locus (SD2). The research[4,5] on the HL-A antigens of the Kel Kummer have shown that they possess only 21 haplotypes, two of which exist at high frequencies, namely: HL-A11/W21, 19%; W28/HL-A7, 17%.

Among the 106 individuals whose genotypes (the two haplotypes) are known, only one is a possible homozygote; but this cannot be ascertained since he may have a blank allele at both loci; a study of his genealogy gives the probability 0.50 of homozygosity.

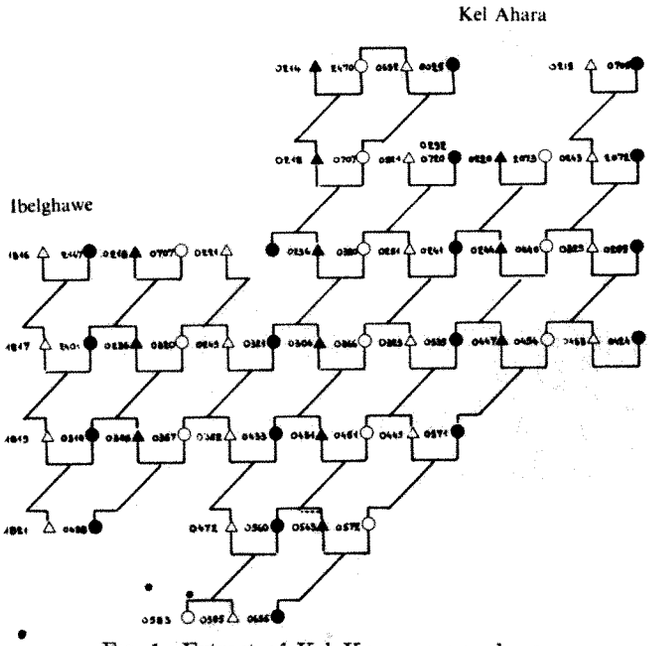


FIG. 1 Extract of Kel Kummer genealogy.

TABLE 1 Genetic make-up of the Kel Kummer tribe

Founder No.	Proportion of the genetic make-up (%)	Cumulative
1	10.6	
2–7	41.8	52.4
8–12	19.4	71.8
13–22	17.4	89.3
23–97	10.7	100

The number of homozygotes we expected is, however, significantly higher. If the population were in Hardy-Weinberg equilibrium for the HL-A loci, the total frequency of homozygotes, equal to the sum Σp_i^2 of the haplotypes' squared frequencies, would be 8.4%. Moreover we have seen that the rules of choice of mate are very strict and so the prohibition of sib-mating, which entails a small 'negative inbreeding', is largely counterbalanced by the preference for cousin mating (30% of the marriages) or for mating with a cousin once removed (15%). The mating behaviour for the whole tribe corresponds to a deviation from random mating measured by the δ coefficient[3], such that $\delta = + 0.01$. The frequency of homozygotes, knowing the present genetic structure and the mating behaviour should therefore be

$$h = \delta + (1 - \delta)\ \Sigma p_i^2 = 9.3\%$$

Under the hypothesis that the observed sample is a random sample, the expected number of homozygotes among the 106 tested individuals, is a random variable with expected value $E = 106 \times 0.093 = 9.86$ and standard deviation $\sigma = (106 \times 0.093 \times 0.907) = 2.99$. The observed number (1 or maybe 0) is thus largely outside the confidence interval ($P < 0.005$).

The mean inbreeding coefficient of the tested individuals is $F = 0.1108$. Each of them has therefore a probability of greater than 0.11 of being a homozygote. Because of the non-independence of the genes of the various individuals, one cannot consider the number of individuals with two identical genes as a binomial variable. There is an inconsistency between the expected number of such individuals ($0.1108 \times 106 = 11.7$) and the observed number of homozygotes.

This observation supports the hypothesis that the HL-A chromosome segment is maintained in a polymorphic condition by disadvantages to being a homozygote, whatever genes are possessed. A model of this kind has been proposed for the histocompatibility systems[6], but has not previously been supported by observation. The present data do not enable the role of HL-A system itself to be distinguished from that of a linked locus. It is probable however that HL-A antigens are present, in the haploid state, on the spermatozoa[7]: however, no disturbance on zygotic assortment for the HL-A system has been found in family studies[8,9].

Finally we note that the genes of the main system of histocompatibility, HL-A, are located on a chromosome segment which is very important for many immunological processes (locus of mixed lymphocyte culture, MLC, and probably locus of immune response, Ir). The detection of the presence of foreign cells (alloimmunisation) depends mainly of this segment; it is possible that it includes 'archaic' loci, which have remained unchanged during evolution, together with duplicated loci which have become more and more sophisticated. In plants there exist loci for detection of foreign cells, the 'self-sterility systems', which prohibit homozygous fertilisation and maintain polymorphism. It is possible that such genes still exist in man on the alloimmunisation chromosome segment, contributing to the maintenance of polymorphism. This interpretation of our observations is supported by the following argument: when non-related parents share the same allele for HL-A or for MLC loci, the offspring is homozygote with a frequency close to the expected proportion[10]; consequently the 'prohibition of homozygote' we have observed may correspond to a locus closely linked to HL-A but which is not HL-A itself nor MLC.

This homozygous deficit has not, however been found in studies of other relatively isolated populations[11]. This leads us to believe that the gene preventing homozygosity (which is not HL-A) may be at some distance from the HL-A loci on this chromosome. Nevertheless in the Tuareg tribe, which is extremely inbred, it is always transmitted with the HL-A loci because of the limited recombination opportunities. Another theory would be that the locus linked to HL-A possesses several alleles, not one of which allows homozygosity. This has been proven to be the case of T locus in mice which is linked to the major histocompatibility locus (H-2).

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[1] Svejgaard, A., Bratlie, A., Hedin, P. J., Hogman, C., Jersild, C., Kissmeyer-Nielsen, F., Lindlom, B., Sandburg, L., Lindolm, A., Low, B., Messeter, L., Moller, E., Stajbnielsen, L., and Thorsby, E., *Tissue Antigens*, **1**, 81 (1971).
[2] Chaventre A., *thesis, Univ. Paris V* (1973).
[3] Jacquard, A., *The genetic structure of populations*, (Springer Verlag, New York, 1973).
[4] Colombani, J., Degos, L., Petrignani, C., Chaventre, A., Lefevre-Witier, P., and Jacquard, A., in *Histocompatibility testing (1972)*, 153 (Munksgaard, Copenhagen, 1973).
[5] Degos, L., *Population*, **28**, (in the press).
[6] Clarke, B., and Kirby, D. R. S., *Nature*, **211**, 999 (1966).
[7] Fellous, M., and Dausset, J., *Nature*, **225**, 191 (1971).
[8] Mattiuz, P., Ihde, D., Piazza, A., Ceppellini, R., and Bodmer, W. F., in *Histocompatibility testing (1970)*, 193 (Munksgaard, Copenhagen, 1970).
[9] Dausset, J., Degos, L., Fellous, M., and Legrand, L., *Genetics* (in the press).
[10] Dausset, J., Sasportes, M., and Lebrun, A., *Transplant. Proc.* (in the press).
[11] Bodmer, J. G., Nevo, S., Bodmer, W. F., and Coukell, A., in *Histocompatibility testing (1972)*, 117 (Munksgaard, Copenhagen, 1973).

Stimulation of bone marrow haemopoietic stem cells by a factor from activated T cells

I present here direct evidence that a factor(s) produced by activated thymus-derived lymphocytes (T cells) enhances proliferation of pluripotent bone marrow stem cells.

T cells have been known as a source of humoral mediators which can regulate (enhance or inhibit) antibody response[1–5]. These mediators are released when T cells are stimulated with an antigen[6–9] or with mitogens (phytohaemagglutin (PHA) or concanavalin A) although direct evidence for the latter is not available[10]. With one exception[9], the effect of the mediator was reported to be non-specific, that is, factor that was induced by one antigen stimulated production of antibody to another non-cross-reacting antigen[6–8]. This implies that the factor stimulates proliferation and differentiation of immunocompetent bone-marrow-derived cells (B cells) in general.

TABLE 1 Activation of splenic haemopoietic c.f.u. by PHA *in vitro* and *in vivo*

Source of spleen cells	PHA*	Recipient mice: No. cells† injected, × 10^5	Recipient mice: c.f.u. per spleen‡, individual mice	c.f.u. per 10^6 injected cells (fresh or cultured)	^{3}H-thymidine pulse§ (d.p.m. per 10^6 cells)
Fresh suspension					
normal donors	−	2.0	3,3,4,4,5	19.0 (15–25)¶	
PHA-injected donors	+	2.0	9,12,13,14,15	63.0 (45–75)	
Cell culture	−	15.0	0,0,0,0,1,2,2,2,3	≤1.0 (<1–2)	1,950 (1,900–2,000)Φ
	+	10.0	2,2,3,3,3,3,4,7	3.4 (2–7)	117,500 (94,300–144,000)

Male Balb/c mice (Jackson Laboratory) were both donors (6–8 weeks old) and recipients (10–12 weeks old). Recipients received an X-ray dose of 700 rad (180 kV, 15 mA, 80 rad min^{-1}, with 2 mm Al and half-value layer 0.5 mm Cu, distance 51 cm) 6 h before cell transfer. A cell suspension (pooled from several donors) was prepared by pressing spleens through stainless steel wire screens in cold Puck's saline with antibiotics, followed by washing. Eagle's minimal essential medium was used for suspension cultures, containing 1% non-essential amino acids (100x), 2mM L-glutamine, lmM sodium pyruvate, 1% antibiotic mixture (100x) and 5% foetal calf serum. Cultures (2–3 ml, 10^7 cells per ml), in disposable tissue culture tubes, (Falcon No. 2051) were kept for 3 d at 37°C in air with 5% CO_2. Cells were then washed and injected into irradiated recipients in the numbers indicated. PHA was added at a concentration of 2.5 μg ml^{-1} of medium, at the beginning of cultivation. For *in vivo* experiments, donor mice were injected with 200 μg intraperitoneally 24 h before cell transfer.

† Nucleated cells (viability determined by trypan blue exclusion).

‡ c.f.u. is the number of macroscopic colonies visible on spleen surface 8 d after cell transfer. Colonies were counted using magnifying glasses (10x) after brief immersion of the spleen into acetic acid-ethanol (1:4). No colonies were found in spleens of control irradiated recipients which receive no cell inoculum.

§ Pulse labelling was done in 2 ml cultures containing 10^6 cells per ml of the medium with or without PHA (0.5 μg ml^{-1}). ^{3}H-thymidine (2.5 μ Ci ml^{-1} of culture) was added for 24 h between the second and third days of cultivation. Cells were then precipitated and washed with 0.2 N perchloric acid, the pellet resuspended in 0.1 N NaOH, aliquots (0.1 ml) pipetted into scintillation vials with 10 ml of Aquasol (New England Nuclear Corp.) and radioactivity determined in a Packard scintillation counter.

¶ Average number of c.f.u. per 10^6 of injected cells (range within the group).

Φ Mean of duplicates (range).

An obvious question arises. Is the effect of activated T cell factor(s) limited to the functionally specialised immunocompetent B cells, or could it also stimulate other bone marrow-derived cells and bone marrow cells? Precursors of antibody-forming cells represent only part of the bone marrow population; another large portion comprises undifferentiated stem cells, or cells engaged in haemopoiesis (erythropoiesis and granulopoiesis).

The answer was sought as follows. Activated T cells were obtained from cultures of spleen cells incubated with phytohaemagglutinin (PHA). The stimulatory effect of PHA was measured by pulse labelling with ^{3}H-thymidine. Haemopoietic stem cells were enumerated by the colony-forming unit (c.f.u.) assay[11]. A sample of the cultivated cell suspension in which the stem cells (c.f.u.) were to be detected, was injected into lethally irradiated syngeneic mice. Spleens of recipients were removed 8 d later and macroscopic colonies on their surface were counted. Each colony is a clone arising from a single pluripotent haemopoietic stem cell and contains cells of the same morphological character, either erythrocytic, megakaryocytic or granulocytic[11–14]. No lymphocytic colonies have been reported so far, even in spleens of mice that received cells from PHA-stimulated donors[15]. Colony-forming stem cells can be obtained from bone marrow, spleen, blood and lymph nodes (in decreasing order of frequency) but not from thymus or thoracic duct cells[15–18].

In my first experiment, normal spleen cells were cultivated *in vitro* for 3 d with or without PHA (2.5 μ ml^{-1}) and c.f.u. in the cultures was counted by the transfer of 10^6 cells to irradiated recipients. To verify that the tissue culture reflected the effect of PHA *in vivo*[15] some irradiated recipients were given freshly isolated spleen cells (10^5 to 10^6) of either normal donors or doners injected with PHA 1 d before the transfer. Table 1 shows that stimulation of spleen cells with PHA, either *in vivo* or *in vitro*, increased the number of haemopoietic cells (c.f.u) about three times ($P < 0.01$, by Wilcoxon's unpaired rank test, ref. 19).

The following experiments were performed to determine if the effect of PHA on stem cells is mediated by T cells. Spleen cells were treated either with anti-theta serum plus complement to eliminate T cells, or with normal serum and complement as a control. The cells were washed and cultured for 3 d with or without PHA. c.f.u. were enumerated as previously. Table 2 shows that the stimulation of DNA synthesis and the increase of stem cells (c.f.u.) observed in control cultures (containing T cells) ($P < 0.01$) did not occur in experimental cultures in which T cells were eliminated ($P > 0.05$).

To show that the effect of T cells on c.f.u. is mediated by a factor(s), supernatant fluids from previously described cultures were collected, filtered through 0.45 μm Millipore filters and stored frozen at −20° C. They were then tested

TABLE 2 Role of theta-positive cells (T cells) in increase of splenic colony-forming cells in cultures stimulated with PHA

Cell treatment before culture	PHA*	Recipient mice: No. cells† injected, × 10^5	Recipient mice: c.f.u. per spleen, individual‡	c.f.u. per 10^6 injected cells	^{3}H-thymidine pulse§ (d.p.m. per 10^6 cells)
Normal serum + C′	−	9.0	0,1,1,1,1,2,2,2	1.4 (<1–2)¶	2,955 (2,850–3,080)‖
	+	4.5	1,1,3,3,3,4,5	6.3 (2–11)	103,750 (103,500–104,000)
Anti-theta serum + C′	−	7.2	2,3,3,3,4,5,7,9,9	6.9 (3–12)	2,460 (2,360–2,560)
	+	6.3	2,3,3,5,5,9	7.1 (3–14)	2,310 (1,830–2,790)

Anti-theta serum was prepared by AKR mice given ten weekly injections of C3H thymocytes (10^7 cells per injection, intraperitoneally). 100 μg of a pertussis vaccine (gift from the Massachusetts State Laboratory) was given with the eighth injection. The serum was specific for thymus cells (titre 1:8,000) and killed 40–50% of spleen cells as determined by trypan blue exclusion. Normal guinea pig serum absorbed twice with mouse spleen and liver cells (5:1, v/v) was used as a source of complement (C′). Freshly isolated spleen cells were incubated, under sterile conditions, with anti-theta serum (5 × 10^7 cells per ml of Puck's saline, final dilution of antiserum 1:10) in a water bath at 37°C for 20 min. Guinea pig serum was then added as a source of complement (1:10) and left for another 20 min. Normal mouse serum (absorbed) served as a control. Cells were washed in Puck's saline and cultivated for 3 d as described in Table 1. As anticipated, specific killing of T cells resulted in a relative increase of c.f.u. per 10^6 viable cells remaining in the spleen cell suspension. The increase was expected to be two-fold but was actually higher (compare first and third row in Table 2). The possible stimulating effect of antigen-antibody complex on CFU might be considered.

Footnotes are explained in the legend to Table 1.

TABLE 3 Stimulation of bone marrow c.f.u. with a T cell product *in vitro*

Experiment	Culture medium	Recipients: No. cells† injected, × 10^5	Recipients: c.f.u. per spleen, individual‡	c.f.u. per 10^6 injected cells	^{3}H-thymidine pulse§ (d.p.m. per 10^6 cells)
A	MEM	4.3	3,3,4,4,5,6,6	10.0 (7–14)	10,500 (9,600–11,500)
	MEM + PHA	4.5	4,4,4,5,5,5,6,6	11.0 (9–13)	7,500 (7,500–7,500)
	S_{MEM} 1:20	8.4	4,5,6,6	6.4 (5–7)	12,300 (11,200–13,400)
		16.8	9,9,10		
	S_{PHA} 1:20	4.0	6,7,8,10	20.0 (15–24)	31,450 (31,300–31,600)
		8.0	14,14,16,19		
B	S_{MEM} 1:50	2.5	1,2,2,3,3,4	10.0 (4–16)	12,950 (11,200–14,700)
	S_{PHA} 1:50	2.5	10,10,12,14,15,18	53.0 (40–72)	31,000 (28,000–34,000)
	1:100	2.5	3,4,4,5,6,9	21.0 (12–36)	27,400 (24,000–31,000)
	$S_{anti\text{-}theta.MEM}$	2.5	3,4,4,4,5	16.0 (12–20)	15,500 (13,000–18,000)
	$S_{anti\text{-}theta.PHA}$	2.5	2,2,2,3,3,4,4,5,5	14.0 (8–20)	18,000 (16,000–22,000)

Bone marrow cells (pooled from several donors) were flushed from femurs with cold Puck's saline, washed and resuspended in the culture medium as described in Table 1. Supernatant fluids added to the medium at various concentrations were collected, in previous experiments, from 3-d cultures of spleen cells in the medium without (S_{MEM}) or with (S_{PHA}) PHA. Supernatants added in experiments *A* and *B* came from two different experiments in which the stimulating effect of PHA on splenic c.f.u. in the cultures was determined. '$S_{anti\text{-}theta.MEM}$' and '$S_{anti\text{-}theta.PHA}$' are supernatants from cultures shown in Table 2 (third and fourth row) in which spleen cells were pretreated with anti-theta serum plus C′ and then cultured with or without PHA in the medium. Splenic B cells (obtained by treatment of spleen cell suspension with anti-theta serum and complement before cultivation) were also used as the target for the factor. Results (unpublished) were comparable to those reported here with bone marrow cells.

MEM, minimal essential medium.

Footnotes are explained in the legend to Table 1.

for the presence of a cell-free substance able to stimulate haemopoietic stem cells *in vitro*. Bone marrow cells (which do not respond to PHA), rather than spleen cells, were the source of c.f.u. and the target for the hypothetical factor. This eliminated the possibility of stimulation of T cells by trace amounts of PHA in the supernatant fluids. Bone marrow cells were flushed from femurs of normal donors, washed and resuspended in the culture medium containing various concentrations of either control supernatants from normal, untreated spleen cultures (S_{MEM}) or active supernatants from cells cultured with PHA(S_{PHA}). Another set of control cultures included bone marrow cells with or without PHA in the medium. Cells were tested for the number of c.f.u. after 24 h of cultivation. Some culture tubes were kept for 3 d and were pulse-labelled with ^{3}H-thymidine.

PHA failed to stimulate DNA synthesis and c.f.u. ($P > 0.05$) in bone marrow cell cultures (Table 3, experiment *A*). In contrast, the presence of S_{PHA} in the culture medium increased DNA synthesis in cultures and increased the number of active c.f.u. two to three times compared with control cultures containing S_{MEM} (experiments *A* and *B*). The factor was active up to a dilution of 1:100 in the medium with an optimum concentration of about 1:50. Supernantant fluid from spleen cells pre-treated with anti-theta serum plus complement and then cultured with PHA did not contain any stimulatory activity ($P > 0.05$).

It can be stated that mouse splenic T cells activated with PHA *in vitro* release a substance(s) which can stimulate proliferation of bone marrow cells and activate haemopoietic stem cells *in vitro* and *in vivo*. The possibility of such a mediator was implied by previous observations that the administration of thymus-dependent antigens changed considerably the frequency of haemopoietic stem cells in blood and spleen[20–21]. A similar effect was observed by Micklem[15] after *in vivo* injection of PHA, a specific mitogen of mouse T cells[22–25]. My results provide direct proof of existence of such a factor(s).

The chemical nature of this T cell product is not known. I suggest, however, that it is related to immunoregulating factors induced in T cells by antigen[6–9] rather than to 'thymosine', a soluble substance obtained from normal thymus cells and able to promote immunological maturation of B cells[26–32]. The suggestion is based on the observation that the factor described here can be obtained from stimulated peripheral T cells only. Thymus cells, either normal or incubated with PHA, do not release an active substance that has this effect on stem cells (unpublished results of E. B. Waner and myself).

If upheld, my observations will offer a mechanism by which activated T cells can regulate not only antibody formation in specialised immunocompetent cells, as described by others, but also differentiation of more primitive stem cells and haemopoietic cells in bone marrow. This model will provide another test for comparative analysis of biological functions of humoral products from T cells.

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1 Hartman, K. V., *J. exp. Med.*, **132**, 1267 (1970).
2 Rubin, A. S., and Coons, A. H., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1665 (1971).
3 Dutton, R. W., Falkoff, R., Hirst, J. A., Hoffman, M., Kappler, J. K., Kettman, J. R., Lesley, J. F., and Vann, D., in *Progress in Immunology*, (edit. by Amos, B.), 1, 335 (Academic Press, New York, 1971).
4 Katz, D. A., Paul, W. E., Goidl, E. A., and Benacerraf, B., *J. exp. Med.*, **133**, 169 (1971).
5 Mond, J. J., Takahashi, T., and Thorbecke, G. J., *J. exp. Med.*, **136**, 715 (1972).
6 Schimpl, A., and Wecker, E., *Nature new Biol.*, **237**, 15 (1972).
7 Rubin, A. S., and Coons, A. H., *J. Immun.*, **108**, 1597 (1972).
8 Gorczynski, R. M., Miller, R. G., and Philips, R. A., *J. Immun.* **108**, 547 (1972).
9 Feldman, M., and Basten, A., *Nature new Biol.*, **237**, 13 (1972).
10 Rich, R. R., and Pierce, C. W., *J. exp. Med.*, **137**, 205 (1973).
11 Till, J. E., and McCulloch, E. A., *Radiat. Res.*, **14**, 213 (1961).
12 Becker, A. J., McCulloch, E. A., and Till, J. E., *Nature*, **197**, 452 (1963).
13 Curry, J. L., and Trentin, J. J., *Dev. Biol.*, **15**, 395 (1967).
14 Lewis, J. P., and Trobaugh, jr., F. E., *Nature*, **204**, 589 (1964).
15 Micklem, H. S., *Transplantation*, **4**, 732 (1966).
16 Cole, L., *Am. J. Physiol.*, **204**, 256 (1963).
17 Gesner, B. M., and Gowans, J. L., *Brit. J. exp. Pathol.*, **43**, 431 (1962).
18 Micklem, H. S., Ford, C. E., Evans, E. P., and Gray, J., *Proc. R. Soc.*, B, **165**, 78 (1966).
19 Wilcoxon, F., in *Some rapid approximate statistical procedures*, 3 (American Cyanamid Company New York, 1949).
20 Barnes, D. W. H., and Loutit, J. F., *Lancet*, ii, 1138 (1967).

[21] Cerny, J., and Viklicky, V., *Adv. exp. med. Biol.*, New York, **5**, 257 (1969).
[22] Dukor, P., and Dietrich, F. M., *Int. Arch. Allergy*, **32**, 521 (1967).
[23] Rieke, W. O., *Science*, **152**, 535 (1966).
[24] Stobo, J., *Transplant. Rev.*, **11**, 60 (1972).
[25] Greaves, M., and Janossy, G., *Transplant. Rev.*, **11**, 87 (1972).
[26] Roberts, S., and White, A., *J. biol. Chem.*, **128**, 151 (1949).
[27] Metcalf, D., *Brit. J. Cancer*, **10**, 442 (1956).
[28] Goldstein, A., Slater, F. D., and White, A., *Proc. natn. Acad. Sci. U.S.A.*, **56**, 1010 (1966).
[29] Law, L., Trainin, N., Levey, R. H., and Barth, W. F., *Science*, **143**, 1069 (1964).
[30] Hand, T. L., Ceglowski, W. S., Damrongsak, D., and Friedman, H., *J. Immun.*, **105**, 442 (1970).
[31] Nakamura, K., *J. exp. Med.*, **135**, 476 (1972).
[32] Doria, G., Agarrosi, G., and DiPietro, S., *J. Immun.*, **108**, 268 (1972).

Animal longevity and protein turnover rate

THE life spans of various mammalian species differ but the biochemical mechanism of this regularity is unknown. In our opinion there is a sufficient amount of experimental data to solve this problem. We refer to the data presented in twelve investigations by different authors collated into one table (Table 1), and the data of the two works collated in another (Table 2)[1]. In these, the data on the protein plasma turnover for various species of mammals is presented. We have added a column on the maximum life span of the species[2] into each table.

Table 1 shows that the highest rate of protein turnover is observed in the rat, it is lower in the dog, and still lower in human beings. Table 2 shows that the small animals, such as mice, guinea pigs or rabbits, have the highest rate of turnover, and the turnover is lower for dogs, and still lower for cows, monkeys and human beings.

In terms of a life span there is a negative correlation between the longevity of animals and their protein turnover rate. The existence of such a regularity has attracted no attention so far. The protein turnover rate, however, does characterise the intensity of protein synthesis, and this in turn, depends on the intensity of DNA activity.

TABLE 2 Turnover of homologous proteins of various species labelled with ^{131}I (ref. 1, column reference indicates 262 and 263).

Species	Albumins: Time interval (d)	Albumins: Halflife (d)	γ globulin: Time interval (d)	γ globulin: Halflife (d)	Maximum lifespan (yr)
Human	7–28	15.0	4–21	13.1	110–115
Cow	2–30	20.7	6–27	21.2	20*
Monkey			4–24	6.6	30
Dog	7–28	8.2	7–17	8.0	18
Rabbit	4–22	5.7	4–21	4.6	15
Guinea pig			4–13	5.4	7
Mouse	3–7	1.2	1–5	1.9	3
Rabbit		4.5			5

* These data apply to ruminants and herbivores in general.

TABLE 1 Turnover of plasma proteins of various species of mammals.

Protein	Species	Active matter	Method*	Half-life (d)	Reference to literature†	Maximum life-span (yr)
General plasma protein	Rat	^{35}S-DL-methionine	D	2.6	207	
		^{35}S-DL-methionone	D	4.4	222	5
		DL-serine-3	H	3.0	232	
		Rhodospirillum-rubrum-^{14}C	H	3.0	198	
		^{35}S-L-methionine	D	3.5	259	
	Dog	^{35}S-DL-methionine	D	5.2–6.4	115	18
		^{15}N-DL-lysine	H	5.4	230	
		DL-lysine-6	H	5.0	208	
		^{35}S-DL-methionine	H	5.0	115	
		^{35}S-DL-methionine	D	6.6	260	
	Human	^{35}S-DL-methionine	D	9.2	115	110–115
	Human	^{15}N-glycine	D	10.0	261	
	Human (male)	^{15}N-glycine	D	7.0	240	
	Human (female)	^{15}N-glycine	D	9.0	240	
Albumin	Rat	^{35}S-L-methionine	D	5.0	259	
	Dog	DL-lysine-6	H	6.9	208	
	Human (male)	^{15}N-glycine	D	20	240	
	Human (female)	^{15}N-glycine	D	20	240	
Globulin	Rat	^{35}S-L-methionine	D	3.0	259	
	Dog	DL-lysine-6	H	3.3	207	
α_1 globulin	Rat	^{35}S-L-methionine	D	3.0	259	
α_2 globulin	Rat	^{35}S-L-methionine	D	2.0	259	
β globulin	Rat	^{35}S-L-methionine	D	3.0	259	
γ globulin	Rat	^{35}S-L-methionine	D	3.2	259	
Globulins $\beta + \gamma$	Human (male)	^{15}N-glycine	D	12	240	
γ globulin	Human (male)	^{15}N-glycine	D	19	240	
Globulins $\beta + \gamma$	Human (female)	^{15}N-glycine	D	9.0	240	
γ globulin	Human (female)	^{15}N-glycine	D	18	240	
Fibrinogen	Dog	^{35}S	D, CP	4.2	227	
Fibrinogen	Human	^{35}S	D	8.1	227	

* "D", is a direct method used to determine the isotope concentration in protein at some intervals after injection of the labelled material. "H" is (Heveshi's method) introduction of the labelled protein to the recipient animal with subsequent measurement. "CP" is the constant fund method. Half-life span measured by the different methods is approximately the same.
† Column reference indices Sam Tarver[1].

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[1] Tarver, H., in *The Proteins* (edit. by Neurath, H., and Bailey, K.) 1199–1296 (Academic Press, New York, 1954).
[2] Comfort, A., *Ageing: The Biology of Senescence* (Holt, Rinehart and Winston, New York, 1964).

Solubilisation of angiotensin II receptors in rabbit aortae membranes

HORMONE–RECEPTOR interaction is the first step of a chain of cellular processes leading to the hormonal response. Receptor sites are believed to be macromolecular components finite in number, possessing a genetically determined structure and capable of specific recognition and interaction with hormones with a high degree of specificity and affinity. Receptors of steroid hormones are intracellular, whereas those of neurotransmitters and polypeptide hormones are located in the cell membranes[1].

Angiotensin II is a polypeptide hormone which has, among many pharmacological actions, the basic property to contract smooth muscles[2]. The availability of a ^{3}H-angiotensin II having a high specific activity and possessing the full biological activity of the native hormone[3] allowed us to demonstrate the presence of angiotensin receptors in the intact rabbit aorta[4], in the microsomal membranes extracted from homogenates of intimal medial layers of rabbit aortate[5], and in a membrane fraction derived from the plasma membrane[6]. At the various levels of purification the binding sites were considered as hormone receptors, as the kinetic constants of angiotensin binding were identical to those of the biological responses induced by the hormone.

Here we describe the solubilisation of the above described angiotensin-binding components of smooth muscle cells of rabbit aorta, and provide some information about the molecular conformation of angiotensin required for its interaction with receptors.

The microsomal membranes were extracted from homogenates of intima media of rabbit aortae by differential centrifugation[7]. These membranes were considered to be essentially derived from smooth muscle cells because the contribution of intima was less than 1% total mass. Receptors were solubilised by suspending the 100,000*g* microsomal pellet in 50 mM Tris-HCl buffer, *p*H 8.0, containing 100 mM NaCl and deoxycholate (7-deoxycholic acid, Sigma; final concentration 0.2–1.0%). The membrane protein concentration[8] was 4.6 mg ml^{-1}. After a 30 min incubation at 0°C, the membrane suspensions were centrifuged for 1 h at 200,000*g* and the resulting soluble supernatants were assayed for angiotensin binding. After incubation of soluble material with ^{3}H-angiotensin II (specific activity 40 Ci mmol^{-1}), aliquots

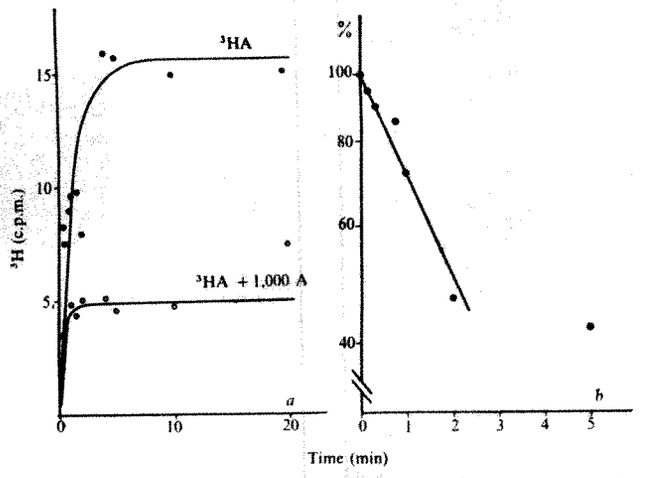


FIG. 1 *a*, Time course of binding of 2.5 × 10^{-8} M of ^{3}H-angiotensin II to solubilised material at 29° C in the absence (^{3}HA) and presence of 1000-fold excess of unlabelled angiotensin (^{3}HA + 1000 A) Results expressed as c.p.m. mg^{-1} (×10^3); *b*, displacement of bound radioactivity induced by addition of 1000-fold excess of unlabelled angiotensin to solubilised material after its incubation with 2.5 × 10^{-8} M of ^{3}H-angiotensin II for 5 min at 29° C. Results are expressed in % of bound radioactivity.

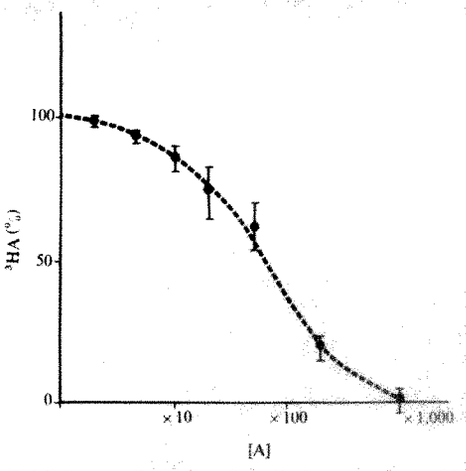


FIG. 2 Inhibitory effect of unlabelled angiotensin II (A) on the binding of 2.5 × 10^{-8} M of ^{3}H-angiotensin II to solubilised material. The various concentrations of unlabelled angiotensin II are indicated on the abscissa (10, 100 and 1000-fold excess compared to the ^{3}H-angiotensin II concentration). ^{3}H-angiotensin II and unlabelled angiotensin II were added simultaneously to the incubation mixture. The nonspecific binding (40% of bound radioactivity) was subtracted from each experimental value. ⧫, the mean and s.d. of 2–15 experiments.

(400 μl) of reaction mixture were placed on the surface of G-50 fine Sephadex columns (14.0 × 0.6 cm) equilibrated and run at 4°C with 50 mM Tris-HCl buffer *p*H 8.0 with or without deoxycholate. The eluates were added to scintillation fluid and counted in a liquid scintillation spectrometer. The validity of Sephadex for the separation of bound and free ^{3}H-angiotensin II was demonstrated in control experiments performed with buffer alone, showing that only 0.01% of ^{3}H-angiotensin II appeared in the void volume. Each experimental sample was corrected for the radioactivity appearing in the void volume in the control.

Treatment of microsomal membranes by deoxycholate solubilised 50% of membrane proteins and solubilisation was maximal at concentrations of deoxycholate greater than 0.2%. After treatment with deoxycholate 80% of the specific binding of ^{3}H-angiotensin II was recovered in the soluble supernatant and 20% in the pellet. The binding of ^{3}H-angiotensin to the solubilised material is proportional to the concentration of protein. Time course studies and concentration-dependent binding curves of ^{3}H-angiotensin II were performed in the presence and absence of 1000-fold excess of unlabelled angiotensin II. The specific binding is considered as the difference between the binding of ^{3}H-angiotensin II measured in the absence of unlabelled angiotensin.

The specific binding of ^{3}H-angiotensin II to the solubilised material reached equilibrium within 5 min at 29°C (Fig. 1). No significant specific binding was observed after 90 min of incubation at 4°C; in contrast the non specific binding was minimally affected by changes in temperature. The association rate constant at 29°C is $K_1 = 2.5 \times 10^5$ mol^{-1} s^{-1}. Dissociation of angiotensin–receptor complex was studied by adding 1000-fold excess native angiotensin to the samples preincubated for 5 min at 29°C with 2.5 × 10^{-8}M of ^{3}H-angiotensin II. The samples were then incubated for varying periods before being assayed for specific angiotensin binding. During the first 2 min dissociation of the ^{3}H-angiotensin binding was a first order kinetic reaction, the rate constant of which being $K_{-1} = 4.1 \times 10^{-3}$ s^{-1} (Fig. 1). A Scatchard plot[9] of the specific binding of ^{3}H-angiotensin II to the deoxycholate-solubilised proteins indicates that a single high affinity receptor site is apparently being measured with ^{3}H-angiotensin II concentrations varying from 2.5 × 10^{-9} M to 10^{-7} M. The number of specific binding sites is 2 pmol per mg of protein, and the apparent dissociation constant

TABLE 1 Inhibitory effect of angiotensin II, angiotensin analogues and fragments, and L-noradrenaline on ^{3}H-angiotensin II specific binding to deoxycholate-solubilised material

	Membranes	Deoxycholate-solubilised material
1 2 3 4 5 6 7 8 NH_2-Asp-Arg-Val-Tyr-Val-His-Pro-Phe-COOH	100	100
———————		45
———————		0
———————	81	100
———————	95	100
———————	39	30
———————	34	50
———————	4	35
——————Phe ——————	92	85
——————————Ile	43	90
——		
	0	0
L-noradrenaline	0	0

The concentrations of ^{3}H-angiotensin II and other drugs were 2.5×10^{-7}M and 5×10^{-7}M, respectively. Conditions of incubation and expressions of results are as in Fig. 2. For each compound, six determinations of ability to compete were made. The elucidation of the structure of angiotensin derivatives has been detailed[15,16]. Data obtained on membranes are from ref. 6.

calculated from the slope of the plot is K_d 29°C $\sim 2.0 \times 10^{-8}$ M. The specificity of the angiotensin binding to the solubilised material was assessed by displacement experiments shown in Fig. 2 and Table 1.

The membraneous hormone receptors which have already been solubilised and partially characterised are those for insulin in liver and fat cells[10], for catecholamines in myocardium[11] and turkey erythrocytes[12], and for acetylcholine in the electric tissue of fish[13]. That the angiotensin receptors of the rabbit aorta smooth muscle membranes were solubilised as described here is supported by several lines of evidence: first, the kinetic constants of ^{3}H-angiotensin II binding obtained in our previous studies on intact aorta and microsomal membranes are similar to those measured on solubilised material (Table 2). These kinetic constants are very close to the kinetic constants of the biological response elicited by angiotensin, that is contraction of intact aorta[4] and release of calcium from microsomes[5,14]. It seems therefore that the binding sites of angiotensin may be considered as the major elements of the receptors responsible for the induction of the biological response. The binding capacity of the solubilised material is lower than that previously measured on microsomal membranes (Table 2). This is partially explained by the dissociation of the angiotensin–receptor complexes which occurs during chromatography on Sephadex: the amount of bound radioactivity was reduced two-fold by an additional gel filtration. Second, only the specific binding of angiotensin to solubilised material was temperature dependent, indicating that the specific binding process possibly involves a variation of conformation of the ligand and/or the binder, and also indicates the possible participation of hydrophobic groups in the binding process.

Third, solubilised receptor–angiotensin interaction is specific; ^{3}H-angiotensin II binding was only inhibited by native angiotensin II and some angiotensin derivatives. As shown in Table 1 the 2–8 and 3–8 angiotensin fragments had an inhibitory effect on ^{3}H-angiotensin II binding identical to that of the unlabelled angiotensin II; the 1–7, 4–8, 5–8 and 6–8 fragments had a partial inhibitory effect; the 1–6 hexapeptide, which has no biological activity, had no effect. The absence of inhibitory effect to the 1–6 fragment is a strong argument for the specificity of the binding as it shows that the binding of angiotensin to the solubilised material is not related to a nonspecific interaction between some hydrophobic part of the angiotensin molecule and deoxycholate molecules which may remain associated to any of the solubilised membrane proteins.

These results agree with those previously observed on membranes[6]. Fermandjian *et al.*[15,16] have demonstrated that the organisation of the angiotensin molecule in concentrated aqueous solutions and polar organic solvents, such as trifluoroethanol, was that of a cross-β type defined by two turns involving the 3, 4, 5 and 6, 7, 8 amino acids respectively, which was illustrated in our previous publication[6]. The present results indicate that binding to receptor sites does not depend upon the N-terminal as long as the amino acids responsible for the two turns are present. These results also indicate that the C-terminal turn alone allows a partial binding. It seems therefore that the interaction of the peptide with its receptor site requires its organisation in a cross-β conformation which has been shown to stem essentially from the His-Pro sequence (D. Greff, S. F., P. F., M. C. Khosla, R. R. Smeby, and F. M. B., unpublished). These considerations illustrate the extreme similarities of the interactions existing between angiotensin and the solvents which have been used in the physical studies, and those occurring between angiotensin and receptor sites. This demonstrates the validity of the angiotensin model established for such solvents. In addition to the cross-β organisation the Phe^8 is of major importance in the binding process: binding is halved by its removal. The maintenance of full competitive activity with ^{3}H-angiotensin binding when Ile^8 replaces Phe^8 may be ex-

TABLE 2 Comparison of the kinetics of ^{3}H-angiotensin II binding to deoxycholate-solubilised receptor, microsomal membranes and intact aorta

	Aorta	Microsomal membranes	Deoxycholate-solubilised material
Specific binding capacity	0.8×10^{-14} mol mg^{-1}	3.5×10^{-11} mol mg^{-1}	2.0×10^{-12} mol mg^{-1}
Dissociation constant	1.3×10^{-8} M	5.5×10^{-8} M	*2.0×10^{-8} M †1.6×10^{-8} M
Biological response (K_m)	‡1.5×10^{-8} M	§2.3×10^{-9} M	

* From Scatchard plots
† From K_{-1}/K_1
‡ Contraction
§ Ca^{2+} release.
Results on aorta and microsomal membranes are from ref 4 and 5.

plained by the similar hydrophobic properties of both terminal amino acids. In solution, however, the intramolecular interactions promoted by Phe[8] and Ile[8] respectively are somewhat different as observed by circular dichroism (D. Greff, S. F., P. F., M. C. Khosla, R. R. Smeby, and F. M. B., unpublished) which underlines the delicate variations of angiotensin II conformation with the environment.

The comparison of the inhibitory effects of the various angiotensin derivatives on the ^{3}H-angiotensin II binding with their biological activities is difficult because only some of them were tested on isolated rabbit aorta[17]. The marked inhibitory effect on ^{3}H-angiotensin II binding of the competitive antagonist (Ile[8])-angiotensin II agrees with its high affinity for receptor sites of rabbit aorta[18]. The inhibitory effects on ^{3}H-angiotensin II binding of the 1–7, 2–8, 3–8 fragments and of the Phe[4] analogue was expected on the basis that they all have some intrinsic contractile activity. At least for these latter two peptides, whose activities were measured on rabbit aorta[19], it seems that their affinity to receptors as measured in our study is higher than that expected from their contractile effect. This was also observed on isolated membranes[6]. A discrepancy between the binding to receptor sites and the contractile response might be easily explained if the biological response is considered as a complex phenomenon depending both on a good adjustment of angiotension to receptor 'cavity' (which would be, as discussed above, ensured by its cross-β organisation) and the interaction of specialised groups of the molecule with membrane components which would trigger the biological response. This discrepancy might also indicate a great complexity of the receptor site. One might speculate that the binding involves more than one macromolecule and the part concentrated in this preparation is specific for the C-terminal sequence of angiotensin II. It is interesting in this respect to note that in the case of catecholamines the part of the molecule responsible for binding was clearly distinguished from that responsible for the activation of adenyl cyclase[20].

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1 Rasmussen, H., in *Protein and Polypeptide Hormones*, (edit. by Margoulies, M., and Greenwood, F. C., (*Excerpta Medica*, **1**, 194, 1971).
2 Page, I. H., and Helmer, O. M., *J. exp. Med.*, **71**, 29 (1940).
3 Morgat, J. L., Lam Thanh Hung, and Fromageot, P., *Biochim. biophys. Acta*, **207**, 374 (1970).
4 Baudouin, M., Meyer, P., and Worcel, M., *Biochem. biophys. Res. Commun.*, **42**, 434 (1971).
5 Baudouin, M., Meyer, P., Fermandjian, S., and Morgat, J. L., *Nature*, **235**, 336 (1972).
6 Devynck, M. A., Pernollet, M. G., Meyer, P., Fermandjian, S., and Fromageot, P., *Nature new Biol.*, **245**, 55 (1973).
7 Verity, A., and Bevan, J. A., *Biochem. Pharmac.*, **18**, 327 (1969).
8 Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, J. R., *J. biol. Chem.*, **193**, 265 (1951).
9 Scatchard, G., *Ann. N.Y. Acad. Sci.*, **51**, 660 (1949).
10 Quatrecasas, P., *J. biol. Chem.*, **247**, 1980 (1972).
11 Lefkowitz, R. J., Haber, E., and O'Hara, D., *Proc. natn. Acad. Sci., U.S.A.*, **69**, 2828 (1972).
12 Bilezikian, J. P., and Aurbach, G. D., *J. biol. Chem.*, **248**, 5584 (1973).
13 Meunier, J. C., Olsen, R. W., Menez, A., Fromageot, P., Boquet, P., and Changeux, J. P., *Biochemistry*, **11**, 1200 (1972).
14 Limas, C. J., and Cohn, J. N., *Nature new Biol.*, **245**, 53 (1973).
15 Fermandjian, S., Morgat, J. L., and Fromageot, P., *Eur. J. Biochem.*, **24**, 252 (1971).
16 Fermandjian, S., Fromageot, P., Titschenko, A. M., Leickman, J. P., and Lutz, M., *Eur. J. Biochem.*, **28**, 174 (1972).
17 Khosla, M. C., Smeby, R. R., and Bumpus, F. M., in *Handbook of Experimental Pharmacology* **37**, 126 (Springer Verlag, 1973).
18 Yamamoto, M., Turker, R. K., Khairallah, P. A., and Bumpus, F. M., *Eur. J. Pharmac.*, **18**, 316 (1972).
19 Papadimitriou, A., and Worcel, M., *Br. J. Pharmac.*, (in the press).
20 Bilezikian, J. P., and Aurbach, G. D., *J. biol. Chem.*, **248**, 5577 (1973).

Electrical coupling between afferent nerve terminal and intrafusal muscle fibre in the frog muscle spindle

FROM electronmicroscopic observations, Katz[1] has pointed out the existence of junctions between the afferent nerve terminal bulbs and the intrafusal muscle fibres in frog muscle spindle. It was suggested that the junction might play an important part in the transducing function of the receptor. Here we give physiological evidence for such a role in the simple-type spindle receptors.

Muscle spindles with intact afferent innervation were isolated from the sartorius muscles of Japanese frogs (*Rana nigromaculata*). The isolated spindle was placed at its *in situ* length in a pool of Ringer's solution (111.3 mM NaCl, 1.88 mM KCl, 1.08 mM $CaCl_2$ and 2.38 mM $NaHCO_3$) on a glass plate. The nerve fibre (2–3 cm) was passed into another Ringer's pool through a liquid paraffin pool. The two Ringer's pools were separated by an embankment of vaseline, which in turn was interrupted at its centre by the 1 mm wide paraffin pool. The pools of Ringer's solution were connected to calomel electrodes through Ringer-agar bridges. The potential between a microelectrode, used for recording from the spindle, and the calomel electrode of the same Ringer's pool, was led to a high input-impedance amplifier and displayed on one beam of a dual beam oscilloscope. The potential across the paraffin gap was fed into a high input-impedance differential amplifier and displayed on the other beam of the oscilloscope. Glass microelectrodes (resistance 20–50 MΩ, filled with 3 M KCl or 2 M NaCl) were manipulated under visual control, using an inverted microscope. Constant current stimuli (pulses of 0.05 ms duration or direct current) were given extracellularly to the axon terminal or intracellularly to the intrafusal muscle fibre.

The simple type spindles which were used had normal innervation: the parent axon divides into two myelinated branches; one of them again subdivides into two short myelinated branches, while the other does not[2]. Our experiments concern only the non-subdivided branch and the intrafusal muscle fibre innervated by it. Propagated and abortive spike discharges were defined, as usual[3,4], by recording across the paraffin gap.

Spontaneous spike discharges were recorded extracellularly by a microelectrode in contact with the surface of the nerve terminal. The amplitude was about 1 mV (negative). As the microelectrode was moved away from the nerve terminal in

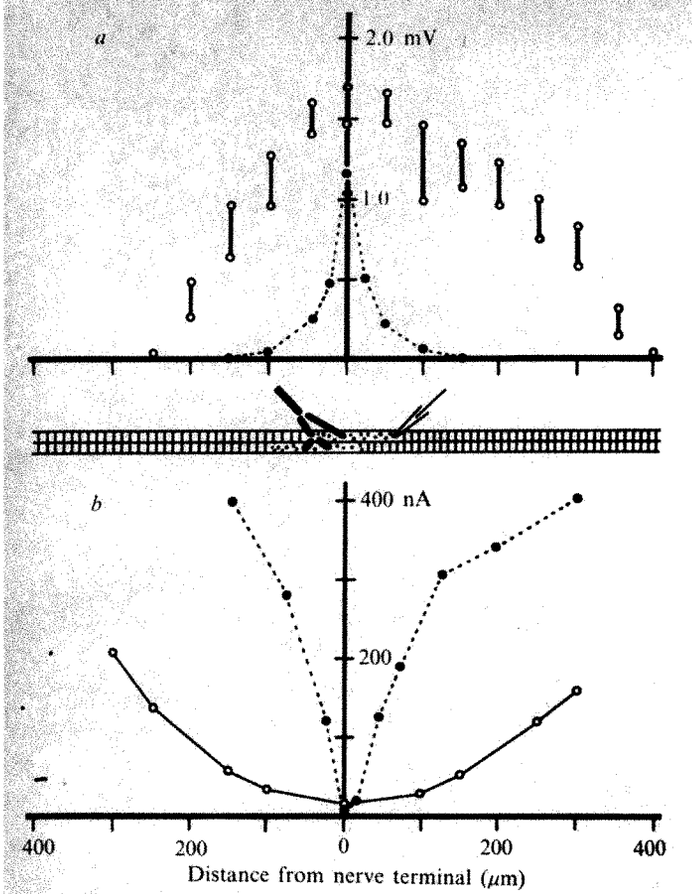


FIG. 1 Decrement of electrical events with increasing distance from nerve terminal. Glass microelectrodes for recording (*a*) and stimulating (*b*) were moved along the longitudinal axis of the intrafusal muscle fibre. ———, Intracellular results; – – – – –, extracellular results. Centre panel shows reticular area (stippled) and polar regions (striated), drawn to scale. *a*, Amplitude of fast potentials recorded from muscle, coincident with spontaneous propagated spikes. Microelectrodes were filled with 3 M KCl for intracellular recording and 2 M NaCl for extracellular recording. *b*, Current strength necessary to excite nerve terminal. Negative pulses were given extra- and intracellularly through glass microelectrodes. Efficacy of positive pulses (data not shown) was about the same.

either direction along the intrafusal muscle fibre, the spike amplitude decreased nearly exponentially, falling to 50% at a distance of 20 μm, and becoming undetectable at a distance of 150 μm from the terminal.

Insertion of the microelectrode into the reticular zone of the intrafusal muscle fibre revealed a resting potential of −50 to −60 mV, and negative spikes which were coincident with the propagated and abortive spikes. These spikes could be recorded intracellularly (solid vertical bars in Fig. 1*a*) at distances as great as 400 μm from the nerve terminal; by that point well-developed striations were present.

These results indicate the existence of a strong coupling between the axon and the intrafusal muscle fibre, allowing fast electrical changes to pass through easily. This coupling seems to have a capacitative component because the above mentioned spikes were transformed as if differentiated (data not shown). Such coupling was also indicated by the following experiments.

Electrical pulses were applied at various points along the surface of the muscle fibre through a microelectrode, and the current intensity necessary to excite the terminal was measured. The dotted line in Fig. 1*b* demonstrates that a negative current pulse of about 2 nA was required at the level of the nerve terminal, and that the current requirement increased steeply with lateral displacement of the stimulating electrode in either direction.

The same experiment was then conducted following penetration of the muscle fibre by the stimulating electrode (solid line in Fig. 1*b*). All stimuli were subthreshold for muscle twitching. When a negative pulse of 3 nA was applied at the level of the nerve terminal, a propagated spike response did occur along the parent axon. No muscle twitching was observed, of course. In sharp contrast to the extracellular stimulation, the current necessary to stimulate the nerve terminal increased only slightly over a longitudinal distance of 100 μm. Even at a distance of 300 μm from the terminal, a pulse of only 150 nA was still sufficient to excite the terminal. Electrical pulses of 100–200 nA, applied intracellularly to the polar region of the intrafusal muscle fibre, occasionally provoked muscle impulses and twitching.

Depolarisation of the intrafusal muscle fibre by means of current injected through an intracellular microelectrode increased the rate of spontaneous afferent discharge, and hyperpolarisation decreased it (Fig. 2). A quasilinear relationship between the current and the discharge rate was obtained in the range of +20 to −20 nA, during which no contraction could be observed. This suggests that the axon terminal may be coupled with the intrafusal muscle fibre by a relatively low resistance.

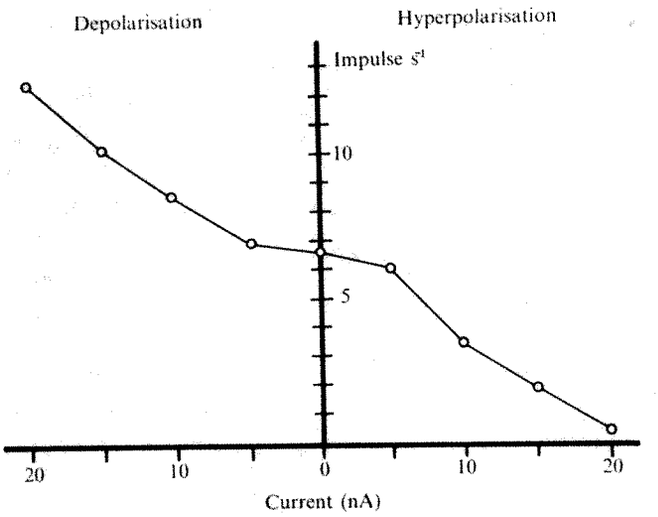


FIG. 2 Effect of direct current injection into muscle fibre upon propagated spike discharge rate of afferent nerve. The microelectrode was inserted into intrafusal muscle fibre 50 μm from nerve terminal.

In the muscle spindle, the afferent nerve terminal and the intrafusal muscle fibre may have electrical coupling through capacitance and resistance, arranged in parallel. Such an electrical coupling must contribute to the transmission of events from the axon terminal to the intrafusal muscle fibre and *vice versa*. It may play an important role in the transduction mechanism of the receptor. It is likely that the sensory nerve terminal is also activated by conducted muscle fibre impulses; it has been found that these 'early discharges' in turn precede the twitch of the intrafusal muscle fibre in mammalian spindles[5,6].

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[1] Katz, B., *Phil. Trans. R. Soc.*, **B243**, 221 (1961).
[2] Kuroda, H., and Ito, F., *Proc. Japan Acad.*, **48**, 206 (1972).
[3] Ito, F., *Jap. J. Physiol.*, **19**, 373 (1969).
[4] Ito, F., and Kanamori, N., *Proc. Japan Acad.*, **48**, 695 (1972).
[5] Hunt, C. C., and Kuffler, S. W., *J. Physiol. Lond.*, **113**, 298 (1951).
[6] Granit, R., Pompeiano, O., and Waltman, B., *J. Physiol. Lond.*, **147**, 399 (1959).

Mouse brain antigen detected by rat anti-C1300 antiserum

THE concept of the cell surface mediating specific cell–cell interactions is pertinent to neurogenesis in which the complex cell interactions known to occur may involve the recognition of specific cell surface components. Characterisation of neuronal cell surfaces is necessary to prove this idea and to elucidate functional mechanisms. Antisera raised against lymphoid cells have provided valuable information on the membrane constituents of lymphoid cells[1–3], and so I have used a similar approach to identify antigenic components expressed on cells of the nervous system of the mouse. The murine neuroblastoma C1300 can, in culture, give rise to cells which display many of the morphological, biochemical, and physiological properties characteristic of neurones[4–6]. Here, I present evidence indicating that rat antisera raised against C1300 tumour cells recognise a tissue antigen which is expressed predominantly on mouse brain.

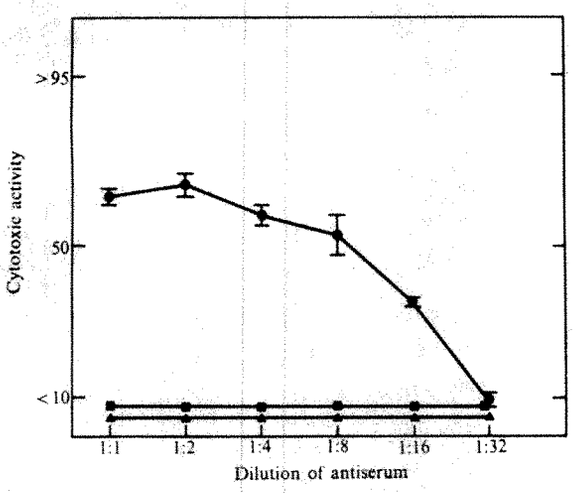


FIG. 1 Titration of rat anti-C1300 antiserum against C1300 tumour cells (●), lymph node cells (LNC) of A mice (■), or LNC of C57 mice (▲). To label the target cells, 0.5 ml of a cell suspension containing 5×10^7 cells ml^{-1} was incubated for 30 min at 37° C with 50 μCi of ^{51}Cr (Na_2CrO_4, Amersham) in medium containing 10% foetal calf serum (FCS). The cells were washed twice and resuspended to 3×10^6 cells ml^{-1} in medium containing FCS. A volume of 20 μl of cell suspension was incubated at room temperature for 30 min with 20 μl of various dilutions of antiserum. The cells were washed with 300 μl of medium and, after centrifugation at 800 r.p.m. for 7 min, the cells were resuspended in 50 μl of a 1:4 dilution of rabbit serum and incubated at room temperature for 30 min. Medium (150 μl) was added and the tubes centrifuged at 1,000 r.p.m. for 10 min. The radioactivity in 100 μl of supernatant from cells exposed to antibody and complement (ab) was measured and compared with that released from cells exposed to detergent (max) or complement alone (comp). Cytotoxic activity was calculated by the formula

$$(ab - comp)/(max - comp) \times 100.$$

In all experiments the absolute release of ^{51}Cr caused by complement was less than 10% of the maximum release. The antiserum tested at a 1:2 dilution in the absence of complement was not toxic. Duplicate or triplicate determinations were made in all cytotoxicity assays.

Although the plateau of maximum cytotoxic activity observed in the ^{51}Cr release cytotoxicity assay was 60% of the amount of ^{51}Cr released by detergent, trypan blue analysis showed that 90%–94% of viable ascites cells were killed by dilutions of antiserum up to 1:4.

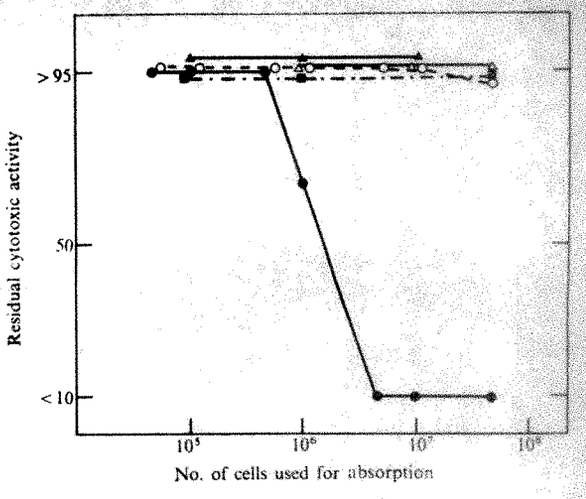


FIG. 2 Residual cytotoxic activity of rat anti-C1300 antiserum after absorption with C1300 ascites tumour cells (●——●), LNC of A mice (○- - -○), thymus cells of A (■-·-■), AKR (▲——▲), or 129 mice (△——△). Volumes (50 μl) of a 1:4 dilution of antiserum were incubated with varying numbers of cells for 30 min at room temperature. Volumes (20 μl) of antisera were assayed in duplicate as described in the legend to Fig. 1. The residual cytotoxic activity was calculated by the formula

(% lysis by absorbed antiserum/% lysis by unabsorbed antiserum) × 100.

The C1300 tumour is maintained in several laboratories by subcutaneous passage in strain A mice. For this study, the tumour was converted to an ascites form by repeated intraperitoneal passage of tumour cells into A_{sirong} mice, referred to subsequently as A mice. Two weeks after the intraperitoneal injection of 3×10^6 C1300 tumour cells, approximately 2×10^7 ascites cells could be obtained per mouse. The ascites cells used in these experiments were between 90% and 95% viable. Of the viable cells, more than 90% were C1300 tumour cells.

Antiserum was raised in adult Wistar rats by six intraperitoneal injections of 10^7 viable ascites tumour cells, at intervals of 14 days. The rats were bled 2 weeks after the last injection. Before absorption with mouse lymphoid cells the antiserum had a cytotoxic titre of 1:300 against both C1300 tumour cells and lymph node cells of A mice. The antiserum was absorbed five times for 30 min at room temperature with equal volumes of a mixture of spleen and thymus cells obtained from A, C3H/He, BALB/c, AKR, and C57BL (C57) mice. The absorbed antiserum was not cytotoxic for LNC of A or C57 mice. When tested against C1300 tumour cells, the antiserum had a cytotoxic titre of 1:16 (Fig. 1). In indirect immunofluorescence studies, using fluoresceinated sheep anti-rat immunoglobulin antibody, 93% of the viable ascites cells incubated with a 1:4 dilution of rat anti-C1300 antiserum demonstrated bright ring fluorescence. C1300 cells incubated with normal rat serum did not fluoresce. Less than 1% of LNC, thymus cells, or spleen cells of A mice were fluorescent after incubation with either rat anti-C1300 antiserum or normal rat serum. Aliquots of a 1:4 dilution of rat anti-C1300 antiserum were absorbed with varying numbers of C1300 ascites tumour cells, LNC of A mice or thymus cells of A, AKR, or 129 mice and tested for cytotoxic activity against C1300 tumour cells. A sample of 5×10^6 C1300 cells

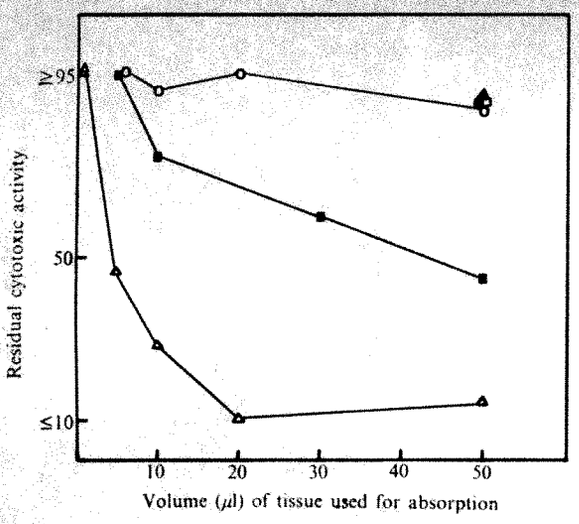


FIG. 3 Residual cytotoxic activity of rat anti-C1300 antiserum after absorption with various amounts of brain (△), kidney (■), liver (○), muscle (●), spleen (▲) and testes (□) of A mice. Volumes (50 μl) of a 1:4 dilution of antiserum were incubated with a measured volume of tissue at room temperature for 30 min. Volumes (20 μl) of absorbed antisera were assayed in duplicate as described in the legend to Fig. 1. The residual cytotoxic activity was calculated as described in the legend to Fig. 2.

removed all cytotoxic activity. No significant activity was removed by any of the other cell types (Fig. 2).

To investigate whether the antigen recognised by the rat anti-C1300 antiserum was a normal tissue antigen of A mice, aliquots of a 1:4 dilution of the antiserum were absorbed with various tissues of A mice and tested for residual cytotoxic activity against C1300 tumour cells. A putative anti-H-2^a antiserum raised in C57 mice by three intraperitoneal injections of 2 × 10^6 spleen cells of A mice was absorbed and tested in parallel. To control for dilution and possible non-specific absorption by the tissues, aliquots of the rat anti-C1300 and the C57 anti-A antisera were also absorbed with syngeneic rat tissues. The cytotoxic activity of the rat anti-C1300 antiserum was removed by brain and, to a lesser extent, by kidney of A mice. Brain tissue of A mice did not absorb appreciable amounts of antibody activity from the anti-H-2^a antiserum. Liver, lung, spleen, and testes of A mice, could remove cytotoxic activity from the anti-H-2^a antiserum, but did not remove a significant amount of cytotoxic activity from the rat anti-C1300 antiserum. Absorption with muscle from A mice did not significantly lower the cytotoxicity of either antiserum (Table 1). In further experiments, aliquots of antiserum absorbed at a 1:4 dilution with either brain, kidney, liver, muscle, or spleen were tested for residual cytotoxic activity at 1:4, 1:8, 1:16, and 1:32. Compared with Wistar rat tissues, only brain and kidney of A mice removed significant amounts of cytotoxic activity from the antiserum. To estimate the relative amounts of the antigen expressed on brain and kidney of A mice, 50 μl volumes of a 1:4 dilution of the rat anti-C1300 antiserum were absorbed with various amounts of these tissues. The results of these experiments indicated that there was at least 10 times as much of the antigen on brain as on kidney of A mice. Liver, muscle, spleen, and testes did not absorb appreciable amounts of the cytotoxic activity (Fig. 3). The antigen recognised by

TABLE 1 Residual cytotoxicity of absorbed antiserum

	Rat anti-C1300 antiserum		C57 anti-A antiserum	
Tissues used for absorption	A (mouse) tissues	Wistar (rat) tissues	A (mouse) tissues	C57 (mouse) tissues
Brain	8	83	88	NT
Kidney	45	78	35	77
Liver	86	84	10	77
Lung	84	NT	30	96
Muscle	88	87	80	89
Spleen	89	88	0	NT
Testes	88	NT	58	80

Residual cytotoxic activity of rat anti-C1300 and C57 anti-A antisera absorbed with various tissues. Organs were removed from exsanguinated animals and chopped into pieces of roughly 1 mm^3. The chopped tissues were pressed through a 333 mesh nylon net and the filtered suspensions washed four to seven times with medium containing 10% FCS. Diluted antiserum (50 μl) was absorbed with an equal volume of packed tissue for 30 min at room temperature. Volumes (20 μl) of absorbed antisera were tested as described in the legend to Fig. 1. The residual cytotoxic activity was calculated as described in the legend to Fig. 2. The titre of the C57 anti-A antiserum was 1:40 against both C1300 tumour cells and LNC of A mice. A 1:10 dilution of the antiserum was used in the absorption experiments. NT, not tested.

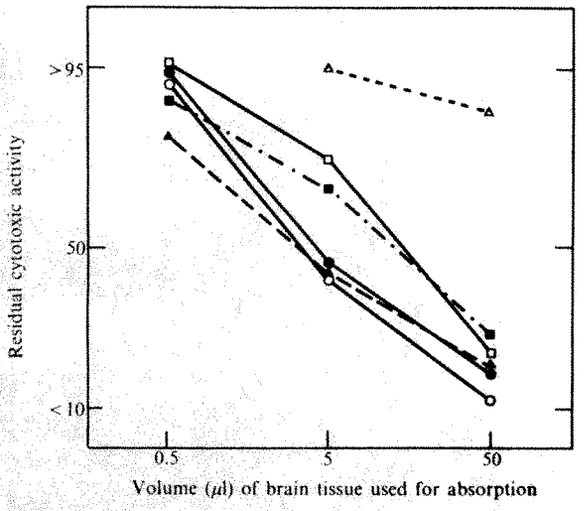


FIG. 4 Residual cytotoxic activity of rat anti-C1300 antiserum absorbed with various volumes of packed brain tissue of A (●——●), C57 (■-·-■), C3H/He (○——○), CBA (▲- - -▲), or DBA (□——□) mice or Wistar rats (△- - -△). The brain tissues were prepared as described in the legend to Table 1. Brains were removed from animals between 3 and 4 months of age. The residual cytotoxic activity was calculated as described in the legend to Fig. 2.

rat anti-C1300 antiserum was expressed in comparable amounts on the brains of A, C57, C3H/He, CBA, and DBA mice (Fig. 4).

C1300 tumour cells express several cell surface antigens present on brain as well as other tissues of certain mouse strains[10]. These antigens include H-2^a, the major histocompatability antigen of A mice; the θ-C3H alloantigen; Pc-1, the plasma cell alloantigen; GCSA, the Gross cell surface antigen; Sk-1, the skin alloantigen; and the NZB autoantigen. None of these alloantigens is expressed predominantly on nervous tissue or present on the nervous tissue of all strains of mice. The inability of LNC or thymus cells of A mice to absorb the cytotoxic activity of the rat anti-C1300 antiserum indicates that the antigen recognised by the antiserum is neither H-2^a, θ-C3H nor the NZB autoantigen. The inability of thymus cells of AKR or 129 mice to absorb the cytotoxic activity of the antiserum eliminates the possibility that the antigen is either GCSA or GIX[11], Gross virus-related antigens. The antigen detected by the rat anti-C1300 antiserum is not Pc-1 since Pc-1 is present on liver and spleen as well as brain and kidney of A mice. Nor is the antiserum directed against the serologically defined Sk-1 antigen expressed on the brain tissue of A mice since a different Sk antigen is expressed on the brain tissue of C57

mice. In addition, Sk has not been reported to be present on kidney tissue. Thus, the antigen recognised by the rat anti-C1300 antiserum is a normal tissue antigen of A mice present on brain and, to a lesser extent, on kidney, but not on liver, lung, muscle, spleen or testes and distinct from the alloantigens previously determined to be present on both C1300 tumour cells and A brain. In subsequent communications, this antigen will be referred to as mouse brain antigen-1 (MBA-1).

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1 Boyse, E. A., and Old, L. J., *A. Rev. Genet.*, **3**, 269 (1969).
2 Boyse, E. A., Miyazawa, M., Aoki, T., and Old, L. J., *Proc. R. Soc.* **B170**, 175 (1968).
3 Raff, M. C., Nase, S., and Mitchison, N. A., *Nature*, **230**, 50 (1971).
4 Amano, T., Richelson, E., and Nirenberg, M., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 258 (1972).
5 Harris, A. J., and Dennin, M. J., *Science*, **167**, 1253 (1970).
6 Harris, A. J., Heinemann, S., Schubert, D., and Tarikas, H., *Nature*, **231**, 296 (1971).
7 Blume, A. J., Gilbert, F., Wilson, S., Farber, J., Rosemberg, R., and Nirenberg, M., *Proc. natn. Acad. Sci. U.S.A.*, **67**, 786 (1970).
8 Nelson, P. G., Peacock, J. H., Amano, T., and Minna, J., *J. cell. Physiol.*, **77**, 337 (1971).
9 Schubert, D., Tarikas, H., Harris, A. J., and Heinemann, S., *Nature new Biol.*, **233**, 79 (1971).
10 Schachner, M., *Nature new Biol.*, **243**, 117 (1973).
11 Stockert, E., Old, L. J., and Boyse, E. A., *J. exp. Med.*, **133**, 1334 (1971).

Flagellar rotation and the mechanism of bacterial motility

BACTERIAL flagella are generally composed of three morphologically distinguishable regions: (*a*) the long flagellar filament which accounts for more than 95% of the flagellar protein; (*b*) the hook, which is generally 80–90 nm long and has a characteristic shape, and (*c*) the basal structure which is composed of an intricate set of disks and rods attaching the hook to the cell membrane and cell wall[1–3].

Explanations of how the flagella move[4] include the suggestion that helical waves are propagated along the filament[5,6], or that the filament behaves as a semrigid helical rotor[4,7,8]. Berg and Anderson[9] concluded that the evidence ". . . favours a model in which each filament rotates". The data presented here adds strong support to the existing evidence and provides a way to follow flagellar function that is independent of translational motility.

The methods that have been used to study motility depend on observation of the behaviour of the whole organism. This is a complex result of a series of events and is thus, at times, difficult to analyse and interpret. It would be useful to be able to follow the motion of a single flagellum or to assay for its activity in a way that does not depend on chemataxis or cell motility. To do this, a series of experiments was designed based on the observation that bacteria can be found which appear to be 'tethered' by their flagellum to a particle of debris or to the surface of the microscope slide[7]. These bacteria rotate rapidly. The rotation could reflect the motion of the tethered flagellum or it could result because the remaining untethered flagella cause the cell to move. To examine this motion in greater detail, we used 'polyhook' mutants of *E. coli*[10], which carry two mutations, one in the *hag* gene which eliminates the formation of the flagellar filament and another in the *flaE* gene which causes the loss of a function necessary to terminate the assembly of the hook structure. These cells make continuous polyhooks which can be 1–2 μm long and they are nonmotile. However, when dilute anti-polyhook antibody is added to a suspension of cells, they form clumps and begin to rotate rapidly. Several situations have been observed: the bacteria seem to be trapped or attached to the slide; the bacteria are bound to each other in pairs and counter-rotate or one rotates while the other is stationary; large groups of bacteria are cross linked and rotate or move with a jerking type of motion. Ten to twenty per cent of the bacteria in a microscope field can be found to be rotating. Figures 1 and 2 illustrate the first two situations. In Fig. 1, the cell seems to be attached to the surface of the microscope slide. It rotates 360° in a counterclockwise direction around an axis through one end of the cell body. The speed of rotation of an individual cell varies from two to nine revolutions per second. The cells can be observed to modulate their spinning in three ways: (*a*) by continuing to spin counterclockwise; (*b*) by stopping and then restarting in the same direction, or (*c*) by changing direction and spinning clockwise. The cells generally spin

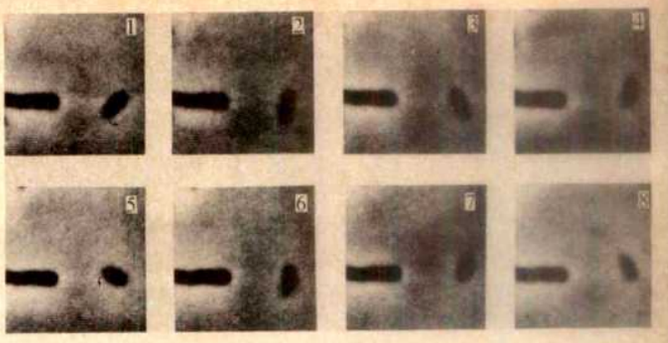


FIG. 1 The rotation of a cell bound to the microscope slide. *E. coli* strain MS1381 was grown on minimal medium with glycerol as the carbon source. Cells were put on a microscope slide and an equal volume of antipolyhook antibody diluted to 1:200 was added. After a 10 m lag, the cells began to spin. Their behaviour was recorded through a Zeiss phase contrast microscope onto video tape and then transferred to film. The pictures presented represent frames taken at intervals of 1/12 of a second. The cell at the left did not move during the sequence and it provides a reference point.

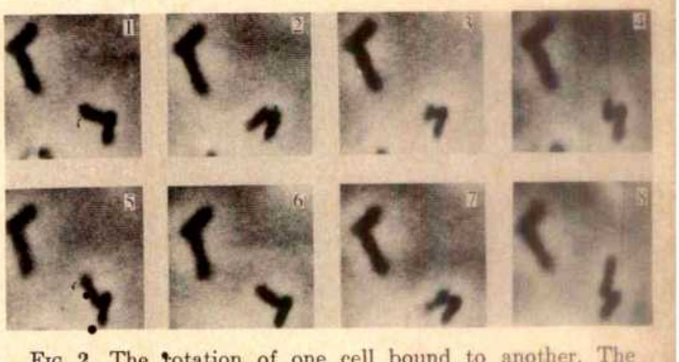


FIG. 2 The rotation of one cell bound to another. The preparation was the same as that described in the legend to Fig. 1. The cells in the upper left corner serve as reference.

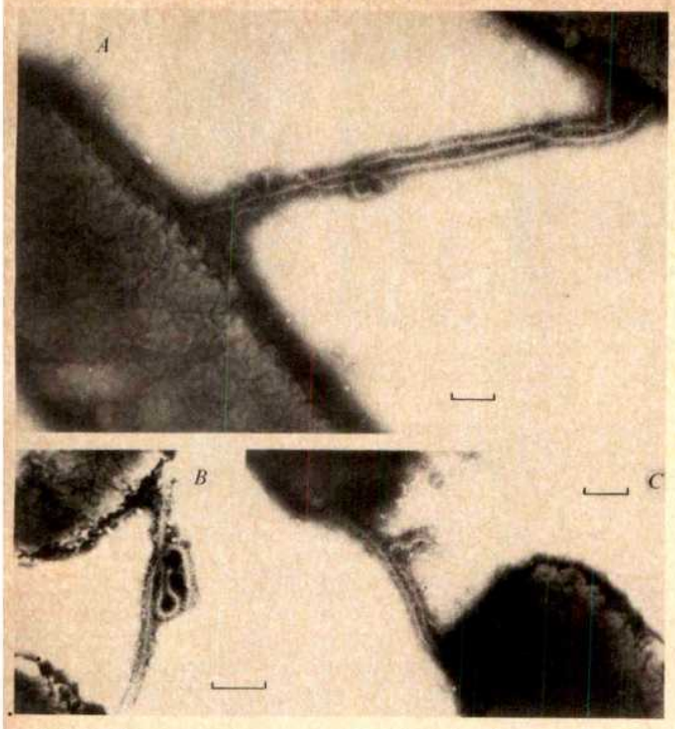


FIG. 3 Electron microscopy of cells bound together via their polyhooks. The cells were prepared as described in Fig. 1. They were placed on copper grids covered with carbon coated formover and stained with 0.5% phosphotungstic acid. The polyhook structure generally appears to be helical with a diameter of about 16 nm (ref. 10). These, however, are straightened and thickened as a result of the antibody molecules bound to them. The line in the photograph represents 0.2 nm. *A*, two cells held together by antibody bound to three polyhooks, *B* and *C*, cells held together by antibody bound to polyhooks derived from each of them.

counterclockwise and occasionally stop or reverse direction for one or two turns and then resume spinning counterclockwise. Individual cells tethered in this way have been observed to spin with intermittent short pauses for more than 30 m. Figure 2 illustrates the motion observed with a pair of tethered bacteria. Again the observations are most consistently interpreted as resulting from the complete rotation of one of the cells about a point of attachment to the other cell. The attachment appears as a narrow gap between the cells (Fig. 2, frame 4 and frame 8) which could correspond to the polyhook-antibody complex.

Thus, it is clear that the cells can rotate. However, it is possible that they rotate around the point of attachment, using the antibody as a swivel rather than rotating around the point of insertion of the basal structure into the cell body. Figure 3 shows electron micrographs of the tethered cells. It is clear that the polyhook structures are coated with antibody molecules, they bind the structures together at many points. The polyhooks have lost some of their characteristic helical appearance and are straightened, suggesting that they have some structural flexibility. The multiple attachment points along the surface of the polyhook appear to stitch them together and make it seem highly unlikely that the cells could rotate around the attachment site.

While these experiments were all done using the polyhook mutant, similar experiments were possible with bacteria that had flagellar filaments. *E. coli* strain W3110 which we used carries a mutation in the *hag* gene that results in the synthesis of straight flagellar filaments lacking the characteristic helical appearance. The cells are nonmotile. Nonetheless, indirect evidence suggests that the flagella are active[11]. To decrease the number of flagella per cell so that they would not tangle and crosslink, the bacteria were grown in minimal medium with glucose as a carbon source[12,13]. When dilute antiflagellar filament antibody was added to these cells they were tethered to the glass and to each other. They began to rotate in the same manner as the polyhook mutants. Thus, even with the straight flagellar filament the cell rotation is observed.

All these observations are concerned with the rotation of the cell, and the rotation of the filament is inferred. To observe the activity of the filaments themselves, we used small latex beads 0.7 μm in diameter (Dow), which were incubated with a 1:1,000 dilution of anti-flagellar antibody and then washed in 0.01 M Tris buffer, *p*H 7.8, and 0.1 M sodium chloride. They were added to suspensions of *E. coli* W3110 that had been grown in minimal medium with glucose. Beads were found attached 1–2 μm from the bacterium and they rotated very rapidly. In the same way, beads could be attached to flagella on wild-type bacteria and these were again observed to rotate rapidly, trailing behind motile bacteria.

All these observations, taken together, can be explained best if the hook is driven in a rotary fashion, probably by a mechanism anchored to the cell body at the base of the flagellum. This results in the rotation of the flagellar filament. Furthermore, the cell has the capacity to vary the speed and direction of rotation as well as the frequency of stopping and restarting. The ability to modulate the rotation of flagella may be the basis for the mechanism of chemotaxis.

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1 Dimmitt, K., and Simon, M., *J. Bact.*, **105**, 369–375 (1971).
2 Depamphilis, M., and Adler, J., *J. Bact.*, **105**, 376–383 (1971).
3 Abram, D., Koffler, H., and Vatter, A., *J. Bact.*, **90**, 1337–1354 (1965).
4 Doetsch, R. N., and Hageage, G. F., *Biol. Rev.*, **43**, 317–362 (1968).
5 Doetsch, R. N., *J. theor. Biol.*, **11**, 411–421 (1966).
6 Klug, A., *Symp. int. Soc. Cell Biol.*, **6**, 18–39 (1967).
7 Stocker, B. A. D., *Symp. Soc gen. Microbiol.*, **6**, 19–40 (1956).
8 Iino, T., *Behaviour of Micro-organisms*, Proc. Tenth int. Cong. Microbiol., Mexico City, 1970 (edit. by Pérez-Miravete, A.), 205–213 (Plenum, London, New York, 1973).
9 Berg, H., and Anderson, R., *Nature*, **245**, 380–383 (1973).
10 Silverman, M., and Simon, M. I., *J. Bact.*, **112**, 986–993 (1972).
11 Raimondo, L. M., Lundh, N. P., and Martinez, R. J., *J. Virol.*, **2**, 256–264 (1968).
12 Adler, J., and Tempelton, B., *J. gen. Microbiol.*, **46**, 175–184 (1967).
13 Yokota, T., and Gots, J., *J. Bact.*, **103**, 513–516 (1970).

Change in direction of flagellar rotation is the basis of the chemotactic response in *Escherichia coli*

BERG and Anderson[1] recently argued from existing evidence that bacteria swim by rotation of their helical flagella. Silverman and Simon[2] have now provided a clear demonstration of this. By means of antibodies specific for flagellar components, they tethered cells to microscope slides or to each other and observed rotation of the cell bodies. The cells were able to stop and to rotate in either direction. It seemed possible, as they proposed[2], that cessation or reversal of flagellar rotation might be involved in bacterial chemotaxis. Accordingly, we used wild-type and chemotaxis-defective mutant cells of

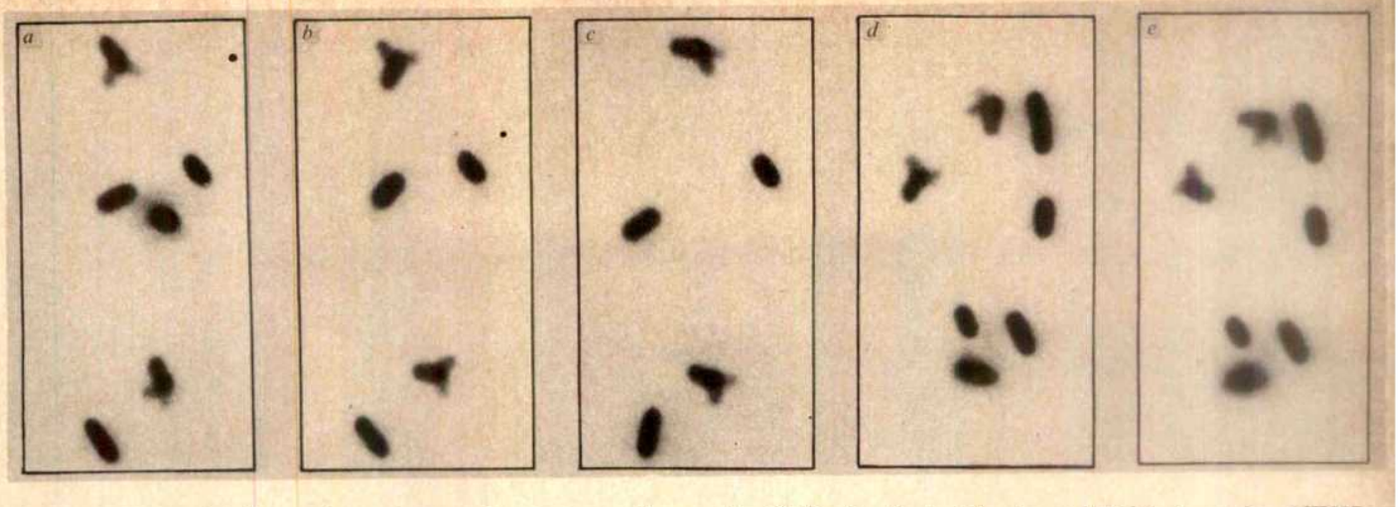


FIG. 1 Tethered cells rotating and responding to temporal chemical gradients. Rotating cells were adhered to glass slides by antibody-mediated fixation of a single flagellar filament. Bacteria were grown in glucose to reduce the number of flagella per cell[2,9,10]. (About 30% of cells had flagella with an average number of 1.5 per cell compound with 4.3 for glycerol-grown cells as counted by electron microscopy.) This was necessary for reproducible results, presumably because additional flagella could tangle and crosslink[2], or cause additional torque on the cells. Bacteria, grown in liquid glucose minimal medium[4] to logarithmic phase, were washed twice by centrifugation in chemotaxis medium (10 mM potassium phosphate, 0.1 mM potassium EDTA, *p*H 7.0), and resuspended in the same. A sample of bacterial suspension was mixed with 0.003 volumes of anti-filament antibody in chemotaxis medium (an amount just sufficient to produce rotation of cells), and then immediately placed on a glass microscope slide. After 2 min untethered cells were rinsed off with chemotaxis medium to improve the dark-field image of rotating cells. Addition of antibody eliminated the small proportion of free swimming cells; furthermore, less than 5% as many rotating cells were found in the absence of antibody as in its presence, and these readily washed away. A 5μl sample of chemotactic agent in chemotaxis medium was then added to the approximately 50 μl drop remaining over the tethered cells, using Drummond microcapillary pipettes. Photomicrographs were taken with three exposures, each of two-fold decreasing duration 1/20 s apart, generated by three slits of decreasing width in a rotating disk in the light beam. Negative prints of dark-field images are shown. Therefore, to determine directions of rotation, follow cell bodies from darkest, to intermediate, to most faint exposure. The same field of wild type (AW405) is shown with (*a*) no addition, (*b*) addition of 0.15 M L-leucine, and (*c*) subsequent addition of 0.1 mM L-serine. The same field of the tumbling mutant AW620 is shown with (*d*) no addition, and (*e*) addition of 0.1 mM L-serine. (Approximate magnification is ×2,600.)

Escherichia coli tethered to microscope slides in a manner similar to that of Silverman and Simon[2], and stimulated them by sudden increases of chemotactic agents. We found that changes in the direction of flagellar rotation indeed constitute the basis of chemotaxis: addition of attractants causes counter clockwise (CCW) rotation, whereas repellents cause clockwise (CW) rotation.

Historically bacterial chemotaxis has been identified[3] and measured[4] using an assay in which bacteria swim into a capillary tube containing attractant. More recently, chemotaxis has been studied by analysing the frequency of abrupt changes in direction of swimming (tumbles) of individual cells of *E. coli*[5,6] and *Salmonella typhimurium*[7,8] when they are presented with chemical gradients. Each tumble results in a new randomly chosen swimming direction[5] such that variation of the frequency of tumbles causes a net migration of cells during a spatial chemotactic response. In this report we conclude that tumbles are caused by CW flagellar rotation whereas smooth swimming is caused by CCW rotation.

In the absence of added attractants or repellents, rotation of tethered wild-type cells as viewed from above is biased to the CCW direction[2] (Fig. 1*a* and Table 1, strains AW607, AW405 and AW574). CCW rotation of the cell body corresponds to CCW rotation of the flagellum if viewed along the helix axis toward the point of insertion in the cell.

Changes in the direction of flagellar rotation are shown to be involved in chemotaxis by the following evidence. (1) Repellent additions (L-leucine) cause CW rotation of wild-type cells (Fig. 1*b* and Table 1). Note that mutants unable to respond to L-leucine in conventional tests for chemotaxis (AW608 and AW518) show no significant change. (2) Attractant additions (L-serine or L-aspartate) cause CCW rotation of wild type cells, and can even reverse CW rotation initiated by previous L-leucine addition (Table 1 and Fig. 1*a*, *b* and *c*). However, mutants with defective responses to L-serine or L-aspartate (AW518 and AW539 respectively)[11] show no response to a corresponding chemotactic stimulus (Table 1). These mutants respond normally to other chemicals (data not shown). (3) The generally non-chemotactic mutants (unable to respond to any known chemicals)[12,13], *cheA*593, *cheB*590, and *cheC*497, rotate only in the CCW direction, even following stimulation by L-leucine (Table 1). (4) Mutants which tumble incessantly when suspended in chemotaxis medium (for example, AW620), almost always rotate CW (Fig. 1*d* and Table 1). This tumbling mutant is capable of a subnormal but significant response to L-serine in a capillary assay (data not shown), and tethered cells reverse to predominantly CCW rotation when stimulated with L-serine (Fig. 1*e* and Table 1). (5) By adding chemotaxis medium to a drop containing tethered cells (AW405), we found that a forty-fold dilution of L-serine (from 20 μM) reversed the cells from CCW to predominantly CW rotation for 1–2 min. A similar dilution from 20 mM L-leucine immediately reversed their previous L-leucine-stimulated CW rotation. Thus decreasing temporal gradients have the opposite effect on rotation to that of increasing gradients.

The following experiments (data not shown) were performed to substantiate the above evidence. (1) Chemotactically inert agents (chemotaxis medium, 20 mM glycerol, 20 mM D-gluconate or 20 mM DL-lactate)[11,14] had no effect on the direction of rotation. (2) Other attractants (1 mM D-glucose, 20 mM glycine or 20 mM L-methionine)[11,14] also cause CCW rotation. (3) Other repellents (20 mM L-isoleucine, 20 mM L-phenylalanine or 0.4 mM potassium salicylate[14a] caused predominantly CW rotation. (4) Non-metabolisable attractants (20 mM α-aminoisobutyrate or 2 mM DL-α-methylaspartate)[11] elicited CCW rotation. Hence, availability of energy or metabolism of chemotactic agents is neither involved in chemotaxis[11,15] nor in the rotational changes we observe. (5) The effects with both attractants (even the non-metabolisable analogues) and repellents were observed to decay to the original state of rotation at times ranging from one-half to several minutes after the stimulus. Thus the effects are due to temporal stimulation and not simply

TABLE 1 Rotation of tethered wild-type and taxis-defective cells before and after chemotactic stimulation

Line No.	Strains* Parent	Strains* Mutant	Pertinent taxis properties	Stimulus, final concentration†	No. of cells timed	% of time in CCW rotation‡	Fisher t test of means Line No. compared with:	P
1	AW607§		Wild type	None	10	88.2 ± 15.6		
2				30 mM L-leucine	11	0.2 ± 0.6	1	<0.001
3		AW608§	Leucine	None	7	94.6 ± 6.5	1	0.3
4			taxis mutant	30 mM L-leucine	8	93.2 ± 8.9	3	0.7
5	AW405[12]		Wild type	None	30	73.0 ± 20.0		
6				3 mM L-leucine	11	20.6 ± 14.8	5	<0.001
7				30 mM L-leucine	9	3.8 ± 5.7	5	<0.001
8				20 μM L-serine	8	100.0 ± 0.0	5	<0.001
9				2 μM L-aspartate	17	100.0 ± 0.0	5	<0.001
10		AW518[11]	Deficient in serine and	None	14	72.6 ± 29.4	5	>0.9
11			leucine taxis§	3 mM L-leucine	8	65.6 ± 26.2	10	0.6
12				20 μM L-serine	7	78.3 ± 8.8	10	0.6
13		AW539[11]	Defective in aspartate	None	11	87.9 ± 9.4	5	0.03
14			taxis	2 μM L-aspartate	9	85.3 ± 11.2	13	0.6
15		*cheA*593[13]	Generally non-	None	18	100.0 ± 0.0	5	<0.001
16			chemotactic	30 mM L-leucine	14	100.0 ± 0.0		
17		*cheB*590[13]	Generally non-	None	29	100.0 ± 0.0	5	<0.001
18			chemotactic	30 mM L-leucine	8	100.0 ± 0.0		
19		*cheC*497[13]	Generally non-	None	10	100.0 ± 0.0	5	<0.001
20			chemotactic	30 mM L-leucine	6	100.0 ± 0.0		
21	AW574¶		Wild type	None	23	88.3 ± 9.9		
22				20 μM L-serine	11	100.0 ± 0.0	21	<0.001
23		AW620	Tumbling motility	None	19	2.3 ± 3.6	21	<0.001
24				20 μM L-serine	10	100.0 ± 0.0	23	<0.001

Cells were grown and prepared for observation as described in Fig. 1, except that 'untethered' cells were not rinsed from the slide. Rinsing is recommended when increased reproducibility is required. Occasionally, a cell would appear to become stuck, or to change its centre of rotation during timing. Data from such cells were not included.

* All strains are derivatives of *E. coli* K12.

† Five or 10 μl of stimulant in chemotaxis medium were added to 20 μl drops covering tethered cells by using Drummond microcapillary pipettes.

‡ Each cell was observed for 30 s beginning immediately after stimulation. Times were determined manually with one stop clock for CW and another for CCW rotation. The infrequently observed short periods of no rotation were not included in the calculations.

§ See ref. 14a

¶ A *gal*$^+$, *su*$^-$ derivative of AW405.

to presence of the chemical. (6) Rotational responses of several strains before and after stimulation were confirmed by high speed microcinematography. (7) Tethered cells of the polyhook mutant[2] showed rotational responses to stimulation identical to wild-type cells. (8) Tethered cells of a paralysed mutant (flagellated but non-motile)[13] did not rotate even in response to attractants or repellents.

From the above results we conclude that changes in direction of flagellar rotation are the basis of both positive (toward attractants) and negative (away from repellents) chemotactic responses.

As mentioned above, chemotaxis occurs by means of variation of the tumbling frequency. Increasing attractant concentrations (whether encountered as temporal stimuli, or while swimming in spatial gradients) cause cells to tumble less frequently[5–8], while increasing repellent concentrations cause more tumbling[8]. On the other hand, decreasing concentrations cause the opposite effect (more tumbling for attractants and less tumbling for repellents)[7,8]. What, then, is the relationship between direction of flagellar rotation and tumbles?

The generally non-chemotactic mutants swim smoothly with virtually no tumbles[10] and, when tethered, never rotate in the CW direction (Table 1). On the other hand, mutants which tumble continually rotate their filaments in the CW direction most of the time (Table 1). Furthermore, these mutants swim smoothly and rotate their filaments predominantly in the CCW direction only when stimulated by attractants. From these observations, as well as the effects of attractants and repellents on rotation of wild-type cells (Fig. 1*a*, *b* and *c*, and Table 1), we conclude that tumbling occurs as a result of CW rotation of flagella.

Peritrichously flagellated bacteria swim by coordination of several helical filaments in a bundle behind the cell[16]. Bacteria with predominantly smooth swimming (wild-type, generally non-chemotactic mutants, and responsive strains when attractant was added) all rotate their filaments predominantly in the CCW direction. Conversely, bacteria induced to tumble rotate their filaments CW. We therefore conclude that to obtain the thrust required for smooth swimming from CCW rotating filaments in a bundle, the helical sense of the filaments of *E. coli* must be left-handed. It is significant that the helix of filaments of *Proteus vulgaris*, another enteric Gram negative peritrichously flagellated bacterium, is also left-handed[17,18].

Although it is possible that cessation of rotation or differential rates of CCW rotation of filaments could cause a tumble, we believe that CW rotation is the most likely, cause, because tethered cells of tumbling mutants rarely stop rotating and seldom rotate CCW; whereas, when not tethered, they tumble very frequently. The same can also be said for cells responding to repellents. Thus cessation of rotation does not correlate with tumbling. Although we have no evidence against involvement of differential rates of flagellar rotation in tumbling, it is not necessary to invoke them in the mechanism of chemotaxis. Clockwise rotation of one or more left-handed helical filaments can lead to tangling or, more probably, dissociation of flagellar bundles, and thereby generate tumbles.

Several interesting questions arise now that it is known that flagella can rotate in either direction[2] and that this ability is basic to chemotactic responses. As proposed by Berg and Anderson[1], the morphology of the flagellar basal complex and its association with the cell envelope[19,20] is suggestive of a cylindrically symmetrical rotary 'motor'. Are there separate motors for CCW and CW rotation, or does a single motor reverse? Do the motors rotate by step-wise molecular interactions, or as 'turbines' driven by ion fluxes? How do chemotactic stimuli, acting through chemoreceptors[15], control the direction of rotation? Do chemoreceptors communicate only with a nearby flagellum, or simultaneously with all flagella?

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1 Berg, H. C., and Anderson, R. A., *Nature*, **245**, 380–382 (1973).
2 Silverman, M. R., and Simon, M. I., *Nature*, **249**, 73–74 (1974).
3 Pfeffer, W., *Untersuch. Bot. Inst. Tübingen*, **1**, 363–482 (1884); *ibid.*, **2**, 582–661 (1888).
4 Adler, J., *J. gen. Microbiol.*, **74**, 77–91 (1973).
5 Berg, H. C., and Brown, D. A., *Nature*, **239**, 500–504 (1972).
6 Brown, D. A., and Berg, H. C., *Proc. natn. Acad. Sci. U.S.A.* (in the press).
7 Macnab, R. M., and Koshland, jr., D. E., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2509–2512 (1972).
8 Tsang, N., Macnab, R., and Koshland, jr., D. E., *Science*, **181**, 60–63 (1973).
9 Adler, J., and Templeton, B., *J. gen. Microbiol.*, **46**, 175–184 (1967).
10 Yokota, T., and Gots, J. S., *J. Bact.*, **103**, 513–516 (1970).
11 Mesibov, R., and Adler, J., *J. Bact.*, **112**, 315–326 (1972).
12 Armstrong, J. B., Adler, J., and Dahl, M. M., *J. Bact.*, **93**, 390–398 (1967).
13 Armstrong, J. B., and Adler, J., *Genetics*, **61**, 61–66 (1969).
14 Adler, J., Hazelbauer, G. L., and Dahl, M. M., *J. Bact.*, **115**, 824–847 (1973).
14a Tso, W-W., and Adler, J., *J. Bact.* (in the press).
15 Adler, J. *Science*, **166**, 1588–1597 (1969).
16 Weibull, C., *The Bacteria*, I (edit. by Gunsalus, I. C., and Stanier, R. Y.), 153–205 (Academic Press, New York and London, 1962).
17 Weibull, C., *Ark. Kemi*, **1**, 573–575 (1949).
18 Astbury, W. T., Beighton, E., and Weibull, C., *Symp. Soc. expl. Biol.*, **9**, 282–305 (1955).
19 Dimmitt, K., and Simon, M., *J. Bact.*, **105**, 369–375 (1971).
20 DePamphilis, M. L., and Adler, J., *J. Bact.*, **105**, 396–407 (1971).

Dynamic properties of bacterial flagellar motors

TETHERED bacteria[1] rotate at the angular velocity at which the torque generated by the flagellar motor[2] is balanced by the torque due to the viscous drag. In general, $M = b\eta\Omega$, where M is the torque, η is the viscosity, Ω is the angular velocity, and b is a coefficient which depends on the size and the shape of the cell, the position of the axis of rotation, and the distance between the cell and the wall. For a sphere of radius a (not too close to the wall) $M = 8\pi\eta a^3\Omega$ (ref. 3). Viscous forces are so large in comparison with inertial forces[4] that Ω will change with M virtually instantaneously; any discontinuities in the one will be evident in the other. Consider a cell of radius a and uniform density ρ rotating at an angular velocity Ω_0; if its motor is suddenly disengaged, Ω will decay exponentially to 0 with a time constant $\rho a^2/15\eta$, and the cell will stop in $\Omega_0\rho a^2/15\eta$ radians. For *Escherichia coli* this is less than a millionth of a revolution. The cell also is subject to rotational diffusion, but this will be evident only if the coupling between the flagellum and the body of the cell is fluid. The root-mean-square deviation in the angular position is $(2Dt)^{1/2}$, where D is the rotational diffusion constant and t is the time. For a cell which can rotate freely, $D = kT/b\eta$, where k is Boltzmann's constant and T is the absolute temperature.

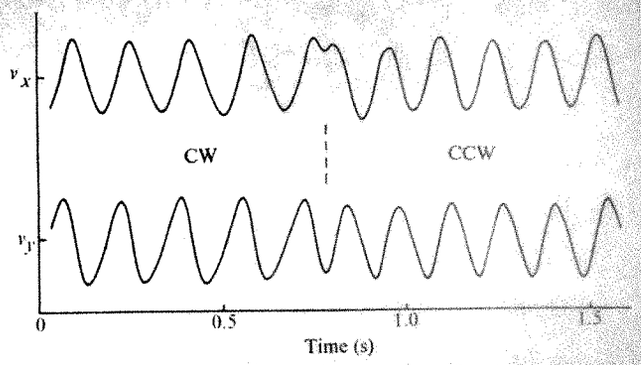


FIG. 1 The rotation of a polyhook mutant bound to the top window of the tracking chamber. The cell rotated clockwise (CW) at 6.2 r.p.s. and then counterclockwise (CCW) at 7.2 r.p.s. (The x velocity lags the y velocity for CW rotation but leads it for CCW rotation; the phase shift is 90°.) *E. coli* strain MS1381 was grown on minimal medium containing glycerol as described by Silverman and Simon[6], washed twice with a solution containing 10^{-2} M potassium phosphate (pH 7.0) and 10^{-4} M ethylenediamine tetraacetate, and suspended in a similar medium containing 0.067 M sodium chloride (10^8 cells ml^{-1}). Antipolyhook antibody was added to a dilution of 1:20,000, and the window was immersed in this mixture. It was removed 20 min later and inverted onto the top of a tracking chamber (filled with the suspension medium). The cells were tracked at 32°C. The microscope readout voltages (ref. 5, Figs 1 and 5) were amplified, differentiated, and displayed on a strip-chart recorder. The frequency response of this system was checked with sine waves of amplitude 0.05 and frequency 20 times that of the signals which generated the recording shown in the figure; the test signals were readily detectable. Note that the signals generated by the rotation of the cell are completely smooth.

The rotation of *E. coli* can be monitored with the tracking microscope[5], which follows the point on the surface of the cell closest to the centre of the laboratory reference frame. If the cell rotates counterclockwise ($\Omega > 0$), the transducer traces out a counterclockwise path. If the cell is seen from the side and is tethered near one end, the x and y velocities of the tracker vary with time roughly as $\Omega\sin\Omega t$ and $-\Omega\cos\Omega t$ (Figs 1 and 2). The exact shape of the waveform depends on the size and the shape of the cell and on the position of the rotation axis.

The motion can be remarkably smooth. If the motor makes one revolution in n discrete steps, I conclude from data of the kind shown in Fig. 1 (for cells rotating as slowly as 4 r.p.s.) that $n \gtrsim 25$. This probably rules out the mechanism envisaged earlier[2], in which a cycle is executed when each of three (or more) cross bridges takes nine steps. This mechanism could explain the data only if the motions of the different cross bridges were highly synchronised. If the response time of the tracking and recording system is τ, then the root-mean-square deviation in Ω due to rotational diffusion should be of order $(2D/\tau)^{1/2}$. Under the conditions of the experiment ($\tau \simeq 10^{-2}$ s), the value of the deviation expected for a freely rotating object the size of *E. coli* is about 6 s^{-1}. The data are much smoother than this (Fig. 1), so the coupling between the flagellum and the body of the cell is not fluid.

Changes in the direction of rotation[1] occur smoothly, yet abruptly (Figs 1 and 2), and they occur at different points in the cycle (Fig. 2 and data obtained with the polyhook mutant, not shown). The data at hand are consistent with a model in which the reversals occur at random[9]. The angular velocities for clockwise and counterclockwise rotation are generally different (Figs 1 and 2), but this does not necessarily mean that the dirve is asymmetric. When the motor reverses, the torque applied to the hook and the filament changes

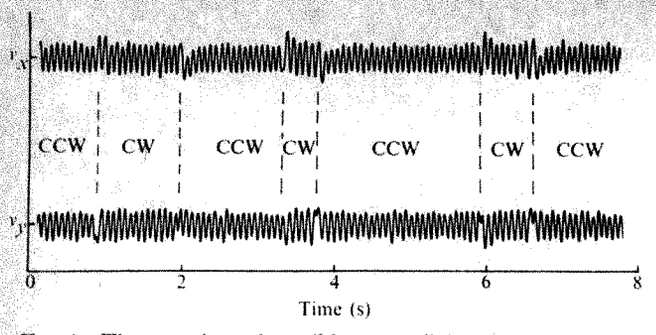


FIG. 2 The rotation of a wild type cell bound to the top window of the tracking chamber. In the interval shown the cell changed direction six times; it ran 71 cycles CCW at 13.2 ± 0.2 r.p.s. and 28 cycles CW at 12.4 ± 0.8 r.p.s. The reversals occurred at different points in the cycle. The cell was followed for a total of 6 min; the interval shown is typical of the entire record, except that the cell stopped occasionally for about 0.2 s and then started again in the same direction. *E. coli* strain AW405 (ref. 7) was grown, washed, and linked to the window by the methods of Larsen *et al.*[8] Other conditions were as described in Fig. 1.

sign; if either one changes its shape, the orientation of the cell (and thus the load) may be different. Note that the amplitudes of the signals (Fig. 1 or Fig. 2) are larger when the frquencies are lower.

Cells which rotate with larger radii of gyration tend to rotate more slowly, which implies that the motors slow down with increasing load. This was confirmed quantitatively in experiments with the polyhook mutant in which Ω was measured as a function of η (Table 1). When the motors run more slowly, they deliver somewhat more torque but less power. Since the torque is approximately constant, a cell tethered by an inert structure, such as a pilus (the point of attachment assumed fluid), and driven by a free helical filament (rotating rapidly on the opposite side of the cell) should rotate at about the same rate as a cell tethered by the flagellum itself (the point of attachment assumed rigid). I have seen this with wild type cells: in the absence of antibody, as many as 90% spin the wrong way (at the usual rates but predominantly clockwise on the slide, counterclockwise under the coverslip). As Silverman and Simon[1] note in the accompanying report, a distinction between rotation and other means of propulsion cannot be made with tethered cells unless they are non-motile when free.

Cells rotate perfectly well at high osmotic pressures, even when visibly plasmolysed (experiments with the polyhook mutant in 0.25 and 0.50 M sucrose). When the osmotic strength is increased, some cells slow down, but most continue to rotate rapidly (Ω in the range expected from changes in η). At higher osmotic strengths (0.75 M sucrose) most cells stop. These effects are reversible. The results are consistent with those obtained by Vaituzis and Doetsch[10,11], who found that flagellated spheroplasts swim in a medium isotonic to the cytoplasm provided that they retain remnants of the cell wall. Severe plasmolysis may cause reversible disruption of flagellar basal structures[12].

A model for a flagellar motor is presented in Fig. 3. The motor operates with two rings (the M and S rings), since only two have been found in Gram positive bacteria[13]. Four have been identified in Gram negative bacteria[13], but the outer pair probably serve as part of a bushing required for passage of the rod through the multilayered wall. The M ring has been shown to be associated with the cytoplasmic membrane in both Gram positive and Gram negative cells[14], but specific attachments of the S ring to known cell envelope structures have not been found. The motor must be attached to the wall somewhere, or else the torque which it can generate cannot be applied. If the rings are pulled away from the wall[12] or if the wall is dissolved away[11], the rings may continue to rotate relative to one another, but they will not drive the cell.

Lubrication does not seem to be a problem, the reason being that everything is very small. I have computed the torque due to the viscous shear in the space between the rod and the wall and in the space between the M and the S rings[3] and approximated the torque due to the rotation of the M ring in the membrane by half the torque on a sphere of similar radius. If the passage through the wall is 20 nm long, the gap between the M and the S rings is 0.5 nm thick, and the viscosity is 1 P (see Fig. 3 for the other dimensions), the internal drag is only 3×10^{-4} times as large as the drag on the body of the cell.

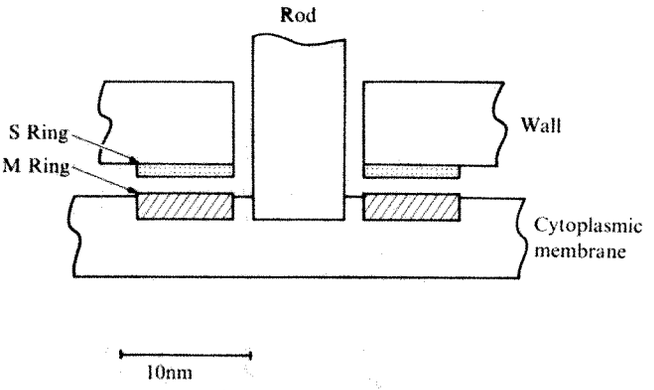


FIG. 3 A schematic representation of a flagellar motor. The rod is connected to a filament by a proximal hook (not shown). The torque is generated between the M ring and the S ring. The M ring, which is mounted rigidly on the rod (connections not shown), rotates freely in the cytoplasmic membrane. The S ring is mounted on the wall. The radius of the rod is 3.5 nm and the inner and outer radii of the rings are 5 nm and 11.2 nm, respectively[13]. (The wall thickness and the space between the M and S rings are not to scale.)

TABLE 1 Changes in angular velocity, torque and power dissipation resulting from changes in viscosity

Relative change in viscosity	2.42*	2.31†
Relative change in angular velocity	0.50 ± 0.08	0.51 ± 0.09
Relative change in external torque	1.21 ± 0.19	1.18 ± 0.21
Relative change in power dissipation	0.61 ± 0.22	0.60 ± 0.19

Cells were tethered to microscope coverslips by the method described in the legend of Fig. 1 and examined manually at 32°C. Cells rotating at uniform rates (0.3–3 r.p.s.) were clocked for about 1 min, the medium was replaced with one containing more (or less) methylcellulose (Dow Methocel 90 HG), and the measurements were repeated. Since the cells had different shapes and sizes and were tethered in different ways, the relative changes in Ω (and hence in the torque $b\eta\Omega$ and in the power dissipation $b\eta\Omega^2$) were computed for each cell separately. The data were obtained from twenty-one paired measurements on ten cells from four cultures. The standard deviations shown in the table were computed by weighting each pair of measurements equally. The viscosities were determined at 32°C with a Cannon-Ubbelohde viscometer.

* 0.776 to 1.88 cP.

† 1.88 to 4.35 cP.

The motor must bear both axial and radial loads, since the rod in a peritrichously flagellated bacterium rarely lies along the direction of motion. The forces are of order $6\pi\eta av$, where a is the radius of the cell (approximated as a sphere) and v is its speed; that is, of order 10^{-8} dynes. When an intact cell swims in a dilute solution, the axial load is more than balanced by osmotic pressure, which forces the M and S rings out against the wall (a force of order 10^{-5} dynes); when a spheroplast swims in a medium isotonic to its cytoplasm, the axial load is not balanced in this manner. As noted above, the coupling between the flagellum and the body of the cell

is not fluid, so the axial and the radial loads can be borne at the point of contact (between the M and the S rings).

How is the torque generated? Larsen *et al.*[15] have found that an intermediate in oxidative phosphorylation (but not ATP) is required for motility in *E. coli*. This suggests a mechanism in which the movement of one molecule down an electrochemical gradient (through the membrane) causes another molecule to exert a force on the S ring in a direction parallel to its face but normal to its radius. A force in the opposite direction could result from a change in the coupling between these molecules or from the action of an independent set. Since the power dissipation for an *E. coli* spinning 10 r.p.s. is of order 10^{-9} erg s^{-1} and the energy which can be gained from the transit of one molecule through the membrane is of order 10^{-13} erg, 10^3 such events could drive the cell through one cycle. The individual steps would be too small to be seen in the present experiments.

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1 Silverman, M., and Simon, M., *Nature*, **249**, 73–74 (1974).
2 Berg, H. C., and Anderson, R. A., *Nature*, **245**, 380 (1973).
3 Landau, L. D., and Lifshitz, E. M., *Fluid Mechanics*, Ch. 2 (Pergamon, London, 1959).
4 Taylor, G., *Proc. R. Soc.*, **A209**, 447 (1951).
5 Berg, H. C., *Rev. Sci. Instrum.*, **42**, 868 (1971).
6 Silverman, M., and Simon, M. I., *J. Bact.*, **112**, 986 (1972).
7 Armstrong, J. B., Adler, J., and Dahl, M. M., *J. Bact.*, **93**, 390 (1967).
8 Larsen, S. H., Reader, R. W., Kort, E. N., Tso, W.-W., and Adler, J., *Nature*, **249**, 74–77 (1974).
9 Berg, H. C., and Brown, D. A., *Nature*, **239**, 500 (1972).
10 Vaituzis, Z., and Doetsch, R. N., *J. Bact.*, **89**, 1586 (1965).
11 Vaituzis, Z., and Doetsch, R. N., *J. Bact.*, **91**, 2103 (1966).
12 Okrend, A. G., and Doetsch, R. N., *Arch. Mikrobiol.*, **69**, 69 (1969).
13 DePamphilis, M. L., and Adler, J., *J. Bact.*, **105**, 384 (1971).
14 DePamphilis, M. L., and Adler, J., *J. Bact.*, **105**, 396 (1971).
15 Larsen, S. H., Adler, J., Gargus, J. J., and Hogg, R. W., *Proc. natn. Acad. Sci. U.S.A.* (in the press).

Presence of HCN in *Chlorella vulgaris* and its possible role in controlling the reduction of nitrate

NITRATE reductase of *Chlorella vulgaris* is present in crude extracts mainly in an inactive form[1–3] which may be converted to an active form by the addition of an artificial oxidant such as ferricyanide[4]. In crude extracts from which low-molecular weight substances have been removed by gel filtration, added NADH (or NADPH) causes a rapid conversion of the active form of nitrate reductase to the inactive form[5]. Losada and coworkers have also observed an inactivation of the nitrate reductase from *Chlorella fusca*[6] and *Chlamydomonas reinhardi*[7] by added NADH. A low-molecular weight fraction separated from crude extracts of *Chlorella vulgaris* also causes an inactivation of nitrate reductase when added back to the extract which had been treated with ferricyanide and passed through a Sephadex column to remove excess reagent[5]. In all cases the enzyme can be reactivated by the addition of the oxidant, ferricyanide[4–8]. On purification of the enzyme, Solomonson[9] found that two substances must be present simultaneously to cause the reversible inactivation: (1) a reducing substance, preferably NADH, and (2) a substance that behaves like HCN. The molar concentration of HCN needed to effect a rapid and reversible inactivation of the enzyme was of about the same magnitude as the enzyme concentration. Thus, activated, suitably purified nitrate reductase with added excess NADH, provides an exceedingly sensitive, though non-specific test for HCN, as shown in Fig. 1.

Although this bioassay could not be applied directly to extracts, it could easily be used to show that distillates prepared from *Chlorella* cells, or from extracts of *Chlorella* cells, contained something which behaved like HCN. The concentration of the unknown substance could be increased by redistillation until it came within the sensitivity range of specific chemical tests, which showed that the substance was, indeed, HCN. After many preliminary experiments, conditions were found for obtaining sufficiently high HCN concentrations in the first distillate to come within the range of a chemical test as well as of the bioassay. The results of a series of such experiments, given in Table 1, show that the amount of HCN which could be obtained from freshly collected *Chlorella* cells was somewhat greater than the amount of nitrate reductase present in the cells. Thus, the quantity of HCN found can account for the fact

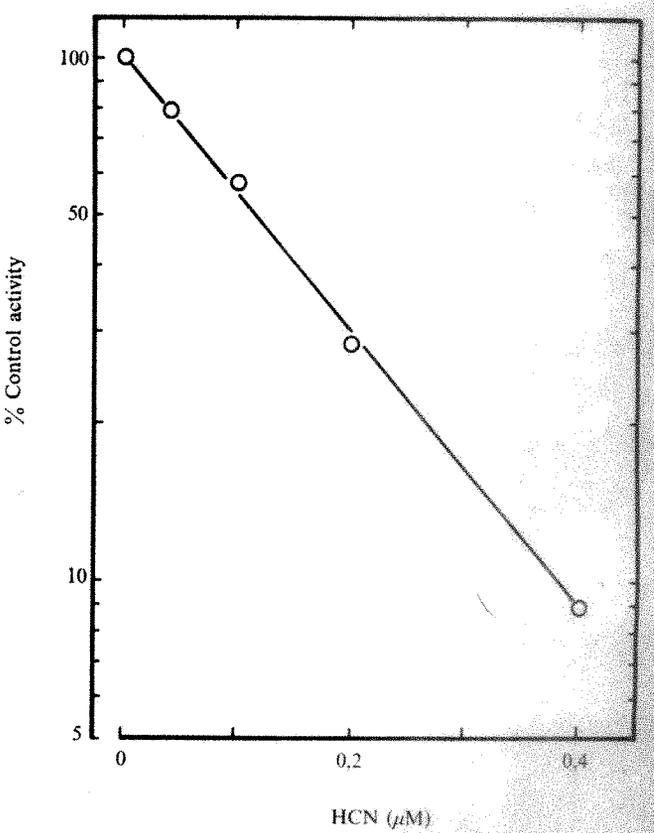


FIG. 1 A calibration curve for the bioassay for HCN. The percentage inactivation of nitrate reductase is plotted on a log scale against the initial HCN concentration. Incubation mixtures containing 0.05 M Na,K phosphate buffer (*p*H 7.6), 0.2 mM NADH, and the indicated concentrations of HCN were brought to 20° C. The inactivation was initiated by addition of activated enzyme to a concentration of 0.86 units ml^{-1}. After 6 min, aliquots were added to the enzyme assay mixture and activity was assayed as previously described[1,9]. A control inactivation mixture without cyanide, gave 95% of the initial activity. This was taken as 100% for calculation of the percentage inactivation. It is essential that all HCN be removed from the enzyme preparation, so that NADH alone gives a negligible inactivation. The preparation of the enzyme is described elsewhere[9]. The sensitivity of this bioassay can be increased by decreasing the concentration of enzyme.

TABLE 1 HCN from *Chlorella* cells

Cells (ml)	HCN (nmol per ml cells) Bioassay	Chemical assay
23.7	1.22	1.38
21.3	1.27	1.62
28.0	1.26	1.39
27.5	2.05	1.75
(Mean)	1.45	1.53

Chlorella vulgaris was grown under the conditions used for the preparation of nitrate reductase[1,5,9,23]. HCN was separated by simple distillation. In the first experiment, 23.7 ml freshly collected, packed cells were washed with 500 ml redistilled water and suspended in a distillation flask in enough water to give 0.5 ml cells per ml suspension. An equal volume of 1 N H_2SO_4 was added, and distillation was begun immediately. The condenser was well cooled, and the receiving flask was held deep in an ice bath. Several fractions of distillate were collected, until no further active material was obtained. Over 90% of the total yield of HCN was obtained in the first 17 ml of distillate. Other experiments were done with minor variations. Control experiments with known HCN showed that there was negligible loss of HCN under these conditions. Another control experiment with tracer $H^{14}CN$ showed that the ^{14}C distributed itself with the chemical assay and the bioassay. Addition of alkali to the receiving flask was unnecessary and was avoided because other substances present in the distillate reacted with the HCN at alkaline pH.

The cells used for these experiments generally gave about 1.4 units of activated enzyme per ml of sonicated extract, corresponding to about 200 μl of cells. This is a yield of 7.0 units per ml cells. From the turnover number[2], we estimate that one unit of enzyme is about 0.09 nmol, which gives 0.63 nmol enzyme per ml cells, compared with 1.2 to 2.0 nmol HCN. The estimate of the amount of enzyme present is not very accurate, since we have no reliable measure of the losses sustained during the extraction procedure.

The chemical assay was carried out according to Guilbault and Kramer[11], without addition of isonitrosobenzoylacetone. Since cyanide acts catalytically in this test, the rate of colour formation was measured at 23° C. HCN (0.1 nmol) added to the test system gave $\Delta A_{560nm} = 0.0365$ in 5 min (d = 1 cm).

that the nitrate reductase present is largely in the inactive form.

Our current procedure for purifying nitrate reductase began with a protamine sulphate fractionation. This was carried out on a small scale, to show that the protamine sulphate precipitate, containing the enzyme largely in inactive form, yielded an amount of HCN which was approximately equivalent to the amount of inactive enzyme present. Thus, 32.5 ml extract (frozen, thawed, and cleared by centrifugation), which is approximately equivalent to 6.5 ml of cells, contained 46.3 units of inactive nitrate reductase (measured after activation of a separate aliquot by ferricyanide) and 1.8 units active nitrate reductase. To this extract, 1 ml of 2% protamine sulphate (*p*H 7.0) was added, and the precipitate obtained was removed by centrifugation. Another 1 ml, and then 0.5 ml of protamine sulphate were added, the precipitate each time being removed by centrifugation. From the supernatant, essentially all of the enzyme was precipitated by further addition of 2 ml 2% protamine sulphate. This precipitate was washed twice with 0.02 M Tris buffer (*p*H 8.1), suspended in 4 ml of 0.1 M phosphate (*p*H 7.2) containing 0.1 mM dithioerythritol and 0.1 mM EDTA, and brought to 10 ml with redistilled water in a 100 ml distillation flask. An equal volume of 1 N H_2SO_4 was added, and distillation was carried out as described in the legend for Table 1. Special care was required because of foaming.

The first fraction of 1.0 ml and a second fraction of 0.9 ml distillate were collected. The bioassay gave 2.86 and 0.45 nmol HCN in the first and second distillates, respectively; the chemical assay gave 2.3 and about 0.36 nmol. The total of 3.3 nmol by bioassay compared with 2.7 nmol by chemical assay. The 46 units of inactive enzyme originally present are equivalent to about 4.2 nmol enzyme, some of which must have been lost or activated during the extensive manipulation. The value of 4 thus compares quite well with the value of 3 nmol HCN recovered by distillation. In a control experiment, an equivalent amount of protamine sulphate gave no detectable HCN.

In another experiment, the final supernatant from the protamine sulphate precipitation was distilled after acidification. Here, the HCN recovered amounted to 3.7 nmol HCN per ml *Chlorella* used to make the extract. Adding the 0.6 nmol HCN removed with the inactive enzyme, this gives a total of 4.3 nmol HCN per ml cells, recovered from the extracts of *Chlorella*, compared with about 1.5 nmol per ml as the average recovered from the intact cells (Table 1). This is presented as evidence that cyanide formation occurs in the extract. In these experiments, distillates were examined by the chemical method of Epstein[10] as well as by the bioassay and by the chemical method of Guilbault and Kramer[11]. All methods gave comparable results. By the use of similar procedures, HCN could also be obtained from spinach leaves and from the blue-green alga, *Plectonema boryanum* (Gewitz and Völker, unpublished). These and further experiments will be described in detail elsewhere.

It has long been a puzzle that cyanide-utilising enzyme systems are present in cells not known to make cyanide[12–14]. Thus, Conn and his associates found in 1963[15], that added $H^{14}CN$ is converted to the β-carboxamide group of asparagine by seedlings of some so-called non-cyanogenic plants and also of some cyanogenic plants. Tschiersch[14] made similar independent observations. Fowden and Bell[16] showed that *Chlorella pyrenoidosa* converted $H^{14}CN$ to β-cyanoalanine, and thence to asparagine or to the peptide γ-glutamyl-β-cyanoalanine. The literature on HCN metabolism has been reviewed by Blumenthal *et al.*[17], who noted particularly that enzymes for the metabolic conversions of HCN are widely distributed in bacteria, fungi, algae and higher plants. Most recently, Taylorson and Hendricks[18] have described a stimulatory effect of HCN on seed germination, and ascribed it to the specific synthesis of asparagine, which may be rate limiting in protein synthesis.

We propose that HCN may play an important part in a delicately balanced system which controls the activity of nitrate reductase. The nature of the chemical interaction between cyanide and nitrate reductase has not been firmly established (see ref. 9 for discussion). The fact that cyanide inhibits the enzyme has long been known[19]. Relimpio *et al.*[20] and Solomonson and Vennesland[2] observed that the inhibition by cyanide was enhanced by NADH. This cyanide inhibition was slowly reversed by nitrate[2] and rapidly reversed by added ferricyanide[5]. The reversal of cyanide inhibition by nitrate had at least a superficial similarity to the activation of nitrate reductase in fresh extracts of *Chlorella vulgaris*[2]. Maranville[21] reported an increase in nitrate reductase activity in extracts from sorghum when nickel salts were added to the extraction buffer. He postulated that the nickel acts by binding cyanide released by the hydrolysis of the cyanogenic glycoside, dhurrin. We also noted a similar increase in activity when $NiSO_4$ was added to fresh extracts of *Chlorella vulgaris*[2] but the stimulation of activity by nickel was small by comparison with activation achieved by other methods.

We note that when a plant is called non-cyanogenic, this only means that the amount of HCN present is less than the amount which can be detected by the test used, or that no cyanogenic glycosides have been found in the material[14,22]. Our experiments show that *Chlorella vulgaris*, previously considered non-cyanogenic, actually contains HCN in bound form, some of it complexed to an inactive form of the enzyme, nitrate reductase. Extracts of these cells contain considerably more HCN, which must be formed from an as yet unidentified precursor.

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1 Vennesland, B., and Jetschmann, K., *Biochim. biophys. Acta,* **227,** 554 (1971).
2 Solomonson, L. P., and Vennesland, B., *Biochim. biophys. Acta,* **267,** 544 (1972).
3 Vennesland, B., and Solomonson, L. P., *Pl. Physiol.,* **49,** 1029 (1972).
4 Jetschmann, K., Solomonson, L. P., and Vennesland, B., *Biochim. biophys. Acta,* **275,** 276 (1972).
5 Solomonson, L. P., Jetschmann, K., and Vennesland, B., *Biochim. biophys. Acta,* **309,** 32 (1973).
6 Moreno, C. G., Aparicio, P. J., Palacian, E., and Losada, M., *FEBS Lett.,* **26,** 11 (1972).
7 Herrera, J., Paneque, A., Maldonado, J. M., Barea, J. L., and Losada, M., *Biochem. biophys. Res. Commun.,* **48,** 996 (1972).
8 Vega, J. M., Herrera, J., Relimpio, A. M., and Aparicio, P. J., *Physiol. Vèg.,* **10,** 637 (1972).
9 Solomonson, L. P., *Biochim. biophys. Acta,* **334,** 297 (1974).
10 Epstein, J., *Analyt. Chem.,* **19,** 272 (1947).
11 Guilbault, G. G., and Kramer, D. N., *Analyt. Chem.,* **38,** 834 (1966).
12 Fowden, L., *A. Rev. Pl. Physiol.,* **18,** 85 (1967).
13 Conn, E. E., *J. agr. Fd Chem.,* **17,** 519 (1969).
14 Tschiersch, B., *Pharmazie,* **22,** 76 (1967).
15 Blumenthal-Goldschmidt, S., Butler, G. W., and Conn, E. E., *Nature,* **197,** 718 (1963).
16 Fowden, L., and Bell, E. A., *Nature,* **206,** 110 (1965).
17 Blumenthal, S. G., Hendrickson, H. R., Abrol, Y. P., and Conn, E. E., *J. biol. Chem.* **243,** 5302 (1968).
18 Taylorson, R. B., and Hendricks, S. B., *Pl. Physiol.,* **52,** 23 (1973).
19 Nicholas, D. J. D., and Nason, A., *J. biol. Chem.,* **207,** 353 (1954).
20 Relimpio, A. M., Aparicio, P. J., Paneque, A., and Losada, M., *FEBS Lett.,* **17,** 226 (1971).
21 Maranville, J. W., *Pl. Physiol.,* **45,** 591 (1970).
22 Eyjolfsson, R., in *Progress in the Chemistry of Organic Natural Products* (edit. by Herz, W., Grisebach, H., and Scott, A. I.) **28,** 74 (1970).
23 Solomonson, L. P. and Vennesland, B., *Pl. Physiol.,* **50,** 421 (1972).

Latex spheres as markers for studies of cell surface receptors by scanning electron microscopy

THE distribution of antigens and carbohydrate residues on the surfaces of cells, notably erythrocytes and lymphocytes, can be determined by binding antibodies or lectins to such macromolecules as ferritin[1,2], haemocyanin[3,4] or peroxidase[2,5], which serve as markers for transmission electron microscopy. Improved techniques in high resolution scanning electron microscopy (SEM), however, make it possible to determine the topographical distribution of molecular receptors on the surfaces of cells and tissues by similar histochemical techniques involving markers resolvable by SEM. LoBuglio *et al.*[6] used commercial polystyrene latex particles, 2,300 Å in diameter, as immunological markers for SEM work. But applications of this reagent are limited because the hydrophobic surface of the particles makes them stick non-specifically to many surfaces and molecules.

We have developed a more useful technique, and report here: (1) the design and synthesis of latex spheres equal to or less than 1,300 Å in diameter; (2) the covalent bonding of antibodies and other molecules to these spheres, and (3) the application of antibody-tagged particles as specific cell surface markers for SEM.

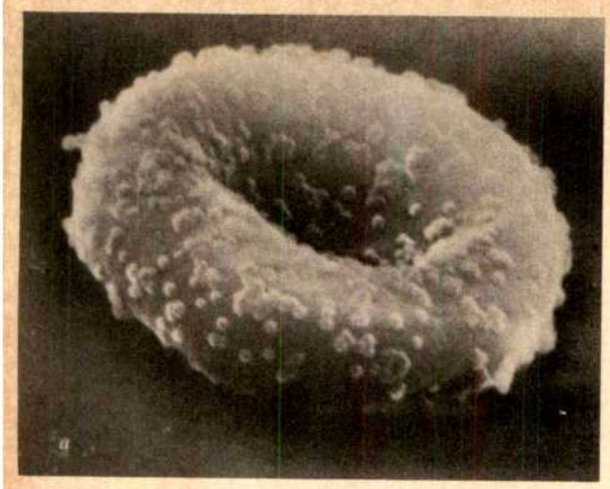
The rationale for the design of polymeric latex is based on the need for small, stable particles which are biocompatible—that is, they do not interact non-specifically with cells or other biological components—and contain functional groups to which specific proteins and other biochemical molecules can be bonded covalently. A crosslinked copolymer of hydroxyethylmethacrylate (HEMA), methacrylic acid and methylmethacrylate has these characteristics: poly HEMA has blood compatible properties and is used in the manufacture of contact lenses[7]. The hydroxyl groups can be activated by cyanogen bromide[8,9] for covalent bonding of proteins to the polymeric latex. Methacrylic acid residues which impart a negative charge onto the particles prevent non-specific binding to cell surfaces and provide carboxyl groups to which various biochemical molecules can be bonded by the carbodiimide method[10]. Cross linking of the polymeric matrix is essential to maintain the stability and size of the particles in both aqueous solutions and organic solvents commonly used in the fixation and dehydration of biological specimens for electron microscopy.

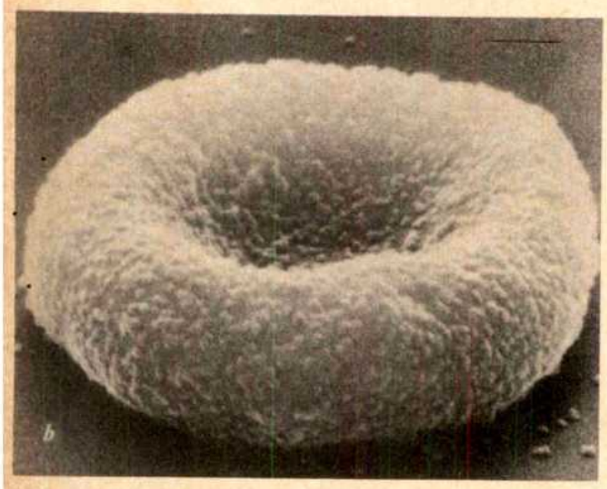
Latex spheres were synthesised by aqueous emulsion copolymerisation of methylmethacrylate (MMA), HEMA and methacrylic acid (MAA) in the presence of the emulsifier sodium dodecylsulphate, the free radical initiator ammonium persulphate, and the cross linking agent trimethylol propane trimethacrylate. Polymerisation was carried out in closed tumbling containers, under argon at 98° ± 2° C, for 6 h. The chemical composition of the latex was: MMA, 57%; HEMA, 30%; MAA, 10%, and crosslinker, 3%. By varying the polymerisation conditions the diameter of the spheres could be changed from 600 to 1,300 Å. The emulsifier and other impurities were removed by passage over a mixed bed ion exchange column.

Proteins and other molecules containing amino groups were bonded covalently to the latex spheres by either the cyanogen bromide[8] or carbodiimide methods[10]. For use as visual markers in fluorescent microscopy or as reagents in quantitative studies, latex spheres (20–50 mg ml^{-1}) activated with cyanogen bromide (50 mg ml^{-1}) were tagged with the fluorescent dye, dansyl-ε-lysine, or ^{3}H-lysine (1 mM). Antibodies (0.2–1 mg ml^{-1}) were covalently bonded to latex spheres previously derivatised with ε-amino caproic acid using the coupling reagent, 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (2 mg ml^{-1}) or spheres derivatised with diaminoheptane using glutaraldehyde (1.25%). Further details of the synthesis and coupling procedures will be described elsewhere.

The application of these immunochemical reagents as visual markers of cell surface components for light and electron microscopy was tested using the indirect or 'sandwich' technique[5]. Purified goat antibodies directed against rabbit γ-globulin (goat anti-rabbit) were coupled to the latex spheres. Unbound antibodies were removed by repeated centrifugation (35,000*g*, 30 min) and redispersion in phosphate-buffered saline (PBS). The immunochemical activity of the latex bound goat anti-rabbit antibody was verified by the agglutination of the latex in the presence of rabbit IgG.

Washed bovine red blood cells (RBC) sensitised with rabbit antiserum against bovine red blood cells (non-specific rabbit antiserum was used in the control) were incubated with goat anti-rabbit–latex conjugates (1–20 mg ml^{-1}) for 20 min at 20° C. After removal of the excess reagent by repeated centrifugation of the cells at 400*g*, the RBC were fixed in 1.25% glutaraldehyde and prepared for examination





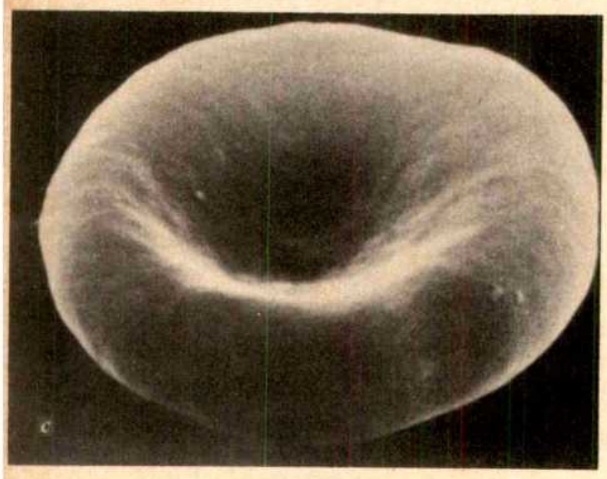




FIG. 1 Scanning electron micrograph of bovine RBC. *a*, Red blood cell labelled with rabbit anti-bovine erythrocyte antiserum followed by goat anti-rabbit antibodies covalently bonded to latex particles 1,300 Å in diameter. Only partial labelling of the cell is observed because of the low concentration of latex conjugate used. *b*, Red blood cell labelled as in (*a*) but with a higher concentration of latex particles 800 Å in diameter. (Horizontal bar represents 1 μm.) *c*, Control experiment. Red blood cell treated with non-specific rabbit antibodies followed by goat anti-rabbit antibodies conjugated to latex particles 800 Å in diameter. *d*, Mouse spleen lymphocyte labelled with rabbit anti-mouse lymphocyte serum followed by goat anti-rabbit antibodies conjugated to 800 Å latex particles. Sodium azide (5 mM) was present during labelling to prevent cap formation.

by SEM[11]. A scanning electron micrograph of a bovine red blood cell specifically labelled with a low concentration of immunolatex particles of average diameter 1,300 Å is shown in Fig. 1*a*. A red blood cell labelled with immunolatex particles of average diameter 800 Å is shown in Fig. 1*b*. The absence of significant non-specific binding of these immunochemical reagents is illustrated in the control experiment (Fig. 1*c*). A mouse spleen lymphocyte settled onto a glass cover slip and labelled first with rabbit anti-mouse lymphocyte serum and then with goat anti-rabbit–latex conjugate (20 mg ml^{-1}) is shown in Fig. 1*d*. The latex spheres coat the surface of the cell and line the microvilli.

These reagents have several advantages for studies in the mapping of specific cell surface components. They can be easily prepared and coupled to proteins and other molecules by standard chemical procedures, they are stable indefinitely, and do not bind non-specifically to cell surfaces. They can be synthesised in various size ranges and chemical compositions to suit particular requirements. For example, smaller spheres in the size range 200–300 Å in diameter can serve as molecular markers (1) in replica and shadowing techniques for high resolution transmission electron microscopy, and (2) in double labelling experiments in conjunction with larger latex particles or with such biological markers as haemocyanin distinguishable by its cylindrical shape[3]. For use with fluorescent microscopy and for quantitative studies, these reagents have the advantage that by binding fluorescent dyes and radioactive molecules to the latex instead of the antibody, a high degree of tagging can be attained without adversely affecting the antibody activity for studies requiring high sensitivity. These particles when tagged with metals or other suitable elements have application in electron beam microprobe analysis and related X-ray techniques.

The application of these multipurpose reagents in studies of antigens on the surface of more complex cell and tissue specimens is being investigated. Latex particles bound to certain hormones, lectins or toxins should prove valuable in the detection and localisation of various specific receptors on cell surfaces.

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[1] Nicolson, G. L., Masouredis, S. P. and Singer, S. J., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1416 (1971)
[2] Wagner, M., *Res. Immunochem. Immunobiol.*, **3**, 185 (1973).
[3] Smith, S. B., and Revel, J.-P., *Devl. Biol.*, **27**, 434 (1972).
[4] Karnovsky, M., and Unanue, E. R., *Fedn. Proc.*, **32**, 55 (1973).
[5] Avrameas, S., *Int. Rev. Cytol.*, **27**, 349 (1970).
[6] LoBuglio, A. F., Rinehart, J. J., and Balcerzak, S. P., in *Scanning Electron Microscopy*, Part II, 313 (ITT Research Institute, Chicago, 1972).
[7] Wichterle, O., in *Encylopedia Polymer Sci. Tech.*, **15**, 27B (Interscience, New York, 1971).
[8] Cuatrecasas, P., *J. biol. Chem.*, **245**, 3059 (1970).
[9] Hoffman, A. S., Schmer, G., Harris, C., and Kraft, W. G., *Trans. Am. Soc. artif. int. Organs*, **18**, 10 (1972).
[10] Goodfriend, T. L., Levine, L., and Fasman, G., *Science*, **144**, 1355 (1964).
[11] Bessis, M., and Weed, R. I., in *Scanning Electron Microscopy*, Part II, 289 (ITT Research Institute, Chicago, 1972).

Calcium uptake by sarcoplasmic reticulum of muscle from vitamin D-deficient rabbits

RECENT rapid advances in understanding the metabolism and action of vitamin D have confirmed its primary effect on intestinal calcium transport and continue to elucidate its action on bone and kidney[1,2]. Biochemical research has, however, largely neglected the well-known clinical observation of proximal muscle weakness in most vitamin D-deficient states[3]. Those studies which have been done provide only indirect evidence of a link between vitamin D and muscle. Thus, autoradiography after pharmacological doses of radioactive vitamin D has shown its localisation under the sarcolemma[4], and when more physiological doses are given to rachitic animals, vitamin D and its metabolites may be found in significant amounts in muscle[5]. Skeletal muscle also contains a protein similar to the binding protein of kidney (but not of plasma) that binds 25-hydroxycholecalciferol[6].

We have recently measured the uptake of calcium by the isolated sarcoplasmic reticulum (SR) of the psoas muscles of vitamin D-deficient and vitamin D-supplemented rabbits and our results provide direct evidence of a biochemical effect of vitamin D deficiency on striated muscle.

Weanling Dutch rabbits were fed a vitamin D-deficient diet (General Biochemicals, Laboratory Park, Chagrin Falls, Ohio) from the age of 4 weeks (D− rabbits). Their litter mates were given the same diet supplemented with vitamin D providing 10–13 IU vitamin D_2 d^{-1} (D+ rabbits, 'controls'). The D− rabbits became weaker than the controls, and showed histological and biochemical evidence of rickets, (plasma calcium 7.9 ± 0.4 mg per 100 ml; control 12.8 ± 0.4; mean ± s.e.m.). At the end of the period on the diet (5–7 weeks), pairs of D− and D+ animals were killed and both psoas muscles were quickly excised from each animal and placed in ice-cold buffer (5 mM histidine, *p*H 7.0; 0.1 M KCl). Individual muscles were homogenised and an SR fraction isolated, sedimenting between 8,000 and 70,000*g*, by differential centrifugation, according to Martonosi, Donley and Halpin[7], except that only one extraction in 0.6 M KCl was carried out. The final SR pellet was resuspended in 5 mM histidine, *p*H 7.0, containing 0.1 M KCl and 5 mM dithiothreitol. Electron microscopy showed little contamination of the vesicular material by either sarcotubular fragments or mitochondria. The rate of calcium uptake by SR was measured on the day after isolation by Millipore filtration assay (Table 1)[8,9]. Preliminary experiments showed that calcium uptake was dependent on ATP. Its rate varied linearly with SR protein concentration (10–40 μg protein ml^{-1}), and with time for the first 90 s of the reaction. The rate of calcium uptake over the first minute by the SR from each muscle was measured in duplicate at three different protein concentrations. As the mean value obtained from the psoas muscle on each side of any individual animal was not significantly different, the result given in Table 1 is the average value for each animal and represents the mean of 12 measurements—6 on each psoas muscle.

TABLE 1 Rate of calcium uptake by SR isolated from the psoas muscles of D+ and D− rabbits

Pair No.	Calcium uptake (μ mole per min per mg SR protein)	
	D+	D−
1	1.94	1.42
2	2.13	1.88
3	1.88	1.68
4	2.06	1.55
5	2.19	1.65
6	1.73	1.23
7	1.74	1.25
8	1.00	0.81
Mean ± s.e.m.	1.83 ± 0.13	1.43 ± 0.12

Calcium uptake was measured in 3 ml of medium containing 20 mM histidine, *p*H 6.4, 100 mM KCl, 5 mM Mg^{2+}, 5 mM oxalate, 5 mM ATP, 100 μM $^{45}CaCl_2$ and 10–40 μg SR protein ml^{-1} at 25 °C. The reaction was started by the adding of $^{45}CaCl_2$ and stopped, after 60 s, by passing an 0.1 ml sample, diluted with 0.5 ml of 5 mM histidine, *p*H 7.0, 0.1 MKCl buffer, through an HA Millipore filter (pore size 0.45 μm). The rate of calcium uptake by the SR was calculated from the ratio of the amounts of ^{45}Ca, determined by liquid scintillation counting, retained on the filter and in the filtrate. Blank values were obtained in the absence of SR protein. The values for each rabbit are the average of twelve separate determinations (see text).

In the D− rabbits the rate of calcium uptake by the SR was lower in all experiments than in the D+ controls. The mean difference between the pairs of D− and D+ rabbits is significantly different from zero ($t = 7.27$; $P < 0.001$), and the difference between the mean rates of calcium uptake for the two groups is also significant ($t = 2.25$; $0.05 > P > 0.02$). In some experiments the SR samples were incubated for varying times at 37° C and the rates of calcium uptake subsequently measured (Fig. 1). The SR from the D−

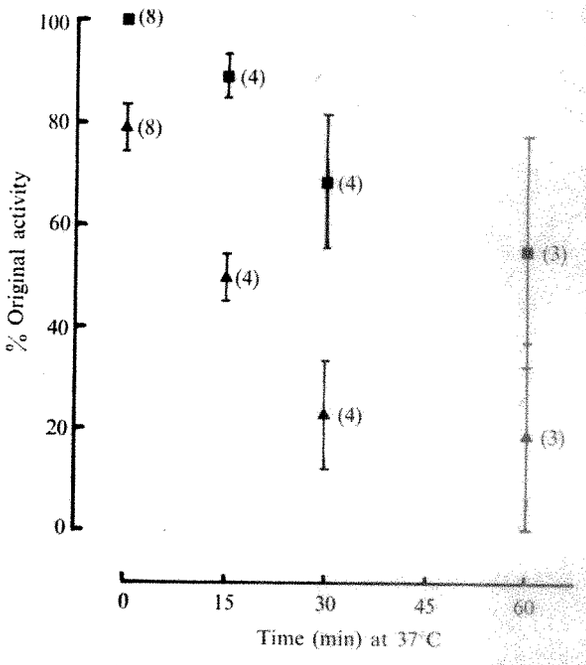


FIG. 1. Percentage of initial rate of calcium uptake by SR after incubation at 37°C. The results are expressed as a mean % ± s.e.m. of the rate of calcium uptake of the paired control when first measured. Numbers of animals are given in brackets. ■, D+; ▲, D−.

animals lost its ability to concentrate calcium more rapidly than that from the D+ controls.

No consistent differences were observed by the linked spectrophotometric assay of Horgan, Tume and Newbold[10] in the rates of either the Mg^{2+} or the Mg^{2+}–Ca^{2+}stimulated ATPase between the SR isolated from the two groups of animals. In an initial repletion experiment three oral daily doses of 100 IU of Vitamin D_3 were given to one of a pair of D− rabbits. The rate of calcium uptake by the SR from the repleted rabbit was higher than that of the D− rabbit (1.39 as against 1.09 μmol Ca per min per mg SR protein). The activities of the Mg^{2+} and Mg^{2+}–Ca^{2+} stimulated ATPase were again similar.

In rabbits up to the age of 8 d it has been shown that both muscular activity and age affect the rate of calcium uptake by SR[11]. The subsequent changes until maturity are not known. In our rabbits the animals in each pair were exactly the same age and this showed no apparent relation to the rate of calcium uptake by the SR; there was also no clear difference in their spontaneous muscle activity although the D− animals were weak and hypotonic. We consider that the difference in calcium uptake between the D− and the D+ animals is due to an effect of vitamin D deficiency on the muscle itself rather than on its nerve supply. In rats denervation may increase (rather than decrease) the rate of active calcium transport by the SR of slow muscle, but not of fast, to which group the psoas belongs[12].

How vitamin D deficiency affects SR is not known. As no differences were seen in the Mg^{2+} and Mg^{2+}–Ca^{2+} stimulated ATPase activities of the SR from the D− and D+ rabbits, the observed alteration in the rate of calcium uptake may be a result of changes in the calcium transport coupling system[11–14], and similar 'inefficient' SR has been isolated from dystrophic muscle[14].

There are clearly many possible calcium dependent mechanisms in muscle upon which vitamin D might have an effect. Our results provide support for the inclusion of muscle as another target organ for vitamin D.

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1 T.C.B.S., *Nature*, **245**, 180–182 (1973).
2 Omdahl, J. L., and DeLuca, H. F., *Physiol. Rev.*, **53**, 327–372 (1973).
3 Smith, R., and Stern, G., *J. neurol. Sci.*, **8**, 511–520 (1969).
4 Kodicek, E., in *Transfer of Calcium and Strontium across biological membranes* (edit. by Wasserman, R. H.) 185–196 (Academic Press, New York, 1963).
5 Neville, P. F., and DeLuca, H. F., *Biochemistry*, **5**, 2201–2207 (1966).
6 Haddad, J. G., and Birge, S. J., *Biochem. biophys. Res. Comm.*, **45**, 829–834 (1971).
7 Martonosi, A., Donley, J. R., and Halpin, R. A., *J. biol. Chem.*, **243**, 61–70 (1968).
8 Martonosi, A., and Feretos, R., *J. biol. Chem.*, **239**, 648–658 (1964).
9 Basten, J. F., *thesis*, Univ. Oxford (1972).
10 Horgan, D. J., Tume, R. K., and Newbold, R. P., *Analyt. Biochem.*, **48**, 147–152 (1972).
11 Holland, D. L., and Perry, S. V., *Biochem. J.*, **114**, 161–170 (1969).
12 Margreth, A., Salviati, G., Di Mauro, S., and Turati, G., *Biochem. J.*, **126**, 1099–1110 (1972).
13 Martonosi, A., Boland, R., and Halpin, R. A., in *Cold Spring Harb. Symp. quant. Biol.*, **37**, 455–468 (1972).
14 Samaha, F. J., and Gergeley, J., *New Engl. J. Med.*, **280**, 184–188 (1969).

Site and mechanism of water vapour uptake from the atmosphere in ixodid ticks

Uptake of water vapour from subsaturated atmospheres has been demonstrated in a variety of terrestrial insects and mites but no previous effort to identify the site and mechanism has been completely conclusive. The tracheal system, the rectum and the general body surface have variously been proposed in specific studies.

Noble-Nesbitt[1] found that occlusion of the anus of the insect *Thermobia domestica* (Packard) prevented recovery of an experimentally imposed body water deficit and he suggested that the site of vapour sorption is in the rectum. The tick *Dermacentor variabilis* (Say), however, was not impaired in this ability by blocking the anus[2,3].

The present investigation deals with the site of water vapour uptake in the ixodid tick *Amblyomma variegatum* (Fabricius) and considers a possible uptake mechanism. Unengorged adults, both males and females, were used between 8 and 20 weeks after ecdysis. During this period there were no waste products; weight and water content (60% of total weight on the average) showed no significant changes at constant conditions of 20° C and 93% relative humidity (r.h.) This humidity was well above the critical equilibrium humidity (found to be between 80% and 85% r.h.) of the adults. At 20° C, ticks could be handled without using carbon dioxide anaesthesia.

Ticks exposed to 33% r.h. and 20° C for 4 weeks lost 17%–19% of their initial weight or 28%–31% of their initial water mass. Subsequently, in groups of 10 ticks each, the following parts of the body were covered with paraffin wax (melting point 42° C–44° C): the anus, the complete dorsal surface and the mouthparts. A control group of 20 ticks remained without paraffin treatment. During the ensuing exposure to air of 93% r.h. and 20° C the body water mass increased at 3.0 ± 0.7% d^{-1} in the control ticks, 3.4 ± 0.6% d^{-1} in those with the anus covered, 3.1 ± 0.8% d^{-1} in those with the dorsal surface covered; those ticks with the mouthparts covered lost 0.3 ± 0.1% d^{-1} of their body water for a period of 4 weeks.

The effect of covering the mouthparts is also evident in hydrated ticks in equilibrium with 93% r.h. Of a group of 20 ticks, those untreated were able to maintain equilibrium weight in air of 93% r.h. and 20° C; but those with the mouthparts covered lost 0.2 ± 0.1% of their body water each day during an observation period of 7 weeks.

To show that the application of paraffin wax and the contact between the mouthparts and the applied wax had no adverse effects on water uptake, the following experiments were performed. In one group of ticks, dehydrated as above, only the distal half of the mouthparts was painted with paraffin wax such that both the dorsal space between the chelicerae and the lateral spaces between the hypostome and the chelicerae provided paths between the surrounding air and the buccal cavity. These ticks were able to increase their body water, comparable with that in the control group, at a rate of 2.9 ± 0.5% d^{-1} in air of 93% r.h. and 20° C. When the wax was removed manually from 10 ticks that had suffered loss of weight with their mouthparts sealed

with wax, vapour sorption commenced immediately in each. Moreover, in most ticks with sealed mouthparts, beginning after approximately 4 weeks the wax cover progressively softened from the inner surface (perhaps because of ejected saliva), gradually detached itself from the mouthparts and after about 5 weeks could be removed in one piece. Accompanying this process, the rate of gain of body water mass increased gradually from $\sim$ 0.5% d^{-1} in the early stages of loosening to $\sim$ 3.0% d^{-1} after the removal of the wax cover. From these experiments we can conclude that blockage of the mouth prevents net water gain from the atmosphere in *A. variegatum*.

That water vapour actually passes through the mouthparts was shown in an experiment which did not require blockage of the mouthparts with paraffin wax. For this purpose the mouthparts of a dehydrated tick were inserted through a wax-coated parafilm membrane up to the base of the palps. This membrane was clamped between the open ends of two glass tubes (inner diameter 5 mm, length 20 mm) such that the mouthparts projected into one tube and the rest of the body into the other. The contact line between the two tubes and the penetration point of the mouthparts through the membrane was carefully sealed with melted paraffin wax. The opposite ends of the glass tubes were closed by parafilm membranes coated with paraffin wax. By injecting the needle of a microlitre syringe through these parafilm membranes, 1 μl of saturated KNO_3 solution (which provided a humidity of 93% at 20° C) was placed on the wall of each glass tube, adjacent to but not touching the tick. The membranes were subsequently repaired. After 28 h the drop of KNO_3 solution adjacent to the mouthparts had changed to a moist crystal of KNO_3, whereas that in the tube containing the rest of the body seemed to be unchanged. The possibility that vapour inadvertently escaped to the surrounding air was completely precluded by holding the assembly in a container over KNO_3 solution. This experiment brought the same result when repeated with five different ticks. The rate of decrease in the the volume of the solution adjacent to the mouthparts was estimated to equal in magnitude the rate of net water gain from air of 93% r.h. and 20° C that had been measured in similarly dehydrated ticks.

Active uptake of water vapour, either to acquire or to maintain equilibrium water mass, takes place through the mouthparts in the ixodid tick *A. variegatum*. Since similar results have been obtained when these experiments were performed on adults of *Rhipicephalus bursa* (Can. et Fanz.), *Hyalomma anatolicum excavatum* (Koch) and *Hyalomma schulzei* (Ol.), it may be suggested that active uptake of water vapour proceeds through the mouthparts in all ixodid ticks.

There is some evidence for a significant role of the saliva in the mechanism of vapour uptake as shown by the following observations. Severely dehydrated adults of *A. variegatum* (desiccated to 50.6 $\pm$ 11.3% of their initial water mass) have been observed to secrete through the mouthparts a clear fluid which accumulated between the hypostome and the palps and quickly dried to a white crystalline solid in the desiccation chamber, but when these ticks were transferred to humidities greater than the critical equilibrium humidity the crystalline solid dissolved in absorbed water and the liquid was subsequently imbibed. These crystals occurred only between the hypostome and the palps and so the passage to the mouth remained open. Moreover, the clear fluid could be observed in the buccal cavity and the salivary ducts of all ticks (regardless of water status) after removal of the chelicerae and the dorsal part of the capitulum.

The crystalline solids, described above, were collected from the mouthparts of dehydrated ticks and exposed to a series of different humidities. The substance remained unchanged in humidities less than 75%; at 75% r.h. it became moist without dissolving, above 80% r.h., however, it took up substantial amounts of water within a few seconds and finally dissolved. Recrystallisation occured when the dissolved substance was transferred to relative humidities less than 75%. It should be noted that the lower limit of water uptake by the crystalline solids corresponds with the critical equilibrium humidity of the species.

Preliminary chemical analysis of the crystalline substance has identified high concentrations of sodium and potassium; anions have not yet been identified. To obtain greater amounts of secretion from the mouthparts 0.5 μl of a 3% isotonic solution of pilocarpin was injected into unengorged adults which were in equilibrium with 93% r.h. at 20° C. This immediately led to copious salivation. The secretion obtained was, however, different from that described above. It lost water only very slowly at low humidities and did not exhibit the dramatic water uptake at high humidities. Furthermore, it contained an immiscible viscous, milky cement substance which has been described for ticks in connection with attachment to a host.

This study shows that active uptake of atmospheric water vapour in ixodid ticks proceeds through the mouth. It may well be related to hygroscopic properties of salivary secretion. Resorption of the absorbed water could possibly take place in the gut, although the remarkable abilities of the salivary glands in handling water[4–8] should be kept in mind. Further experiments on the uptake of water vapour in ticks are in progress.

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1 Noble-Nesbitt, J., *J. exp. Biol.*, **52**, 193 (1970).
2 Knülle, W., and Devine, T. L., *J. Insect Physiol.*, **18**, 1653 (1972).
3 McEnroe, W. D., *Acarologia*, **14**,(4), 542 (1972).
4 Tatchell, R. J., *Nature*, **213**, 940 (1967).
5 Tatchell,R. J., *J. Insect Physiol.*, **15**, 1421 (1969).
6 Kitaoka, S., and Morii, T., *Natn. Inst. Anim. Hlth Q.*, **10**, 34 (1970).
7 Kirkland, W. L., *J. Insect Physiol.*, **17**, 1933 (1971).
8 Kaufman, W. R., and Phillips, J. E., *J. exp. Biol.*, **58**, 523 (1973).

Is texture-density contrast an inhibition or an adaptation?

MACKAY'S recent experiments on texture-density contrast[1] are an interesting contribution to the current, rapidly-growing literature on 'feature-specific inhibition' in the visual system. There have been suggestions, based mainly on psychophysical evidence, that there is mutual inhibition between 'channels' selective for binocular disparity[2], orientation[3,4] and spatial frequency[5,6]. It is known that many neurones in the visual cortex respond selectively to particular values on these three dimensions[7–9] (as well as others, such as direction and velocity). Our knowledge of lateral inhibition at earlier levels in the visual pathway indicates that mutual inhibition between

such multi-channel neurons would account for a wide range of visual effects.

Following a previous suggestion[10], MacKay has proposed a new type of neurone, responding as a monotonic function of texture density, with inhibition between such units playing an important perceptual role in enhancing local density gradients (such as the edge between two surfaces of different material, or at different distances). Before accepting the existence of 'texture-density contrast' as an important phenomenon, however, we must be sure that MacKay's observations cannot be explained in terms of known effects.

Successive contrast between gratings or textures of different densities (the 'spatial frequency shift') is well documented[11–13]. After inspection of one pattern, a subsequently viewed finer pattern looks even finer, a coarser one even coarser. It is particularly important when doing experiments aimed at showing simultaneous contrast to show that the effects obtained are not simply the after effects of adaptation[14,15]. For example, when viewing MacKay's demonstrations[1] (Fig. 5 of ref. 1), if fixation drifted onto the background, even for a second or two, an after effect would be expected when the test pattern was refixated.

MacKay himself reported that the contrast effect was enhanced by moving the figure up and down while the eye was held stationary. This of course suggests an explanation in terms of adaptation, as fixation was alternating between the background and test patterns. Because MacKay presented no formal data, and little information on viewing conditions, it is impossible to assess the likely extent of adaptation in his experiments.

Turning to his demonstrations, the reader is faced with some difficulties in judging the phenomenon. Figure 5*a* cannot properly be assessed, as it contains a serious artefact. The two outermost lines in the left half of the test grating are in contact with lines in the background grating, and so actually are wider! Another error occurs in Fig. 2*a*, which contains three regions of supposedly equal frequency. On taking a ruler to it, one finds 8 cycles cm^{-1} in the central and right-hand regions, but about 8.5 cycles cm in the left-hand region. This difference of 6% is in the same range (5-10%) as the effect claimed. The reader cannot tell whether it is an error in photographic reproduction, or was present in the original display.

I have obtained reports from several observers, who agreed that the dot pattern of Fig. 5*b* gave a noticeable contrast effect on immediate viewing, but most were unable to make a judgment on the random texture of Fig. 5*c*.

About a year ago, I did some experiments also aimed at showing spatial-frequency contrast, with stimuli very similar to MacKay's Fig. 5*a*. The background patterns were vertical, high-contrast sinusoidal grating photographs of 3.5 and 7 cycles per degree. The two test patterns were of much lower contrast, at 5 cycles per degree, each subtending 1.5° × 1.5°, one vertically above the other, separated by a grey strip 0.25° wide. They were presented in a tachistoscope, with the test patterns either both vertical or both horizontal, for 0.5, 1, or 2 s with instructions to fixate a mark centred between the test gratings, and to report whether the upper or lower test grating appeared to have the broader bars and/or wider spacing.

In the first experiment, the background pattern was left unchanged over a sequence of 120 trials for each of four subjects. The result for vertical test gratings was an average of 78% reports in favour of contrast, while for the horizontal test, only 57% (where 50% represented chance).

Although the trials were brief, specifically to avoid adaptation, I then realised that adaptation to the background could have been building up over trials. Five more subjects were run, with 0.5 or 1 s presentations and the background patterns were interchanged after each trial to balance out any spatial adaptation. The results in this series were much more variable, and did not deviate significantly from chance (48% with test vertical, 37% with test horizontal). I concluded that a spatial-frequency, or texture contrast effect did not exist, when adaptation was properly controlled for.

In view of MacKay's demonstration with dot textures, it now seems possible that a texture-contrast effect may exist in some circumstances. Much more controlled experimentation is required, however, to demonstrate that it has any generality, and that it is not due to the after-effects of adaptation to the background pattern. Of course, the spatial-frequency shift (after effect) may itself play a role in enhancing a texture discontinuity, but it does not need a new type of neurone or process to account for it.

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1 MacKay, D. M., *Nature*, **245**, 159 (1973).
2 Richards, W., *Vision Res.*, **12**, 1113 (1972).
3 Blakemore, C., Carpenter, R. H. S., and Georgeson, M. A., *Nature*, **228**, 37 (1970).
4 Blakemore, C. ,and Tobin, E. A., *Expl. Brain Res.*, **15**, 439 (1972).
5 Georgeson, M. A., *Nature*, **245**, 43 (1973).
6 Tolhurst, D. J., *J. Physiol., Lond.*, **226**, 231 (1972).
7 Blakemore, C., *J. Physiol., Lond.*, **209**, 155 (1970).
8 Hubel, D. H., and Wiesel, T. N., *J. Physiol., Lond.*, **195**, 215 (1968).
9 Maffei, L., and Fiorentini, A., *Vision Res.*, **13**, 1255 (1973).
10 Fiorentini, A., and Maffei, L., *Vision Res.*, **11**, 1299 (1971).
11 Blakemore, C., and Sutton, P., *Science*, **166**, 245 (1969).
12 Blakemore, C. ,and Nachmias, J., *J. Physiol.*, **213**, 157 (1971).
13 Anstis, S. M., *Am. J. Psychol.*, (in the press).
14 Coltheart, M., *Nature new Biol.*, **231**, 256 (1971).
15 Blakemore, C., Carpenter, R. H. S., and Georgeson, M. A., *Nature*, **234**, 418 (1971).

PROFESSOR MACKAY REPLIES: Dr Georgeson[1] could easily have disposed of most of his doubts.

The test strips in my demonstrations of simultaneous space-frequency contrast[2] had a subtense of between 1° and 2°. There is no difficulty in keeping one's fixation on the horizontal midline of such a broad strip, yet an effect of about 10% is then measurable even with Fig. 1, which has no background lines at the ends of the strip. Slight vertical oscillation of the figure enhances but is not at all essential to the effect.

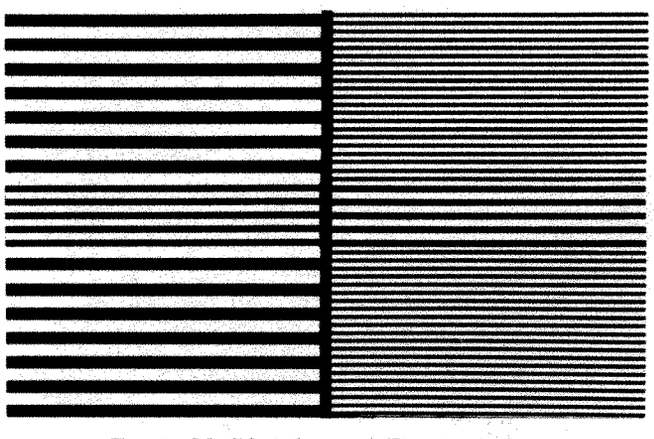


FIG. 1 Modified form of Fig. 5*a* (ref. 2).

The effects for some O's are stronger immediately on viewing than after a minute or so of inspection. This adaptation

is opposite to that which might be expected on Georgeson's hypothesis.

What Georgeson describes as a 'serious artefact' is easily evaluated. With movable test strips, the contrast effect is seen regardless of their location relative to the background stripes, and Fig. 1, free of the allegedly 'serious' artefact, is just as effective as my original figure (Fig. 5*a* ref. 2).

The distortion of the line-spacing on the left of Fig. 2*a* of ref. 2 is regrettable; but it need not prevent the reader from checking the points made, which depend an (i) comparing the perceived spatial frequency of the central panel with that of either side panel, (ii) observing the effects of masking the transition regions, and (iii) comparing Fig. 2*a* with Fig. 2*b*.

(i) Only the left-hand panel was distorted; yet covering over this panel as far as the transition region (or indeed the whole panel) does not abolish the illusory frequency contrast between the other two panels.

(ii) Masking the transition regions, on the other hand, does abolish the illusion.

(iii) In Fig. 2*b*, the centre frequency of the middle panel as printed (8.25 cycles cm^{-1}) is still higher than the average for the other two panels (7.75 cycles cm^{-1}); yet it is perceived as matching them when the transition regions are visible, and not when they are masked.

In view of all this, Dr Georgeson's own negative findings with different stimuli under different conditions scarcely justify his conclusion.

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1 Georgeson, M. A., *Nature*, **249**, 85–86 (1974).
2 MacKay, D. M., *Nature*, **245**, 159 (1973).

Mid-Precambrian prokaryotes(?) from the Belcher Islands, Canada

SPECULATION about the Precambrian biosphere and about evolutionary history is slowly being substantiated and modified by new palaeontological evidence from many regions. I present here more such evidence—a preliminary report of the discovery of a well preserved stromatolitic microflora from cherty dolostones of the Belcher Supergroup in Hudson Bay. This microflora may provide important information about the stage of algal evolution about 2,000 Myr ago, and about stromatolite genesis. Futhermore, the well preserved cellular biofabric facilitates description of the development of primary stromatolitic microstructure permineralised *in situ* by silica, and its subsequent modification (Fig. 1*a*) by carbonate diagenesis into a secondary microfabric, which is important in stromatolite taxonomy.

This fossil microflora contains the oldest known, well preserved palmelloid algal colonies, and their mode of growth and occurrence in stratiform and domanl stromatolites confirm that non-filamentous algae are important in the formation of Aphebian stratiform stromatolites. Cells have well differentiated morphological details, such as protoplast, cell wall, and a laminated envelope (Fig. 1). Moreover, two of the taxa in these colonies contain dark inclusions in the protoplast. In the Bitter Springs microflora[1] and elsewhere similar structures have been interpreted as nuclei, and the fossils as eukaryotic, and the same evidence could be used for a similar interpretation of the Belcher material. On the other hand, the dark bodies may be granular inclusions of prokaryotes, or plasmolytic artefacts[2] of prokaryotes or eukaryotes, or possibly diagenetic relics.

Microfossils and possible microfossils are already known from the Belcher Islands[3,4]. Those reported here occur in black chert layers and pockets in laminated, stromatolitic carbonates of the Kasegalik and McLeary Formations in the lower part of the Belcher Supergroup[5,6]. These rocks form part of an Aphebian miogeoclinal sequence of sedimentary and volcanic rocks belonging to the Belcher Fold Belt, part of the larger Circum-Ungava geosyncline, the principal phase of deformation of which embraces the period of the terminal Aphebian Hudsonian orogeny. The latest available isotopic age determinations[7], based on the Rb-Sr whole-rock isochron method ($\lambda^{87}Rb = 1.39 \times 10^{-11}\ y^{-1}$), indicate a minimum of 1800 Myr for the sequence yielding the microfossils. Basement is not exposed in the Belcher Islands, but correlative units along the Manitounuk-Nastapoka arc to the east lie on Archaean basement which is 2,500 Myr old[8]. The general geology and geological history of the Belchers has been described by Jackson[4,6]. A more detailed study of the stratigraphy, sedimentology and palaeontology of the Belcher rocks is in progress (R. T. Bell and H. J. H., in preparation); this should lead to a better understanding of the Aphebian record there.

The new Belcher microfossils were obtained from the upper parts of the Kasegalik Formation on an unnamed island at the northern end of Churchill Sound (56°29.5′N

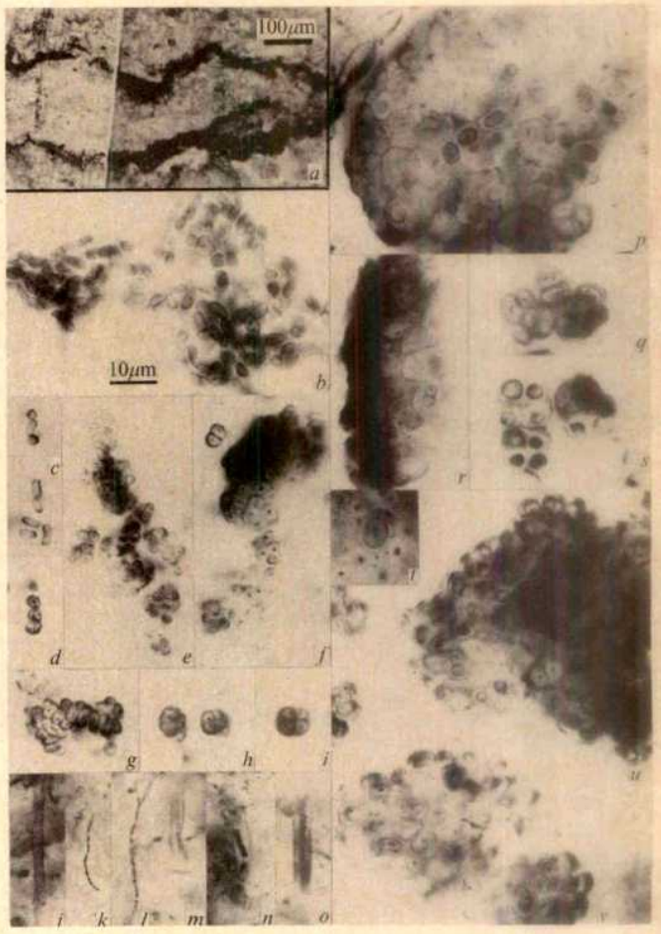


FIG. 1 Optical photomicrographs of stromatolitic lamellae and microfossils in thin sections from the Kasegalik Formation of the Belcher Islands. Compare the effects of carbonate diagenesis on dark, organic lamellae in the left third of (*a*) with the unaffected parts in silica matrix to the right. Magnifications for (*b*)–(*v*) are identical, and are ten times that for (*a*).

79°30.2'W), and from several levels within the McLeary Formation on Flaherty Island, along the south shore of Eskimo Harbour. Both formations show evidence of peritidal deposition. The geological setting of the stratiform and domal algal mats of the two formations, yielding the palmelloid colonies illustrated in Fig. 1, is probably a large sebkha/algal flat/shallow basin complex, as attested by abundant desiccation features, laminated structure and the presence of halite and sulphate casts[9].

In mode of preservation, level of organisation and palaeoenvironmental setting, the new Belcher assemblage compares with other Precambrian assemblages such as those in the Gunflint or Bitter Springs cherts, but it is distinguished from them by its relatively small diversity of species. This probably reflects its mature biocoenotic character of uniform, environmentally restricted algal mats, rather than a lack of diversity of organisms existing about 2,000 Myr ago.

The half-dozen new microfossils and the stromatolites will be described formally elsewhere after further investigations. For this report the microfossils are assigned to the following categories. (1) Loose aggregates of oblong bacterioid cells without inclusions and without envelopes, 1.5–2 μm wide and 3–4 μm long (Fig. 1*b* and *c*). (2) Colonial unicells in loose clumps; composed of spheroidal cells averaging 2 μm in diameter, with single dark inclusion and without envelope (Fig. 1*d–i*); some cells show evidence of division. (3) Compact, coherent colonial clusters and crinkled, pustulose stromatolitic layers composed of ellipsoidal to ovoidal cells with well differentiated protoplast, 3–5 μm in diameter, dark inclusions 0.5 μm across, cell wall, and a thick sheath which may be laminated around some cells and with an external form which is polyhedral because of close packing. (4) *Eomycetopsis*-like tubes 3–4 μm across, possibly representing empty sheaths of filamentous algae (Fig. 1*j*). (5) *Gunflintia*-like trichomes less than 1 μm across and up to 40 μm or more long, made up of a chain of dark spheroidal bodies (Fig. 1*k* and *l*). (6) Filaments composed of a chain of dark bodies 1 μm across, surrounded by a sheath 3–4 μm in diameter (Fig. 1*m-o*). This type may represent more nearly complete individuals composed of forms (5) and (6).

The biological affinities of these fossils are not known, although an algal origin for the colonies seems the most plausible. Nor is it established whether the different unicellular types are really different organisms or whether two or more represent different ontogenetic or degradation stages of the same species.

Of special interest are the types with dark bodies (Fig. 1*d–i*; *p–v*), with well defined protoplasts. Similar inclusions, though in larger microfossils, from the Late Precambrian have commonly been interpreted as nuclei[1]. Without these bodies, the size range and morphology would invite comparison with such modern blue-green algae as *Chroococcus*, *Microcystis*, *Entophysalis* and *Gloeocapsa*. Recent experimental evidence indicates that such dark internal bodies may be entirely plasmolytic artefacts[2]. However, this does not necessarily mean that all microfossils with internal dark spots so far described should be reassigned to the prokaryotes: it provides an alternative explanation. Other possibilities are that the structures are remains of metabolically-functional organelles, vacuoles, intracellular symbionts[10], or diagenetic artefacts. Present evidence, therefore, is inconclusive as to whether the structures are eukaryotic or prokaryotes preserved at different stages of plasmolysis. Evidence against the eukaryotic nature of the microfossils is the small size of the cells, and the lack of other unquestionable eukaryotes from rocks older than 1,000–1,200 Myr, although microfossils with attributes suggestive of eukaryotic organisation have been reported from the Aphebian[4,11]. Nevertheless, it is possible that the evolution of larger, chlorophytic algae with normal eukaryotic organisation began with the development of nuclear bodies and organelles in primitive, cyanophyte-sized, ancestors at least 1,800–2,000 Myr ago; the Belcher microfossils would represent such remains.

I thank Dr R. T. Bell, Brock University, for logistic support and advice in the field, Mr D. Krause, settlement manager of Sanikiluaq, for facilitating work in the Belcher Islands, and Dr S. M. Awramik, Harvard University, for a copy of the text and slides accompanying the abstract cited in ref. 2. This investigation was supported financially by the National Research Council of Canada.

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1 Schopf, J. W., *Biol. Rev. Camb. Phil. Soc.*, **45**, 319 (1970).
2 Awramik, S. M., Golubić, S., and Barghoorn, E. S., *Geol. Soc. Am. Abst. Prog.*, **4**, 438 (1972).
3 Moore, E. S., *J. Geol.*, **26**, 412 (1918).
4 Hofmann, H. J., and Jackson, G. D., *Can. J. Earth Sci.*, **6**, 1137 (1969).
5 Jackson, G. D., *Geol. Survey Canada, Papers*, **60-20** (1960).
6 Dimroth, E., Baragar, W. R. A., Bergeron, R., and Jackson, G. D., *Geol. Survey Canada, Papers*, **70-40**, 45 (1970).
7 Fryer, B. J., *Can. J. Earth Sci.*, **9**, 652 (1972).
8 Douglas, R. J. W., ed., *Geol. Survey Canada, Econ. Geol. Rep.* **1**, 5th ed. (1970).
9 Bell, R. T., and Jackson, G. D., *Can. J. Earth Sci.* (in the press).
10 Margulis, L., *Origin of Eukaryotic Cells* (Yale University Press, New Haven, 1970).
11 Hofmann, H. J., *J. Paleontol.*, **45**, 522 (1971).

Erratum

Unfortunately during preparation for the printer the axes of Fig. 2 were wrongly labelled in the letter "Ion fluxes in disk membranes of retinal rod outer segments" by W. T. Mason, R. S. Fager, and E. W. Abrahamson (*Nature*, **247**, 562; 1974). The corrected figure is now shown.

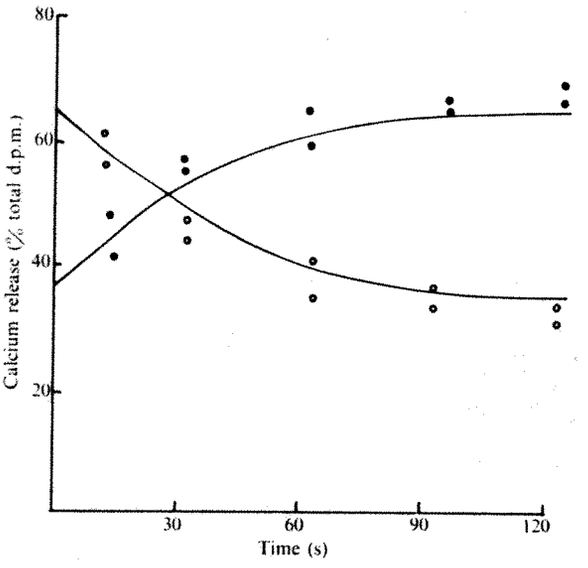


Fig. 2 Light-induced calcium release by sonicated bovine photoreceptor membranes over a time course of 120 s bleaching. Filled circles indicate percentage of Ca^{2+} in the extradisk space and open circles the percentage of Ca^{2+} in the intradisk space.

book reviews

Solid state

Theoretical Solid State Physics. By W. Jones and N. H. March. Vol. 1, *Perfect Lattices in Equilibrium*: pp. xvi+680; vol. 2, *Non-Equilibrium and Disorder*: pp. xv+681–1301. (Interscience Monographs and Texts in Physics and Astronomy.) (Wiley: London and New York, September 1973.) Each volume £14.75.

An introduction to Solid State Physics and its Applications. By R. J. Elliott and A. F. Gibson. Pp. xx+490 (Nature-Macmillan Physics Series) (Macmillan: London and Basingstoke, February 1974.) £5.95.

TEXTS on solid state physics are numerous. It is therefore pleasing to note the appearance of two new works for which substitutes are not already available. The two volumes by Jones and March give a comprehensive coverage of a large area of theoretical solid state physics and are directed toward professional theoreticians and postgraduate students. In contrast, the book by Elliott and Gibson is aimed at the undergraduate in the general area of material science and brings together the theory of solids and device applications.

The first volume of Jones and March includes band theory and crystal symmetry, collective effects in solids, lattice waves, spin and orbital magnetism, interactions of particles with a crystal. The second volume covers transport, optical properties, superconductivity, polarons and excitons, defects and disordered systems. Detail is developed in numerous appendices, and a set of problems and a basic bibliography are given.

In general the material is thoroughly covered and developed as far as more important current research papers. Techniques of many body theory are introduced in the text and developed in appendices, the fullest discussion being centred round the Coulomb interaction, though interactions involving phonons are also treated. The consequences of symmetry are also extensively discussed and an 83-page appendix on group theory is provided.

The chapter on band theory, typically, bridges the gap nicely between the limited treatment of a single volume treatise and the specialised monograph. Inevitably, for some sections, readers will prefer other texts. For an introduction to plasma effects and the electron-phonon interaction, I find Pines's *Elementary Excitations in Solids* clearer, although the present work does, with advantage, include detailed many body theory. Regarding the electron-phonon interaction, Pines's initial general survey establishes greater unity than the present separate discussions under phonons, transport and superconductivity.

A nice perspective is developed in the chapter on magnetism. The starting point is the general interacting electron Hamiltonian which leads to a study of the Heisenberg and Hubbard models and a clear appreciation of the approximations involved. The discussion of spin waves and magnetic phase transitions relates to neutron scattering theory which appears in the useful chapter on particles as probes for the study of solids.

Some topics have of necessity been omitted (polariton theory is one), and others chosen for only brief coverage: for example, infrared absorption under optical properties and the Kondo effect in the chapter on disorder. Otherwise this chapter is a full survey of a wide field of much recent activity.

This work, an extensive account of present understanding of solid state theory, is a valuable addition to the literature. The authors are to be commended for undertaking this mammoth task.

The opening two chapters of Elliott and Gibson discuss basic ideas of crystal structure and excitations. The reciprocal lattice is dealt with only briefly and the beginning student may need to refer to a more discursive text. The next two chapters cover lattice vibrations and electrons in bands. The basic theory of lattice vibrations is introduced, followed by neutron scattering results for various crystals, starting with the 'simple' rare-gas crystal and developing through more complicated types. The classification and comparison over a short space is particularly effective. Being of major importance in applications, the fifth chapter treats imperfections in some detail.

A survey of photon absorption over the range 10^{2}–10^{-3} eV in the section on optical properties puts the various absorption processes into perspective. The chapter finishes with optical emission and non-linear optics. The following two chapters deal with optical and microwave properties of free carriers, and transport which includes superconductivity and its applications. The ninth chapter is entirely device oriented (semiconductor junction devices) and the book concludes with theory and applications of magnetic materials. Problems and answers are provided.

Many students will find this book too demanding because of its conciseness in presentation of some basic theory. Hence it is unlikely to replace books such as those of Kittel or Dekker as a basic teaching source, although I expect it to be used widely in conjunction with these. On the other hand, the terseness in style does give the reader an excellent connected impression of the subject. The wood stands out clearly but the student may have to refer elsewhere for a delineation of some of the trees.

J. A. BLACKMAN

Unicellular eukaryotes

Protozoology. By Karl G. Grell. Pp. viii+554. (Springer-Verlag: Berlin and New York, 1973.) 107 DM; $43.90.

IN former times protozoology was considered to be a suitable subject by which students could be introduced to biology, presumably because, being single-celled animals, protozoa were thought to be simpler than so-called higher organisms, and from an evolutionary point of view they seemed primitive. Extensive courses describing many types of protozoa were commonly given and a number of solid textbooks were available, mainly in German, and largely, of course, of a taxonomic nature. Now all that is changed and few university courses treat protozoology in depth.

Protozoa are, however, important objects of study from a number of points of view. They provide admirable experimental material for many types of work, in genetics, nutrition, sexuality, behaviour, and so on. They combine the advantages of being single cells and also of being undoubted eukaryotes, that is, basically the same as cells of higher organisms. They can be used as models for the formulation and experimental testing of theoretical systems of cell organisation involving nuclei, various cytoplasmic components and the external surface. Finally, some protozoa cause exceedingly unpleasant diseases, some still very common and almost uncontrolled in large regions of the world.

The book by Karl Grell is, therefore, very welcome as it is the only one which attempts to discuss the subject in a modern context. Only about a quarter is given to taxonomy, the remainder

being devoted to a general treatment of morphology, cytology, genetics, nutrition, behaviour and so on. Sometimes indeed it seems that protozoology is really a non-subject, a miscellaneous assortment of different types of study on an extraordinarily diverse collection of organisms.

The excellent section on genetics, for example, is based mainly on studies with *Chlamydomonas* and *Paramecium* and includes a great variety of topics. The only feature which stands out is the relatively large amount of attention given to non-Mendelian heredity, involving chloroplasts, mitochondria, kappa particles and other odds and ends. A particularly detailed account of work on Foraminifera is given, reflecting the special interests of the author. On the other hand, important parasitic protozoa, such as the malaria parasites, trypanosomes, *Leishmania* and so on, seem to get rather less attention than they deserve.

The book is extremely well printed and beautifully illustrated, and can be thoroughly recommended to those who can afford it. English-speaking readers must be grateful to the author for writing the book in their language, (earlier versions were in German), though some, may find themselves, as I was, at times irritated and even baffled by some linguistic idiosyncrasies. On such occasions a little knowledge of German still helps.

G. H. Beale

Atmospheres of planets

Physics of Planetary Ionospheres. By Siegfried J. Bauer: Pp. viii+230. (Physics and Chemistry in Space: vol. 6.) (Springer-Verlag: Berlin and New York, 1973.) 78 DM; $35.10.

Dr Bauer has worked in the field of planetary ionospheres for more than fifteen years. Working at the NASA Goddard Space Flight Center he has been closely associated with many of the recent developments in the subject.

His book, intended as an introduction, develops logically from a discussion of neutral atmospheres, ionising radiations, chemical processes and plasma transport. In this respect it follows the pattern of many recent books on ionospheric physics. It differs from these in that at each stage it extends the discussion to the planets, particularly Mars, Jupiter and Venus. The space devoted to the other planets seems to be related (quite reasonably) to the thickness of their atmospheres.

In later chapters Dr Bauer discusses models of planetary ionospheres, wave propagation and experimental techniques. The treatment of the last two subjects is rather synoptic and the student may find it necessary to consult the many references for enlightenment. The last chapter gives details of the observations of planetary ionospheres and includes electron density profiles for Mars and Venus measured by the Mariner spacecraft. It is perhaps surprising that only nine pages are devoted to the discussion of these interesting results.

This book has many excellent qualities: the diagrams, tables and general layout are attractive and there is a comprehensive list of up to date references. The author is familiar with the Russian literature and his explanation of some of the Russian terminology will be useful to many. It has become standard practice in the United Kingdom to use SI units in scientific texts. Dr Bauer uses a mixture of c.g.s. and practical units but I found this no great disadvantage.

The author intended his book "to be an introduction to the subject for a new generation of space scientists and a compendium for the old". In my view he has been largely successful. But even at today's prices fifteen pounds is inordinately expensive for a book of 230 pages.

A. R. W. Hughes

Atomic time

The Measurement of Frequency and Time Interval. By L. Essen. Pp. v+55. (National Physical Laboratory: Teddington, December, 1973.) £1.

The rapid development of increasingly precise methods for the generation of specific (atomic) frequencies and their measurement has had a resounding impact on a vast range of theoretical disciplines and practical applications. Accurate control of frequency is a fundamental requirement of radio and it makes possible, for example, the Doppler measurement of rate of change of distance required in navigation and space research. Since 1967 the SI unit of time has been defined in terms of the frequency of a particular transition of the caesium atom, thus giving rise to the integrated reference time scale of International Atomic Time (TAI), obtained by the continuous summation of time intervals deduced from measures of frequency. Coordinated Universal Time (UTC), an approximation to UT1 (or GMT) and which differs from TAI by an integral number of seconds, is now the basis for civil time in most of the world; it provides direct synchronisation to about a millisecond, but individual atomic clocks may be compared with much higher precisions (tens of nanoseconds) making possible, for example, the almost direct verification of the predicted relativistic dependence of clock rate on speed and gravitational potential. A rather more practical application is Loran C for navigation.

This monograph, written by the acknowledged pioneer in the field, describes the basic principles and practical applications of the use of atomic transitions (or spectral lines) as standards of frequency. The treatment is simple and straightforward; the descriptions of the methods used at the National Physical Laboratory, given with explanatory diagrams and photographs, are exceptionally clear even to a layman in the field. Determinations of precision and stability are fully covered. Understandably the main emphasis is on the caesium definitive standard, but Dr Essen also covers working standards (commercial standards, which are portable, are now available with precisions of the order of 1 part in 10^{11}), standard frequency radio transmissions, and the techniques of the comparison of frequencies. His final chapter is devoted to the design and performance of resonance (particularly cavity resonator) frequency meters. It thus provides invaluable background information for the many concerned with the applications of these fascinating developments. But it seems that much of the text was written in 1972 so that those more directly interested in the latest work, especially at laboratories other than NPL, should refer to the technical journals.

Dr Essen devotes one short chapter to atomic and astronomical time, and refers briefly to the modified form of UTC introduced as from January 1, 1972. Although he refrains from saying so, it must now be generally agreed that the adoption in 1956 of the ephemeris second as the SI unit of time was a grave mistake: the astronomers were over-optimistic as regards the practical and theoretical difficulties of its determination, whereas the physicists were not agreed as to which of several possible atomic transitions should be used as the standard. Apart from confusion and change, the second of Ephemeris Time (introduced purely as a theoretical astronomical time scale) is not an approximation to the current value of the second of UT (which varies with the speed of rotation of the Earth), so that the preservation of its value in the 1967 unit results in an unnecessarily large rate of departure of IAT from UT. This has perhaps led Dr Essen to imply that the introduction of leap-seconds into UTC on the preferred dates of 30 June and 31 December would enable the modified time signals to be maintained within ± 0.7 s of UT1—so drastically contradicted on January 1, 1973.

In spite of this misunderstanding (due mainly to the shortcomings of CCIR Report 517) Dr Essen has a right to be proud of his contributions and of his readable and informative monograph.

D. H. Sadler

Attacking viruses

Selective Inhibitors of Viral Functions. Edited by William A. Carter, Pp. 376. (CRC: Cleveland, Ohio, 1973.) $39.95.

IT is the opinion of many animal virologists that, with one or two isolated exceptions, the successful treatment of virus diseases with specific anti-viral drugs is a subject which falls into the pipe-dream category. Optimists can be found, however, and the editor of this multi-author work clearly defines himself as one in his introductory remarks. He has assembled here a collection of reviews concerned with both laboratory and clinical aspects of specific anti-viral agents together with accounts of host reactions to viral infections and of immunisation against viral infections.

The natural recovery process from acute viral infections is dealt with comprehensively and concisely by W. E. Stewart. He clearly points out that it is still difficult to assess the relative importance of circulating antibody, cell-mediated immunity, interferon, and non-specific defence mechanisms. In the chapter on immunisation against viral infection, L. W. Marshall briefly discusses theoretical aspects and describes the viral vaccines and passive immunisation procedures currently used in human medicine.

A speculative article "Active Sites of the Animal viruses: Potential Sites of Specific Chemotherapeutic Attack" by W. M. Mitchell follows. I feel that this author's enthusiasm has rather carried him away from reality, and that non-specialist readers might imagine, for example, that the synthesis of biologically active human interferon by solid-phase methods was just around the corner. This impression, is, however, corrected by a chapter on interferon structure by R. Weil and F. Dorner. Other chapters on the effects of interferon, the molecular requirements for interferon induction, interferon induction by polynucleotides and non-nucleotide interferon inducers are contributed by E. De Clerq and W. E. Stewart, M. D. Johnston and D. C. Burke, A. K. Field and E. De Clerq respectively. It is apparent that one of the most important aims of interferon research—its purification to homogeneity—is proving an intractable problem.

The remainder of the book is concerned largely with synthetic and semi-synthetic anti-viral compounds and antibiotics. Amantadine hydrochloride and related compounds, arabinosyl nucleosides and nucleotides, and halogenated pyrimidines are discussed, largely in a clinical context, by C. E. Hoffmann, L. T. Chien, F. M. Schabel and C. A. Alford, and J. Sugar and H. E. Kaufman, and comprehensive accounts of the anti-viral properties of thiosemicarbazones, guanidine and 2-(α hydroxybenzyl) benzimidazole, rifamycin SV derivatives, and streptovaricins are given by W. Levinson, L. A. Caliguiri and I. Tamm, B. Moss, and D. M. Byrd and W. Carter. The book concludes with a short article by P. M. Pitha on the possibility of inhibiting virus growth by the use of viral genome analogues.

The quality of the contributions in this volume is generally high, although one or two early chapters show evidence of sloppy proof reading and reference checking. The book can be recommended as an up to date account of its subject, the comprehensive bibliographies which conclude most chapters being particularly useful.

T. H. PENNINGTON

Food for ruminants

Chemistry and Biochemistry of Herbage. Edited by G. W. Butler and R. W. Bailey. Vol. 1: pp. xx+639; vol. 2: pp. xiii+455; vol. 3: pp. xii+295. (Academic: London and New York, October 1973 and January 1974.) Vol. 1: £12; $36; vol. 2: £8; $25; vol. 3: £6; $17.

THESE volumes provide for the first time a comprehensive source of information on the chemistry and biochemistry of herbage defined as plant material excluding seeds or roots grown for feeding herbivorous animals.

Chapters 1-11 deal mainly with organic constituents of plants, free and bound amino acids, amines and ureides; proteins and nucleic acids; non-structural carbohydrates; structural carbohydrates; lipids; plant phenolics; lignin; alkaloids; cyanogenetic glycosides and glucosinolates; chlorophyll, carotenoid pigments and sterols; forage saponins. Most chapters follow a similar pattern describing chemical nature, classification, extraction from tissues and estimation of these compounds, amounts present in plants and their component parts, biosynthesis and amounts of components, for example amino acids in proteins and fatty acids in lipids.

Perhaps it would have been more logical to include chapters 1 and 2 of volume 2 on vitamins, and water in herbage, in volume 1, and chapters 12 and 13 in volume 1 on mineral composition of herbage and nutritional aspects of soil ingestion by grazing animals in volume 2, along with four chapters on mineral absorption and composition of herbage, mineral biochemistry of herbage, organic acids and their role in ion uptake, and cycling of minerals in ecosystems.

Commendably, a good deal of attention has been given to linking treatment of subject matter, for example in the above chapters on minerals, and in chapters on physiology of light utilisation by swards, growth senescence and stress, and organic reserves and regrowth. Further chapters in volume 2 deal with the biochemistry of photosynthesis, symbiotic nitrogen fixation by legumes, the nitrogen cycle of pasture ecosystems and genetic variation in herbage constituents, this last chapter making a fitting end to volume 2 and prelude to volume 3, in its consideration of problems encountered in breeding improved varieties and emphasis on the need to evaluate results against animal performance.

The more applied chapters of volume 3 deal with conservation of herbage as hay and silage, leaf protein as an animal and human foodstuff, digestion of pastures by ruminants and other herbivores, the feeding value of herbage and its evaluation by laboratory methods, and limitation to the productivity of the herbage-fed ruminant that arise from diet, including the effects of toxic components and metabolic diseases.

Advanced students, teachers and research workers will find here an invaluable source of information, for example on assessment of methods of extraction of compounds from tissues, and on areas where, because of paucity of information on herbage plants, recourse has been made to non-herbage tissues, thus pointing to the need for further research. Examples of this are the description of biosynthesis of lipid components in spinach, chorella and bacteria, detailed studies of isolated cellulose from wood and commercial fibres, the need to assume that *in vivo* formation and interconversion of carbohydrates in leaves and stems are probably similar to those in seeds and tubers, and the belief that protein synthesis in plant cells is essentially similar to the mechanism worked out for bacteria and mammalian cells. Attention is constantly focused on the need for further research, for example, in comparing information available on temperate and tropical herbage and in the search for methods of measuring the nutritive value of herbage.

An attractive feature of the work is that agronomists and plant breeders will find useful biochemical treatment of several subjects such as ensilage and ruminant digestion, biochemists will find limitless avenues for research, while the ruminant, the ultimate beneficiary, is frequently brought into the picture. A worthwhile attempt, attended by considerable success, has been made to bridge the all-too-wide gap which frequently separates pure biochemists and workers in agricultural science.

On the whole the work is readable and, bearing in mind the diverse nature of topics covered in what are, for the most part, review chapters with appropriate lists of references, a reasonably even standard of treatment is achieved.

A good deal more attention could have been given to some topics however. The vitamins, for example are covered in only 11 pages, with no mention of important modern aspects such as vitamin D metabolites and the tocotrienols, and little consistency in description of symptoms of deficiencies of individual vitamins. Again, although commendable efforts have been made to take examples from many countries, this has not always been achieved: in chapter 27 all tables of hay composition are taken from the United States.

The volumes are attractively produced and the text contains few typographical errors. Minor blemishes noted in volume 2 include reference to "some mineral, possibly pectin" (page 110) and an obvious omission on page 119 (par. 2). Arrangements of some tables could also be improved.

W. M. Ashton

NMR for enzymes

Nuclear Magnetic Resonance in Biochemistry: Applications to Enzyme Systems. By Raymond A. Dwek. Pp. xviii+395. (Clarendon: Oxford; Oxford University: London, December 1973.) £8.

During the past ten years many attempts have been made to apply nuclear magnetic resonance (NMR) techniques to problems of biological interest and the subject has reached the stage when a comprehensive textbook is required to summarise and appraise the advances in this area. Thus Dr Dwek's book is opportune and is all the more welcome because this first comprehensive text on the subject is also a very good book.

In the chapters devoted to protein spectra, protein-ligand binding and the use of paramagnetic probes in such systems, the book indicates the extent to which NMR spectroscopy can be used to obtain three-dimensional structural information in enzymes, to identify functional groups involved in enzymatic reactions and to investigate the role of metal ions and coenzymes in enzyme systems. Dr Dwek's book attempts to provide a balanced view of the usefulness of the NMR technique in some of these areas by considering both its possibilities and limitations.

Adequate background NMR theory is given to allow for the understanding of the many applications considered. Emphasis is given to the theory of relaxation, exchange and paramagnetic shifts and relaxation because many of the more successful applications rely heavily on these aspects of magnetic resonance. Fortunately, Dr Dwek's research interests are in these areas and this is evident in his authoratitive coverage of relaxation effects related to paramagnetic metals and spin-label probes.

The book is well illustrated throughout with clearly labelled diagrams (although the labelling on the AMX spectrum in Fig. 2.14 is incorrect).

It is a pity that the chapter on protein NMR spectra was not introduced with a detailed section on amino acids and peptides. The problems encountered in trying to determine conformational information for small peptides would have put into perspective Dr Dwek's statement that "in principle, it should be possible to obtain from the spectrum the complete structure of a protein in solution".

The chapter on carbon-13 studies neglected to indicate the potential for studying strongly bound ligands in slow exchange (using ^{13}C enriched ligands to overcome the protein background spectrum) and also the advantages of studying ^{13}C relaxation times.

These are however relatively minor criticisms of an excellent book which can be recommended to anyone interested in learning more about enzyme systems.

J. Feeney

Echo technology

Radar, Sonar and Holography. By Winson E. Kock. Pp. xvii+140. (Academic: New York and London, December 1973.) $9.50; £4.45.

This book is intended to introduce high school and college students to the technology of radar and sonar, the principles of holography, and the impact of holography on echolocation. Its explanations successfully avoid the use of mathematical concepts or formulations. The opening chapters introduce waves, coherence, interference, diffraction, near and far fields and zone plates, radiators and collimators. But the author does not, for example, derive the diffraction beamwidth of a slit from interference between its left and right halves, or explain the Rayleigh distance as the intersection of the near-field projected aperture and the divergent far-field beam.

Next the book outlines the principles of echolocation, and gives examples of conventional radars and sonars. There is, however, no mention of surface ducts, self or radiated noise, reverberation, or the uses of Doppler in sonar. There is a chapter on Doppler radar, but the Doppler effect is not explained. Though no reference is made to normal pulse-Doppler radars, dual-frequency systems are mentioned—without indicating that their Doppler effect is determined by the difference frequency.

Next the book introduces monochromatic optical, acoustic holograms, lucidly explained as a generalisations of the zone plate. This leads on to synthetic-aperture side-scanning aircraft radars, which are well described by analogy to sequentially-generated holograms. The basic commonality of different configurations could, however, with advantage, have been explained in terms of reciprocity and symmetry. The use of coherent optics to synthesise the high-resolution near-field radar picture is clearly delineated.

Thereafter the author introduces some rather dubious material: he implies that large-aperture real arrays are a special case of synthetic-aperture systems, and have become possible only with the advent of holographic processing. He suggests that the number of elements in a given array aperture can be reduced without loss. (A reduced hologram can cover the same total picture, but only with an appropriately reduced resolution and/or contrast.) He fails to equate the gain from a synthetic-aperture end-fire system to that from a conventional coherent Doppler integrator; indeed there is no reference to the equivalence of coherent integration in the space and time domains. After recognising the equivalence between moving target indication (that is velocity null generation) and end-fire directivity (that is, rearward directional null generation), the author spoils it by stating that MTI is restricted to targets moving directly towards the radar.

The book describes pulse compression —but fails to distinguish between power and energy, or to indicate how 'chirp' frequency modulation is handled in practice. The elegant technique of 'zone-plate' amplitude modulation for optical pulse compression is explained, but not the more usual pseudo-random modulation and correlation detection. In discussing phased-array systems, the author extols holographic techniques for receiver beam forming, without reference to the range dimension (echo delay), within-pulse scanning, real-time processing, or adaptive beam steering.

Though open to such criticisms in detail, the book is very readable and well illustrated. It gives clear concise explanations of some modern concepts which are not well covered in popular technical literature. There are a few slips in proof reading, editing, history and theory—but probably no more than usual.

I feel the link between holography and echolocation has been overemphasised. I would also have opted for a less catholic coverage of 'with-it' technology and assorted applications, to make room for more explanation of physical principles and practical constraints. But this may merely reflect my preference for a book aimed at a somewhat more sophisticated readership. The author has probably met his own objectives and those of his senior high school readers well enough.

Ralph Benjamin

matters arising

Calibration of the radiocarbon time scale

SIR,—The recently published paper by Clark and Renfrew[1] on calibration of radiocarbon dates is the latest in a series on this topic[2–5] by archaeologists who are rightly concerned by the uncertainties now involved in interpretation of data. Central to those papers has been the attempt to 'smooth' the bristlecone calibration curve of Suess[6] to negate 'kinks' and thereby ease interconversion of radiocarbon and calendar ages. Here I sound a cautionary note on such practices and question the validity of the smoothing methods. There is no dispute here over the detailed statistical techniques invoked by the various authors. The issue in question is the overworking of experimental data. There is little doubt that the methods employed are justified neither by the quality of the ^{14}C results nor by the present understanding of ^{14}C geochemistry.

The key issue is centred on the vital difference between the absolute accuracy and analytical precision off the ^{14}C measurements which comprise the bristlecone pine calibration curve. It is important to consider the inequality of these parameters. The error terms reported with ^{14}C data are derived essentially from the statistical ^{14}C counting error alone. To assess the real accuracy of the measurements, however, requires an answer to each of the following questions:

(1) Is the ^{14}C level of wood grown at an altitude around 8000 feet indicative of concentrations at sea level and at all other latitudes?

(2) Is the ^{14}C content of wood fractions other than the cellulose component necessarily a precise measure of atmospheric ^{14}C levels during wood growth?

(3) Can experimental unknowns, such as counter behaviour, contamination effects, human error, standardisation and half life uncertainty, be totally neglected? Furthermore, are systematic differences between ^{14}C laboratories possible?

To each of these questions there can, as yet, be no ideal answer. Comment can, however, be made:

(1) At the extreme altitudes where the Bristlecone Pine grows, the ambient cosmic ray neutron flux is approximately one order of magnitude higher than at sea level. Since these neutrons are the source of ^{14}C through the reaction $n + {}^{14}N \rightarrow {}^{14}C + p$, it is feasible that bristlecone pine wood could be non-representative of normal organic material. Whether through *in situ* ^{14}C production or variable atmospheric mixing, bristlecone pine ^{14}C levels could experience localised effects. Furthermore, there is already preliminary evidence that atmospheric ^{14}C levels may be latitudinally dependent. Thus Jansen[7] and Lerman *et al.*[8] have observed depletions of 0.5% to 2% in ^{14}C concentrations of southern relative to northern hemisphere wood.

(2) It has been shown with fair certainty that some chemical components of wood can be transported between tree rings[9]. Cellulose is chemically the most inert constituent of wood and, to minimise cross contamination, should be selected for precise ^{14}C analyses. Perhaps due to the limited size of samples, the major American laboratories engaged in time scale calibration do not take this precaution.

(3) It would be unrealistic to imagine that either systematic differences between laboratories or occasional freak results within one laboratory do not occur. There are many potential causes of such deviations.

The factors discussed here cannot be quantitatively assessed at present because of incomplete evidence. Only future research can clarify the situation and, for this reason, independent calibrations of the radiocarbon time scale, such as now being developed at Queen's University, Belfast, are of major significance. In the meantime a realistic approach to the available ^{14}C data is essential. In other words, besides the analytical error reported for each bristlecone pine ^{14}C analysis, there exists a further major error term to be considered. At present we cannot quantify it. Nevertheless, in view of the geochemical aspects of the factors mentioned, it would be unwise to attribute a degree of absolute accuracy less than $\pm$ 2% (2σ) to the bristlecone calibration curve (unless the archaeological specimen in question is a pinewood sample from an American mountain top and is pretreated and analysed by Suess). A 1% error in ^{14}C content corresponds to a radiocarbon age error of 80 yr so that, including reported analytical errors, it seems at present unjustified to attribute an accuracy of less than $\pm$ 200 yr to the calibration curve. In essence then the curve should be imagined not as a line but as a probability band of considerable spread (400 yr). Through this approach a realistic interpretation can be performed. Admittedly, the value of the calibration curve is severely reduced but this is the real situation which no mathematical manoeuvring should obscure.

Finally, I note that the simple approach of Suess, in adjoining ^{14}C data by hand, is in many respects preferable to smoothing operations by computer. Our need at present is for awareness of the limits of available data and for an increased availability of such results. Smoothing operations, in their role of rendering the experimental data remote, are contrary to this requirement. In addition, given the major error terms inherent in the curve, there is little reason for sophisticated mathematics. Kinks may well exist. The real existence of one such anomaly is beyond dispute[10]. It is questionable therefore whether at this time it is objective to smooth them out.

So I urge patience and restraint. Calibration, if performed at all, cannot yet be as quantitative as would be desired. There can be no justification for the delusion that major uncertainties do not exist. Over interpretation of the calibration curve for archaeological purposes is, for the time being, unwarranted.

Yours faithfully,

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1 Clark, R. M., and Renfrew, C., *Nature*, **243**, 266 (1973).
2 McKerrell, H., *Scottish Archaeological Forum*, **3**, 73 (1971).
3 Renfrew, C., *World Archaeology*, **2**, 199 (1970).
4 McKerrell, H., *Proc. prehist. Soc.*, **38**, 286 (1972).
5 Clark, R. M., and Renfrew, C., *Archaeometry*, **14**, 5 (1972).
6 Suess, H. E., in *Radiocarbon Variations and Absolute Chronology* (edit. by Olsson, I. U.), Plate I (Wiley, New York, 1970).
7 Jansen, H. S., *ibid.*, 261.
8 Lerman, J. C., Mook, W. G., Vogel, J. C., and De Waard, H., *Science*, **165**, 1123 (1969).
9 Fairhall, A. W., and Young, J. A., in *Radionuclides in the Environment* (edit. by Gould, R. F.), 401 (Am. Chem. Soc., Washington, 1970).
10 Dr Vries, H., *Koninkl. Ned. Akad. Wetenschap., Proc. Ser. B*, **61**, 94 (1958).

SIR,—Clark and Renfrew[1] state that "the hypothesis of a common calibration curve for the bristlecone pine and Egyptian data over the time period 3100 BC to 1800 BC is not contradicted and suggestions to the contrary are not well founded". But according to Fig. 2 of ref. 1, all but two of the 14 Egyptian data points for the period 2200 BC to 1800 BC indicate ^{14}C dates that are less recent than indicated by the curve shown for the bristlecone pine, on average by one or two centuries. Consequently the statistical conclusion is difficult to accept; this viewpoint was put to one of the authors of ref. 1 by McKerrell at the 1973 Oxford Archaeometry Symposium, in support of his contention[2] that the bristlecone pine calibration is not consistent with the Egyptian data; but no explanation of the apparent paradox was then offered.

A close inspection of the figure gives an explanation. In the period 2100 BC to 1900 BC there is a substantial difference between the bristlecone pine curve and the tree ring data points that were used for the statistical analysis; this difference is in the same sense as for the Egyptian data. So the analysis by Clark and Renfrew cannot be held to contradict McKerrell's contention.

The tree ring data were taken from the unpublished thesis of Houtermans[3] and comparison of these with the data points shown by Suess on the bristlecone pine curve[4] confirms that there are differences between the two sets; since both sets originate from the same measurements one must presume that corrections have been applied to one set which have not been applied to the other. In the absence of other indications it seems reasonable to accept the published data rather than the unpublished. It is also relevant that using either of the bristlecone pine curves put forward by the Pennsylvania[5] and Arizona[6] radiocarbon laboratories (which incorporate the Suess data with later measurements) the Egyptian ^{14}C dates shown for ~ 2000 BC are still significantly less recent than bristlecone pine ^{14}C dates, though by not quite so much.

Yours faithfully,

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Received August 3, 1973.

1 Clark, R. M., and Renfrew, C., *Nature*, **243**, 266 (1973).
2 McKerrell, H., *Proc. prehist. Soc.*, **38**, 286 (1972).
3 Houtermans, J. C., thesis, Univ. Berne (1971).
4 Suess, H. E., in *Radiocarbon Variations and Absolute Chronology* (edit. by Olsson, I. U.), Plate I (Wiley, New York, 1970).
5 Michael, H. N., and Ralph, E. K., *Proc. 8th int. Conf. on Radiocarbon-dating, Lower Hutt City*, (edit. by Rafter, T. A., Grant-Taylor, T.), 28–43 (R. Soc. New Zealand, 1973).
6 Damon, P. E., Lond. A., and Wallick, E. I., *Proc. 8th Int. Conf. on Radiocarbon-dating, Lower Hutt City*, (edit. by Rafter, T. A., Grant-Taylor, T.), 45–59 (R. Soc. New Zealand, 1973).

DRS CLARK AND RENFREW REPLY: We agree with many of the points made by Baxter and in a paper now in the press[1] discuss the substantial statistical problems involved in constructing an adequate calibration curve from the data available. But the construction of a calibration curve was not, as Baxter seems to imply, the purpose of our recent paper[2]. Instead an evaluation was sought of the general applicability of the bristlecone pine data and their associated radiocarbon dates. The radiocarbon dates obtained from dendrochronologically dated bristlecone pine samples will match radiocarbon dates from historically/archaeologically dated Egyptian samples only if satisfactory answers could be given to his three questions: a precise equivalence would give exactly the independent corroboration which he is seeking. In fact, although the two sets do not contradict within the limits of their associated errors, the match is an inexact one because of the magnitude of those associated errors.

There should be no question here of overworking experimental data. The procedure used was to present, apparently for the first time, a formal analysis of the consistency of the determinations from different laboratories, working always through a consideration of the measurement errors involved (both as reported by the laboratories themselves, and as established from replicate analyses). Is this not in fact the only acceptable way of comparing two independent data sets?

The geophysical problems which Baxter indicates are not new, and will only be answered by statistical analysis of existing data and by the gathering of new data in such a way that it will be susceptible to statistical analysis. He is certainly right to stress that the appropriate form of the calibration curve is not yet clear, and that an error of perhaps ±200 yr is to be associated with the curve[3].

Aitken is correct that the data presented by Houtermans and by Suess are not in precise agreement: we chose to use the former because they were more recent and did not have to be read from a graph. Aitken's reference to Fig. 2 of our paper is not, however, to the curves used by us to compare the bristlecone pine and Egyptian data, but to Suess's original curve presented there for purposes of comparison only. If the data points of Fig. 2 are compared with the curves derived from those data, there is no contradiction. Consider, for example, the least-squares straight line. If the hypothesis (of a common calibration curve) is correct, one would expect roughly half of the data points to lie below this line. Inspection of Fig. 2 shows that, between 2200 and 1800 BC: (i) 5 of the 14 Egyptian points are below this line; (ii) 10 of the 19 bristlecone pine points are below this line.

Such a distribution of the 14 Egyptian points is not unusual; the probability of five or fewer points out of 14 being below the line is about 0.20 (from the Binomial distribution), so that there is no reason to doubt on this basis that the Egyptian points satisfy the same calibration relationship as the bristlecone pine data. The same argument could be applied to the entire interval from 3000 to 1800 BC. Figure 2 shows that 23 of the 52 Egyptian points lie below the fitted straight line, whereas 27 of the 57 bristlecone pine points lie below this line. One could hardly expect to get much closer to 50% than that.

It remains our conclusion that, on the basis of the available data considered, and within the error limits discussed (themselves unfortunately wide), there is no contradiction between the Egyptian and bristlecone pine data. This is an important conclusion since for some parts of prehistoric Europe the calibrated radiocarbon chronology differs by as much as 2,000 yr from archaeological chronologies put forward before the application of radiocarbon dating. At the same time, as Baxter rightly stresses, the error limits are large, and much more work will be needed before the form of the appropriate calibration curve will be known with precision.

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1 Renfrew, C., and Clark, R. M., *Archaeometry*, **16**, (1974, in press).
2 Clark, R. M., and Renfrew, C., *Nature*, **243**, 266 (1973).
3 Burleigh, R., Switsur, V. R., and Renfrew, C., *Antiquity*, **47**, 314 (1973).

Na^+ transport defect in cystic fibrosis

SIR—Using the technique of ^3H-ouabain binding to isolated cells Quissell and Pitot concluded that the Na^+–K^+ ATPase functions normally in fibroblasts from cystic fibrosis patients[1]. However this finding may not be very revelant to the Na^+ transport defect occurring in this genetic disease.

The Na^+ transport abnormality in cystic fibrosis seems to be confined to exocrine glands[2]. It is unknown at present whether the defect resides in an active or passive transport step of the cation. At least with isolated sweat

gland tissue[3] and erythrocytes[4] from cystic fibrosis patients, Na^+–K^+ ATPase activity has been found to be normal. However a decrease in the ethacrynic acid-sensitive efflux[5,6], a Na^+ exchange mechanism[5], has been reported in cystic fibrosis erythrocytes[6,7]. These findings suggest that there is no fundamental abnormality in the Na^+–K^+ ATPase enzyme in the disease, but there may be a defect in an ethacrynic acid-sensitive transport step. Therefore in order to obtain a better understanding of the cation transport defect one should measure the binding of labelled ethacrynic-acid rather than ouabain.

It is possible, however, that there is no basic abnormality in the mechanism of Na^+ transport in cystic fibrosis but in the action of agents regulating this process[8,9]. Membrane transport of this cation is controlled by hormones and cyclic AMP in a tissue specific manner[10]. Failure of a Na^+ transporting system in exocrine glands to respond to a specific hormone or cyclic AMP could explain why the defect is confined to these organs. Isolated model systems like fibroblasts and erythrocytes might not therefore show up this defect.

Yours faithfully

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Drs Quissell and Pitot respond:

Due to the pathophysiology of cystic fibrosis, conclusions derived from data obtained from intact patients with the disease or from biopsy material obtained from CF patients must be carefully scrutinized. Several factors, such as anoxia, infection, malabsorption, liver and pulmonary involvement, and the nutritional and hormonal status of the patient complicate the interpretation of these data.

The Na^+ transport defect could be closely related to the genetic defect or the Na^+ transport defect could be secondary. The small salivary glands and the eccrine sweat glands are affected by the electrolyte defect in cystic fibrosis[11] but the submaxillary and parotid glands secrete normal levels of sodium[2,12].

The decrease in the ethacrynic-acid-sensitive efflux observed in cystic fibrosis erythrocytes[6] is difficult to interpret[13]. Lapey and Gardner[7] observed a normal sodium efflux in heterozygotes and in younger females with cystic fibrosis. The mechanism of action of ethacrynic acid on the overall membrane transport process is not known. In fact, the inhibitory process may involve one or several intracellular metabolic events rather than events on the membrane *per se*[14,15]. Ethacrynic acid does not seem to bind specifically to the membrane but to all cellular fractions[16]. Cystic fibrosis patients seem to have normal kidney function except for a lower metabolic clearance rate and a higher plasma aldosterone level which is probably due to the excessive loss of sodium in the sweat and a subsequent decrease in the intravascular volume[17].

If an alteration in the hormonal response in cystic fibrosis exocrine tissue is observed, the alteration could be intimately related to the primary defect in the disease but the alteration could be secondary to its pathophysiology. Careful evaluation will be required but the results should be very interesting.

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1 Quissell, D. O., and Pitot, H. C., *Nature*, **247**, 115 (1974).
2 diSant, Agnese, P. A., and Talamo, R. C., *New Engl. J. Med.*, **277**, 1344 (1967).
3 Gibbs, G., Griffin, G., and Reimer, K., *Pediat. Res.*, **1**, 24 (1967).
4 Horton, C. R., Cole, W. Q., and Bader, H., *Biochem. Biophys. Res. Comm.*, **40**, 505 (1970).
5 Dunn, M. J., *J. clin. Invest.*, **49**, 1815, (1970).
6 Balfe, J. W., Cole, C., and Welt, L. G., *Science*, **162**, 689 (1968).
7 Lapey, A., and Gardner, J. D., *Pediat. Res.*, **5**, 446 (1971).
8 Duffy, M. J., and Schwarz, V., *Lancet*, ii, 136 (1972).
9 Duffy, M. J., and Schwarz, V., *Clin. chim. Acta*, **49**, 397 (1973).
10 Orloff, J., and Handler, J., *Am. J. Med.*, **42**, 757 (1967).
11 Weisman, U. N., Boat, T. F., and diSant' Agnese, P. A., *Lancet*, ii, 510 (1972).
12 Weismann, U. N., Boat, T. F., and diSant' Agnese, P. A., *J. Pediat.*, **76**, 444 (1970).
13 Hadden, J. W., Hansen, L. G., Shapiro, B. L., and Warwick, W. J., *Proc. Soc. exp. Biol. Med.*, **142**, 577 (1973).
14 Epstein, R. W., *Biochim. biophys. Acta*, **274**, 128 (1972).
15 Sato, K., *Pflugers Arch.*, **341**, 233 (1973).
16 Epstein, R. W., *Biochim. biophys. Acta*, **274**, 119 (1972).
17 Simopoulos, A. P., Lapey, A., Boat, T. F., diSant' Agnese, P. A., Bartter, F. C., *Pediat. Res.*, **5**, 626 (1971).

obituary

Sir Charles Dodds

SIR CHARLES DODDS, Bt., biochemist and physician, who died on December 16, 1973, was at the time of his retirement in 1965 the senior professor in the University of London, having been elected at the age of 25 to the newly created Courtauld Professorship of Biochemistry at the Middlesex Hospital Medical School. Two years later, when the Courtauld Institute of Biochemistry was built he was its first Director, an appointment that he was to occupy with the highest distinction for 38 years. His originally tiny staff of three graduates expanded steadily so that due to his stimulating and inspiring leadership the Institute has acquired a fine reputation for both teaching and research.

Edward Charles Dodds was born on October 13, 1899, brought up in Darlington and moved with his parents to London, where he was educated at Harrow County School and the Middlesex Hospital Medical School, supporting himself by various scholarships and, later by tutorials. He was entirely a 'Middlesex man', who never seemed to wish to work elsewhere. Very early he showed his life-long interest in hormones, developing a method which proved useful for preparing insulin for the Hospital.

When they were both 25, he and F. Dickens wrote *Chemical and Physiological Properties of the Internal Secretions*. Dodds meanwhile was in charge of the Chemical Pathology and pioneered in this country the colorimetric methods of analysis which Folin had recently introduced in the United States. He was called in during the illness of King George V and, then aged 29, received the MVO. With George Beaumont he produced *Recent Advances in Medicine*

which ran to 14 editions. In more recent years, he was one of the first to introduce automation into clinical biochemical analysis in this country.

Dodds own research reached its highest point in the discovery of the highly potent artificial oestrogenic hormone, diethyl stilboestrol and its relatives. With Wilfrid Lawson, he explored the weak activity which they had detected in a range of simple non-steroidal compounds, until they found a highly active by-product arising in the demethylation of the simple phenolic ether anethole. They discovered the structure and synthesis of the compound, in work with Sir Robert Robinson, in 1938. Stilboestrol was even more active than the natural hormone, could be manufactured in quantity, and was effective when given orally. In addition to its value in gynaecology, it proved, following the lead of Charles Huggins, to be the first oral drug to be able to control a form of cancer—carcinoma of the prostate—even when the disease was disseminated.

The honours and decorations conferred on Dodds over the years occupy a whole column of Who's Who, but among these were his Knighthood in 1954 and Baronetcy 1964. He was elected FRSE. 1941; FRS 1942 (Vice-President 1957-9); FRCP 1933 (Harveian Librarian later) and President of the Royal College of Physicians in 1962—unique for a professor of biochemistry to be so honoured. He held honorary degrees from universities all over the world, was widely travelled and had lectured—though this did not come easily to him—in many countries. He succeeded Lord Horder as Chairman of the Scientific Committee of the Cancer Research Campaign; an excellent chairman, he was in constant demand in this capacity, serving on numerous Government and other scientific committees. His medical school owes to him the revolutionary reorganisation of the teaching of biochemistry, and of chemistry, which he insisted should be integrated subject; he built up a devoted staff of gifted teachers.

Though somewhat shy by nature, he was never aloof with his colleagues, always ready to discuss their problems, tempering his shrewd advice with a nice touch of humour. It delighted him when he became Master of the Society of Apothecaries in 1947–9, and nothing pleased him better than entertaining his many friends to good food and good wine, on which he was something of an authority.

His marriage in 1923 to Constance Elizabeth Jordan of Darlington was a happy one and he never seemed to his close friends quite to recover from her death in 1969. Their only son Ralph succeeds to the baronetcy.

Announcements

Corrigendum

In the article "Heavy ion transfer reactions in nuclear astrophysical processes" by T. W. Conlon (*Nature*, **247**, 268; 1974) the third paragraph from the end should read as follows. "A study of the α-transfer reaction $^{12}C(^{16}O,^{20}Ne)^{8}Be$ at energies in the region of the Coulomb barrier has been made possible by the development of a particle identification system based on position-sensitive counters mounted in the focal plane of a Buechner spectrograph. Preliminary results indicate that significant amounts of ^{20}Ne are produced with respect to ^{24}Mg, which results from the principal compound nucleus channel (Table 2)."

In the same article, the following corrections should have been made; para. 1, line 12 should read "corresponds to the transfer of an α particle, . . ."; para. 10, line 5, "available for later evolutionary . . ." and the last column in Table 1 should be "Q optimum (MeV) corresponding to (E_{min} to E_{max})".

International meetings

May 14, **Our Environment—Museum or Habitat?** (Lisa Strange, The Ship and Boatbuilders' National Federation, 31 Great Queen Street, Kingsway, London WC2B 5AD)

May 15, **The Possible Role of Anti-Tumour Immunity in Radiotherapy** (The General Secretary, British Institute of Radiology, 32 Welbeck Street, London W1M 7PG)

May 16, **Friction, Wear and Lubrication** (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX)

May 19–22, **Second International Banff Conference: Man and His Environment** (Miss E. M. Buchanan, Conference Supervisor, Division of Continuing Education, The University of Calgary, Calgary, Alberta T2N 1N4)

May 20–23, **The Impact of Offshore Oil Operations** (Mrs Jill de Wardener, The Institute of Petroleum, 61 New Cavendish Street, London W1M 8AR)

May 20–23, **5th National Convention of the Royal Australian Chemical Institute** (Professor B. J. Ralph, School of Biological Technology, University of New South Wales, P.O. Box 1, Kensington N.S.W. 2033, Australia)

May 20–24, **2nd International Symposium on Biotelemetry** (ETHZ Turnen and Sport, Labor. für Biomechanik, Plattenstrasse 26, CH-8032 Zürich, Switzerland)

May 20–25, **7th World Congress on Occupational Safety and Health** (World Congress Secretary, Ansley House, Dublin 4, Ireland)

May 21–23, **Rubber Processing 1974** (Martin J. Quinlan, Publications Officer, The Institution of the Rubber Industry, 4 Kensington Palace Gardens, London W8 4QR)

May 21–24, **The Science and Technology of Powders and Fine Particle Systems** (Institute for Fine Particles Research, Summer School, 1974, Laurentian University, Sudbury, Ontario, Canada P3E 2C6)

May 22–24, **Workshop/Symposium on Compliance with Therapeutic Regimens** (David L. Sackett, Room 3H2, McMaster University Medical Centre, 1200 Main Street West, Hamilton, Ontario, Canada L8S 4J9)

May 23, **Structure and Function of Intracellular Junctions** (R. C. Hughes, National Institute for Medical Research, Mill Hill, London NW7 1AA)

May 24, **The Future of the Polytechnics** (The Secretary and Registrar, The Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend-on-Sea, Essex SS1 2JY, UK)

May 29–31, **28th Annual Symposium on Frequency Control** (Commander, US Army Electronics Command, Attention: AMSEL-TL-MF, (J. R. Vig), Fort Monmouth, New Jersey 07703)

May 29–31, **26th Annual Brookhaven Symposium in Biology: Processing of RNA** (John J. Dunn, Biology Department, Brookhaven National Laboratory, Upton, New York 11973)

May 29–June 1, **Electronic Properties of Oxides: Applications and Science** (Professor R. W. Vest, School of Materials Engineering, Purdue University, West Lafayatte, Indiana 47907)

May 29–June 2, **5th International Congress of Cytology** (Alexander Meisels, Secretary General, 5th International Congress of Cytology, 1050, Chemin Ste. Foy, Quebec 6, PQ, Canada)

May 31–June 1, **International Symposium on Jaundice** (Gordon Awade, Executive Director, The Canadian Hepatic Foundation, 65 Queen Street West, Toronto, Ontario, Canada)

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APPOINTMENTS VACANT

KENNEDY INSTITUTE OF RHEUMATOLOGY

Have you seen our notice on page xv?

(1606)

PAPUA NEW GUINEA UNIVERSITY OF TECHNOLOGY

LECTURER IN CIVIL ENGINEERING

Applications are invited for the above-mentioned position in the Department of Civil Engineering. The work of the Department is organised into the sub-disciplines of Structures, Soils, Transport, Hydraulics, Public Health and Local Government Engineering.

Applicants must possess a good first degree and, preferably, a higher degree together with appropriate professional experience. The successful applicant in addition to lecturing duties will be required to undertake research and consultative work in one or more of the above-mentioned fields.

The academic establishment of the Department is 10 staff from the level of Technical Instructor to Professor. Laboratories are equipped to Australian University standards and each has supporting technical staff. Computer facilities are available in the form of two Nova 1200 series mini-computers for batch and time share processing and an ICL 2903 computer is to be ordered.

The Department is involved in consultancy, has a substantial practice in commercial testing and is becoming increasingly active in development projects to benefit small communities and small industries in this developing country.

Salaries within the range $A7,570 to $A10,205. In addition $A360 per annum is normally paid to a married male member of staff. Moderate rental housing is provided by the University. Superannuation may be provided.

Applications in duplicate should include particulars of age, nationality, marital status, family, if any, qualifications, experience, present post and the names and addresses of three referees from whom confidential enquiries may be made. They are required by July 1, 1974, and should be forwarded to The Registrar, The Papua New Guinea University of Technology, P.O. Box 793 LAE, P.N.G. An additional copy should be sent to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, from whom further particulars may be obtained. (1693)

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF GENETICS

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Applications, including a curriculum vitae and the names of three referees, should be sent to the Staff Appointments Officer, University of Nottingham, University Park, Nottingham NG7 2RD, before Friday, May 17, 1974. (1712)

The Delft University of Technology

Chair in Biology

Applications are invited for a vacant chair in biology in the Department of Chemistry and Chemical Engineering. Appointee will be trusted with the following duties:

Teaching: The organization of an introductory course in biology for all students of the department and a course in computer simulation of biological systems.

Research: The initiation and direction of research devoted to one or both of the following themes:

(i) energy balances in biological systems

(ii) mixed cultures of two or more organisms.

Applicant should have experience in the formulation and experimental verification of quantitative models of biological systems. He should be willing to co-operate with biological and technological research groups in the department.

Applications, including biography, giving full details of qualification, experience, publications and names of two referees should be addressed to: Prof. L. L. van Reigen, c/o Bureau van de Afdeling der Scheikundige Technologie, 136 Julianalaan, Delft-2208, The Netherlands.

(1658)

NEW ZEALAND

Department of Scientific and Industrial Research

Applications are invited for the undermentioned vacancies:

SCIENTISTS

Botany Division of the Department of Scientific and Industrial Research, Lincoln, has vacancies for two Scientists.

(a) The Department is currently engaged in taxonomic work in all plant groups except fungi. Two volumes of the "Flora of New Zealand" have been published and other volumes are in preparation. A scientist is required to conduct taxonomic revisions of Dicotyledons in New Zealand Flora.

Qualifications: Honours degree or Ph.D.

(b) A botanist is required for the general services section to undertake taxonomic and ecological work on adventive flora in New Zealand. Knowledge of aquatic plants an advantage.

Qualifications: Honours degree in Science of Agriculture, Ph.D. or equivalent experience.

Salary for both positions: up to NZ $10,232 p.a. depending on qualifications and experience.

Passages: Fares for appointee and his wife and family, if married, will be paid.

Incidental expenses: Up to $120 for a single man and $800 for a married man can be claimed to cover the cost of taking personal effects to New Zealand.

Application forms and general information are available from the **High Commissioner for New Zealand, New Zealand House, Haymarket, London, SW1Y 4TQ,** *with whom applications will close on 13 June 1974.*

Please quote reference 2/151 when enquiring.

(1646)

Graduates and Technicians– Anti-Viral Research

G. D. Searle & Co. Ltd., is a research orientated ethical pharmaceutical organisation, based at High Wycombe in Buckinghamshire. The Research and Development Division is engaged on fundamental work in the fields of biochemistry, biophysics and immunology. Currently there is an expansion of our Anti-Viral Research programmes, creating vacancies for both scientists and technicians.

The scientific posts will be filled by graduates holding a Microbiology, Biochemistry, Zoology or Virology degree. Technicians should have at least an HNC in a Biological Science (or equivalent), coupled with laboratory experience in biochemical techniques. Attractive commencing salaries will be offered, and conditions of employment include 4 weeks' holiday, Pension Scheme and active Social Club. Assistance with relocation expenditure will be given in appropriate circumstances.

Initially please contact Mr H. W. Cooke, Personnel Manager, Research and Development Division, G. D. Searle & Co. Ltd., Lane End Road, High Wycombe or telephone High Wycombe 21124 extension 130 quoting reference ST/129.

SEARLE

(1690)

SOUTH GLAMORGAN AREA HEALTH AUTHORITY

TWO RESEARCH TECHNICIANS

Whitchurch Hospital, Cardiff

Applications are invited for the above two posts in the Addiction Unit Research Laboratory of the above hospital, to assist Dr M. Evans and Dr A. Badawy in investigating tryptophan metabolism in animals and man under conditions of drug dependence and treatment with other drugs. The laboratory is undertaking an interesting programme with both national and international activities, and the successful applicants will, therefore, be expected to show competent technical skill. They must be interested in biochemistry, and to preferably possess experience in enzymology, liver and brain biochemistry, chromatography, spectroscopic techniques or cell fractionation and tissue handling in general.

The posts are:

(a) JUNIOR TECHNICIAN/TECHNICIAN, on the N.H.S. scale of £708 to £2,292 per annum. Initial placing is according to age, qualifications and experience.

(b) TECHNICAL ASSISTANT for THREE YEARS ONLY, supported by a grant from the Medical Research Council carrying a salary of £1,263 rising in the third year to £1,395.

Application forms are obtainable from The Administrator and should be completed and returned to him within 14 days from the date of appearance of this advertisement.

(1708)

SOUTH-WEST THAMES REGIONAL HEALTH AUTHORITY IN CO-OPERATION WITH THE UNIVERSITY OF SURREY

offers training posts as BASIC GRADE BIOCHEMISTS with the opportunity to study for an M.Sc. in Clinical Biochemistry by means of a 2-year sandwich course commencing October 1, 1974. Applicants should have a degree in Biochemistry or Chemistry (or its equivalent).

During the parts of the course spent away from the University, students will work in a specified hospital laboratory within the Region. Probationary scale for new entrants to the service, £1,797 or £1,497, depending on class of degree, plus London Weighting Allowance where applicable rising by annual increments. Successful in-service applicants will retain their existing conditions of service. Students will be responsible for meeting the cost of University fees, currently £114 payable in three instalments during the two years, after which period they should be willing to serve a further year within the Region if posts are available.

Interviews are expected to be held in London in late May.

Further details and application forms obtainable from the Academic Registrar, University of Surrey, Guildford, Surrey GU2 5XH, to whom they should be returned by May 10, 1974. (1695)

SOUTH-EAST STAFFORDSHIRE HEALTH DISTRICT

THE GENERAL HOSPITAL

BIOCHEMIST (Basic Grade) required to work under the Principal Biochemist in well equipped laboratory at the above hospital. Attractive bed-sitting room available for female applicants.

Further information from the Principal Biochemist, The General Hospital, Burton-on-Trent.

Applications, stating experience and naming two referees, to Personnel Officer, Burton District Hospital Centre, Belvedere Road, Burton-on-Trent DE13 0RB. (1696)

NORTH-EAST LONDON POLYTECHNIC

Enquiries are invited for the post of:

SENIOR TECHNICIAN IN PHYSIOLOGY T4

in the Department of Biological Science based at our West Ham Precinct, Romford Road, Stratford E15 4LZ.

The Physiology Section of the Department comprises a wide range of laboratories and specialist rooms dealing with teaching to honours degree level and research in physiology. The successful candidate will be second in charge of the section and applicants must have appropriate qualifications and experience at a responsible level in the field of physiology.

Salary on a scale ranging from £1,749 to £2,031 per annum inclusive.

Further details and application forms from the Staffing Office, Ref. S/CF 129, North East London Polytechnic, West Ham Precinct, Romford Road, Stratford E15 4LZ. Telephone: 01-555 0811.

Please contact: Technicians Unit, Ext. 33.

Closing date May 17, 1974. (1694)

UNIVERSITY OF SOUTHAMPTON

FACULTY OF MEDICINE

LECTURESHIP IN HUMAN MORPHOLOGY

Applications are invited from biological graduates, preferably with a Ph.D., or from medical graduates for a teaching and research appointment in Human Morphology. Previous teaching experience is not essential; good research facilities are available in a variety of fields and experience in electron microscopy would be an advantage. Salary scale £1,929-£4,548 per annum (by 16 increments) subject to review. Initial salary will depend upon qualifications and experience. Enquiries should be directed to Professor D. Bulmer or Professor D. Mayor, Medical and Biological Sciences Building, Bassett Crescent East, Southampton, from whom further particulars may also be obtained.

Applications should be addressed to the Deputy Secretary (Ext. 2804), The University, Southampton SO9 4NH (7 copies from applicants resident in U.K. and one from others) not later than May 31, 1974. Ref. Na. (1637)

UNIVERSITY OF MALAYA

DEPARTMENT OF GEOGRAPHY

Applications are invited for the post of ASSOCIATE PROFESSOR in the Department of Geography. Candidates should have high academic qualifications and wide experience in teaching and research in the field of either Physical *or* Human Geography with special reference to South-East Asia. Subject to academic suitability and experience preference will be given to candidates who are competent in Bahasa Malaysia (Malay).

Salary scale (approximately sterling equivalent) £2,278 by £89 to £2,814/RP/£2,919 per annum. In addition the following allowances are payable: Variable Allowance £243 min., £649 max. per annum; Supplementary Housing Allowance £503 per annum and medical benefits.

Further particulars and application forms are obtainable from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF.

The closing date for the receipt of applications is **May 31, 1974.**

(1701)

AGRICULTURAL RESEARCH COUNCIL

POULTRY RESEARCH CENTRE

Applications are invited for a post within the SCIENTIFIC/HIGHER SCIENTIFIC OFFICER GRADES in the Ethology Section of the Poultry Research Centre, for work on reproductive behaviour. The project involves a certain amount of surgical work and the person appointed should be prepared to supervise technical staff.

Candidates should have a pass degree or H.N.C. in an appropriate biological subject, but specialisation in Ethology is not a necessity.

Salary within the scales £1,435 to £2,329 or £2,221 to £2,854 per annum, with placement according to qualifications and experience. A minimum of five years' post-qualifying experience is required for appointment in the higher grade. The post is superannuable with a non-pensionable allowance to offset contributions.

Application forms, which may be obtained from the Secretary, Poultry Research Centre, King's Buildings, West Mains Road, Edinburgh EH9 3JS, should be completed and returned to him not later than May 21, 1974.

(1680)

WELSH NATIONAL SCHOOL OF MEDICINE

(UNIVERSITY OF WALES)

DEPARTMENT OF MEDICAL MICROBIOLOGY

Applications are invited for a

RESEARCH ASSISTANT

for Professor J. F. Watkins (who will be taking up his appointment on October 1, 1974). The appointee will work on the Cell Biology of Tumours and Tumour Viruses.

Previous experience not essential. Post suitable for a new graduate in biological sciences who would not wish to work for a higher degree.

Salary either on the scale £1,380 to £2,112 or on the technical scales depending on qualifications and experience.

Applications, with the names of two referees, to the Registrar, The Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN, within three weeks of the appearance of this advertisement. (1670)

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL

LONDON W1P 5PR

COURTAULD INSTITUTE OF BIOCHEMISTRY

A position as RESEARCH ASSISTANT in the Steroid Unit is vacant. Applicants should be at Doctoral level and should have a sound basis in Chemistry and Biochemistry.

Applications giving details of academic achievement and the names and addresses of two referees should reach the director of the Institute at the above address not later than Friday, May 17, 1974.

Salary, according to age, qualifications and experience, up to the maximum of £4,548 per annum plus £162 per annum London allowance plus superannuation under F.S.S.U. The appointment will be for a period of three years in the first instance. (1663)

JAMES COOK UNIVERSITY OF NORTH QUEENSLAND

LECTURER IN CIVIL ENGINEERING

Applicants should possess a higher degree and have teaching and research experience in the field of structural engineering; experience in wind engineering and an interest in low rise buildings and environmental aspects would be an advantage.

Salary range $A8,698 to $A11,982 per annum plus locality allowance of $A142 per annum for a married male, or $A71 for a single appointee. Conditions of appointment include superannuation, five weeks annual leave and an allowance for travelling and removal expenses.

Additional information and application forms may be obtained from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF.

Applications close on **May 24, 1974.** (1664)

RESEARCH AND DEVELOPMENT

New opportunities in Ulster

The R. & D. Division of Gallaher Limited, based in Belfast, is carrying out vital work in improving the health characteristics of smoking. Increasing the impetus of this work creates a number of new posts for able scientists, who will have excellent facilities for the work and opportunities for rapid personal development. The Laboratories work a 4½ day week.

GROUP LEADER—BIOLOGY GROUP

The Group of about fifteen people works broadly in Biochemistry, Toxicology and Cell Biology. It devises and develops biological systems for assessing the properties of new materials that may be smoked and for predicting their long-term effects. The Group Leader reports directly to the Research Manager. He must have a good Honours Degree and relevant biological research experience. He will also be involved in co-ordinating research projects in Europe. Therefore knowledge of French and/or German would be advantageous. We seek an exceptional scientist (age group 30–40) and offer him or her an exceptional opportunity.

BIOLOGICAL SCIENTISTS

Two new posts for graduates in their twenties are available in the above Biology Group, one in biochemistry, the other in tissue culture.

PHYSICISTS AND CHEMISTS

Several posts are now available for scientists interested in

The physical chemistry of combustion
Materials science of combustible materials (including tobacco)
Synthetic chemistry and
Analytical chemistry

Both long term and development problems are dealt with in inter-disciplinary groups. Applicants should be keen to apply scientific skill to commercial problems.

STATISTICIAN/BIOMETRICIAN

An Honours Graduate in mathematics, statistics and/or computing to develop techniques for the collation, analysis and interpretation of research data, particularly those from biological evaluations.

The Company offers first class conditions of service and initial salaries will be negotiable.

Applicants, male or female, may be graduates with suitable experience. For posts other than that of Group Leader—Biology Group, 1974 graduates may apply. Please write giving details of relevant academic and practical experience to :—

W. H. Forsythe, Manager, R. & D. Laboratories,
GALLAHER LIMITED, R. & D. Division,
Virginia House, Henry Street, Belfast BT15 1JE.

(1651)

UNIVERSITY OF TASMANIA

LECTURER IN AGRICULTURAL MICROBIOLOGY

Applications are invited for the above-mentioned appointment within the Faculty of Agricultural Science. The University would particularly welcome applications from people with training and experience in soil microbiology but the appointee will be expected to participate in teaching of general microbiology. He will be required to participate in supervision of honours and postgraduate students, and to undertake a personal research programme.

The salary scales are: Grade I $A10,575 to $469 by 3 to $11,982: Grade II $A8,698 to $470 to 1 to $469 by 2 to $10,106. Appointment will be offered within these grades according to qualifications and experience.

Further particulars and application forms from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF.

Applications close on **May 24, 1974.** (1673)

UNIVERSITY OF NEW SOUTH WALES

SCHOOL OF ELECTRICAL ENGINEERING

LECTURER

In field of communications. High academic qualifications in area of either high-speed digital systems or circuit applications of solid state devices. The School has well equipped research laboratories and particular research areas are optical communications, UHF and microwaves, communications systems, solid state devices and circuit applications, acoustics and biomedical engineering.

Salary: $A8,698 range $A11,982 per annum. Commencing salary according to qualifications and experience.

Details of appointment, including superannuation, study leave and housing scheme, may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close June 28, 1974. (1691)

Wellcome

Senior Technician for Pharmaceutical Research

The Clinical Pharmacology Departments is looking for a Senior Technician to support an integrated clinical research group.

The work will initially involve drug assays primarily utilising gas chromatographic methods. An awareness of modern laboratory methodology and a good sense of organisation especially in the day-to-day running of an efficient laboratory will be necessary. Applicants should have a biochemical background and practical laboratory experience at H.N.C. or graduate level.

Our laboratories are situated in pleasant parkland surroundings within easy reach of Bromley and Beckenham and about 12 miles from Charing Cross.

Conditions of employment are attractive.

Please write quoting reference U.451 and giving brief details of qualifications and experience to the

Senior Personnel Officer,
THE WELLCOME RESEARCH LABORATORIES,
Langley Court, Beckenham, Kent BR3 3BS.

(1699)

UNIVERSITY OF SOUTHAMPTON

DEPARTMENT OF MEDICINE

Two posts: **SENIOR TECHNICIAN** and **TECHNICIAN/GRADUATE RESEARCH ASSISTANTS** for the Immunology Laboratory, Professorial Medical Unit. Responsibility will be in general laboratory immunology and will include service, research and teaching.

Applications, in writing, as soon as possible to Mr. R. S. Lloyd, Senior Chief Technician, Professorial Medical Unit, Royal South Hants Hospital, Fanshawe Street, Southampton SO9 4PE.
Tel. Southampton 34288, Ext. 336.

(1649)

OXFORD UNIVERSITY

THE DYSON PERRINS LABORATORY
(ORGANIC CHEMISTRY)

Applications from CHEMISTRY GRADUATES are invited for two S.R.C.-C.A.S.E. Studentships, tenable from next September, for research (leading to the degree of D.Phil.) on the synthesis of peptides of pharmacological interest, under the academic supervision of Dr. G. T. Young and in collaboration with industrial pharmaceutical research laboratories. The terms and conditions are similar to those for S.R.C. Research Studentships; undergraduates taking Finals this summer are eligible.

Apply immediately to Dr. G. T. Young, The Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY. (1697)

Biochemist

A leading British pharmaceutical Research Organisation has a vacancy for a Senior Biochemist to initiate and direct research projects in the areas of pharmacokinetics, drug metabolism, molecular pharmacology and enzymology in association with the design and development of new medicines.

Persons with appropriate experience are invited to apply in writing for further details to: The Secretary, Biorex Laboratories Ltd., Biorex House, Canonbury Villas, London N1 2HB, marking the envelope CONFIDENTIAL.

(1688)

North East London Polytechnic

Applications are invited for the post of:

Senior Technician T4 (3 posts)

based at our West Ham Precinct.

Psychology

This post in the Department of Psychology involves working with children and parents at the Department's Child Study Centre and responsibilities include security and maintenance of equipment in the Centre and care of a small animal house.
Candidates should have some relevant experience and preferably a knowledge of recording equipment.

Biochemistry

This position in the Department of Applied Biology involves responsibility for the satisfactory servicing of degree level practical classes in biochemistry including supervision and training of junior staff. Candidates should possess Final City and Guilds, HNC or equivalent. Experience of the use of advanced biochemical instrumentation will be considered an advantage.

Physiology

The physiology section of the Department of Biological Science comprises a wide range of laboratories and specialist rooms dealing with teaching to honours degree level and research in Physiology.
The successful candidate will be second in charge of the section and applicants must have appropriate qualifications to HNC or equivalent level and experience at a responsible level in one or more areas of Physiology

Salary on a scale ranging from £1,749 to £2,031 per annum inclusive.

The West Ham Precinct houses the Faculties of Science and Human Sciences and provides an extensive range of advanced facilities for teaching and research. Sports, canteen, library and other facilities are also available.

Further details and application forms from:
The Staffing Office, Ref: S/CF128
North East London Polytechnic,
West Ham Precinct,
Romford Road, Stratford E15 4LZ
Telephone: 01–555 0811

Please contact: Technicians Unit Ext. 33

Closing date 10th May, 1974. (1674)

OXFORD UNIVERSITY

Applications are invited from organic chemists for POSTDOCTORAL RESEARCH ASSISTANTSHIPS to work in the Dyson Perrins Laboratory with Drs. J. A. Barltrop and A. C. Day on the photochemistry of heterocyclic aromatic systems. Two such appointments are offered, each for a maximum period of 3 years, commencing not later than October 1, 1974. Previous photochemical experience is not essential. Applications will be accepted from those expecting to complete doctoral work this summer. The salary will be in the range £1,821-£2,223 plus F.S.S.U.

Applications, including curriculum vitae and the names of two referees, should reach the Administrator, The Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY, as soon as possible, and not later than May 31, 1974. (1566)

UNIVERSITY OF GLASGOW

DEPARTMENT OF ZOOLOGY

Applications are invited for the post of RESEARCH ASSISTANT to work under Dr. P. Calow on triclad energetics. The appointment will be for not more than 3 years. Salary will be £1,475 (by £75) to £1,625 per annum.

Applications, with the names of two referees, should be sent, not later than June 30, 1974, to the Secretary, Zoology Department, The University, Glasgow G12 8QQ, from whom further particulars can be obtained. In reply please quote Ref. No. 3447M. (1686)

SCIENTIFIC SECRETARY

A part-time position of Scientific Secretary to the Eugenics Society will become available in August. The Society is concerned broadly with the biosocial aspects of human populations and the post would be suitable for a social scientist with an awareness of biological problems or a natural scientist with inter-disciplinary interests.

This new position will give the individual time for developing his own research interests, as well as being involved in the organisation of annual symposia and the Society's quarterly publication. Encouragement will be given to the person appointed to extend the scientific work of the Society. Support would also be given in any application for funds to transform the position into a full-time appointment at the Society's premises. Previous administrative experience would be an advantage. Salary according to age and qualifications within the range £1,500 to £2,500 for 3 days per week or the equivalent and superannuation cover will be arranged.

Further details may be obtained from the General Secretary, The Eugenics Society, 69 Eccleston Square, London SW1V 1PJ, to whom applications should be submitted not later than May 31. (1668)

THE WELSH NATIONAL SCHOOL OF MEDICINE

DEPARTMENT OF HAEMATOLOGY

TECHNICIAN OR SCIENTIFIC OFFICER

Technician or Scientific Officer required in the above Department to perform established tests of neutrophil function in leukaemia and allied disorders and to develop new techniques in this field.

Candidates should possess B.Sc. of H.N.C./A.I.M.L.T. in Haematology.

Salary according to qualifications but in the range £1,380 to £2,517.

Application forms available from the Registrar, The Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN, to whom they should be returned two weeks after the appearance of this advertisement.

(1709)

UNIVERSITY OF MALAYA

FACULTY OF SCIENCE

Applications are invited for the posts of LECTURERS/ASSISTANT LECTURERS in the Departments listed below. Subject to academic suitability and experience, preference will be given to candidates who are competent in Bahasa Malaysia (Malay). Candidates should have at least a good Honours degree with suitable teaching and research experience. For a Lectureship, a doctorate is normally required.

DEPARTMENT OF MATHEMATICS: Vacancies exist in the fields of Pure Mathematics, Applied Mathematics, and Statistics.

DEPARTMENT OF PHYSICS (two posts).

Salary scales (approximately sterling equivalents): Lecturer £1,524 to £2,441; Assistant Lecturer £1,330 to £1,460 per annum. In addition the following allowances are payable: Variable Allowance £243 min., £649 max. per annum; Supplementary Housing Allowance £503 per annum and medical benefits.

Further particulars and application forms are obtainable from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF.

The closing date for the receipt of applications is **May 31, 1974.** (1700)

UNIVERSITY OF OTTAWA

Applications are invited for a staff position at the associate or assistant professor level from individuals having a strong background in research in Organometallic Chemistry. Applicants with research interests in a related interdisciplinary field will also be considered. The applicant will be expected to participate in the teaching of General Chemistry as well as giving instruction at the advanced undergraduate and graduate levels. Ability to lecture in French, in addition to English, would be a distinct advantage. Salary will be up to $17,500 depending on the applicant's experience. The date of appointment is negotiable but September 1, 1974, is preferred.

Applicants should send a complete curriculum vitae and addresses of referees to: Professor H. H. Baer, Chairman, Department of Chemistry, University of Ottawa, Ottawa, Canada K1N 6N5. (1520)

Council for Scientific and Industrial Research South Africa

The Council for Scientific and Industrial Research consists of 15 national research laboratories and institutes. The CSIR undertakes and promotes scientific and industrial research into matters affecting the utilisation of the natural resources of the Republic; on the development of its industries and on the proper co-ordination and employment of scientific research to these ends.

The following vacancies are available in its NATIONAL CHEMICAL RESEARCH LABORATORY, PRETORIA.

BIOCHEMISTS

(ref 73/60/289) required for research on structure-function relationships of biologically active macromolecules. The programme envisaged includes the interaction of phospholipase A, cytotoxins, toxalbumins and other lectins with biological membranes. Excellent facilities and expertise in protein isolation, structure determination and physico-chemical characterisation are already available.

Posts are available on two levels, according to qualifications and experience:

Salary A: Between R8 300 and R10 700 (£5500 and £7150 approx) p.a.

Salary B: Between R5 000 and R7 950 (£3300 and £5300 approx) p.a.

Applicants with extensive experience in leading a research team will be considered for a position on the level of salary scale A.

CONDITIONS OF EMPLOYMENT: The many benefits include medical benefit scheme, assisted housing loan scheme, housing subsidy scheme, annual leave bonus, pension and group insurance scheme and annual leave up to 35 days. Successful candidates will receive assistance with travelling expenses and a contribution towards transportation of household effects. They will be required to make use of the Government immigration scheme and to serve the Council for two years in return for financial assistance.

Please apply to the following address:
The Office of the Scientific Counsellor,
Chichester House, 278 High Holborn,
London WC1V 7HE.
Telephone: 01-242 9641

Further details and application forms may also be obtained from this address.

(1661)

STATISTICIAN

FOR A MEDICAL RESEARCH COUNCIL UNIT IN EDINBURGH

A statistician, who will be senior amongst several on the scientific staff of the Unit, is required to work on the design and analysis of experiments in cell biology and genetic linkage, to advise on the biomathematical aspects of cell behaviour and to assist in studies on the epidemiology of human chromosome abnormalities.

Substantial in-house computing facilities, a high speed link to the Edinburgh Regional Computer Centre and programming support are available.

Applicants should have suitable academic qualifications and considerable experience in applied statistics, preferably in the biological sciences. The position offers excellent opportunities for collaborative work in a wide range of biological and medical projects, especially for someone whose interests include the biomedical field as well as statistical theory and technique. Commencing salary in the range £1,830 to £4,548 with possible promotion to Senior Grades.

To apply, or for further information, please contact the Director, MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU. (1627)

We require a

RESEARCH ASSISTANT

to work in the

Chemotherapy and Biologics Department of our Central Research Laboratories

This is a new vacancy arising from expansion of our research activities. We are looking for someone to join the Antimicrobials project team. This team is involved in an increasing range of Bacteriological techniques including diagnostic work, tissue level studies etc. Candidates should have considerable experience in Bacteriological work and the ability to supervise junior staff.

This job would probably suit someone who has recently passed the final examination in Bacteriological techniques of the IMLT. Conditions of employment include non-contributory pension and death benefit schemes, canteen and social club facilities. Assisted travel is available from towns in the surrounding area. Our research Laboratories are situated on the Kent coast with easy access to London and the Continent.

Pfizer

If you are interested in this position please write for an application form to: G. Marshall, Personnel Officer, Central Research, Pfizer Limited, Sandwich, Kent.

(1669)

MINISTRY OF AGRICULTURE AND FISHERIES

SCIENTIST

WHATAWHATA HILL COUNTRY RESEARCH STATION, HAMILTON

NEW ZEALAND

Salary payable up to $11,986 depending on qualifications and experience.

Duties: Studies in the reproductive physiology of sheep and beef cattle with particular reference to the influence of nutritional and management factors affecting the fertility of hill country flocks and herds. Large numbers of genetically different sheep and cattle are available for study.

Qualifications desired: Masters or higher degree in agricultural or veterinary science, preferably with research experience in the general field of reproductive physiology.

Passages: Fares may be paid for appointee and if married for his wife and children to New Zealand.

Incidental Expenses: Up to NZ $120 for a single man and NZ $800 for a married man to cover the cost of taking personal effects to New Zealand.

Application forms and general information are available from the:
High Commissioner for New Zealand, New Zealand House, Haymarket, London, SW1Y 4TQ
with whom applications will close on 13 June 1974.

Please quote reference 2/14/59 when enquiring.

(1648)

UNIVERSITY OF BATH
SCHOOL OF BIOLOGICAL SCIENCES
POSTDOCTORAL RESEARCH FELLOW
RESEARCH ASSISTANT

Applications are invited for the above posts for work on the fish populations of the Severn Estuary supported by the Leverhulme Trust and the C.E.G.B., and based mainly on sampling from Power Station intakes.

The Postdoctoral Fellow will be expected to carry out a critical examination of sampling techniques and the analysis of seasonal changes in terms of environmental factors. In addition, studies will be carried out on the biology of the more important estuarine species and the successful candidate will be expected to initiate laboratory studies where these may be relevant to an interpretation of field data. Adequate training in statistical techniques and the computerised handling of data is desirable in addition to suitable research experience in the general field of freshwater or marine ecology.

The duties of the Research Assistant will include the collection of Power Station samples, the identification, examination and storage of specimens. Candidates should have an honours degree in zoology and preferably some experience of ecological and statistical techniques. A current driving licence is essential.

Both appointments are for a three-year period and the salary ranges are: Postdoctoral Fellow, £1,929 to £2,553; Research Assistant, £1,440 to £1,608.

Application forms, available from Registrar (S), The University, Bath BA2 7AY, quoting reference number 74/57, should be returned before May 22, 1974. (1666)

UNIVERSITY OF SOUTHAMPTON
FACULTY OF MEDICINE
PHARMACOLOGY—TEMPORARY LECTURESHIPS

Applications are requested from registered medical practitioners or from science graduates with appropriate qualifications for two Temporary Lectureships in Pharmacology on a full- or part-time basis for the academic year 1974/75. Experience in teaching medical students an advantage. Salary within range £3,588 to £5,091 (clinical) or £1,929 to £4,548 (non-clinical). Superannuation.

Further particulars may be obtained from the Deputy Secretary, The University, Southampton SO9 5NH, to whom applications (seven copies from U.K. applicants and one from others) should be sent not later than May 24, 1974. (1657)

THE EDINBURGH SCHOOL OF AGRICULTURE

POST-DOCTORAL RESEARCH ASSISTANTSHIP

Applications are invited for a research assistantship in the Department of Agricultural Biochemistry for work on embryo development in higher plants. Candidates should be qualified in biochemistry or a related discipline.

The appointment, financed by the A.R.C. (salary scale £2,221 to £2,413, with F.S.S.U. benefits), is tenable for three years from October 1, 1974, and will be associated with a small group working on seed development and maturation.

Further details and application form from the Secretary, Edinburgh School of Agriculture, West Mains Road, Edinburgh EH9 3JG. (1683)

UNIVERSITY OF TASMANIA

LECTURER IN AGRONOMY

Applications are invited for the above-mentioned appointment within the Faculty of Agricultural Science.

The salary scales are: Grade I $A10,575 to $469 by 3 to $11,982; Grade II $A8,698 to $470 by 1 to $469 by 2 to $10,106. Appointment will be offered within these grades according to qualifications and experience.

The particular field of interest of the Senior Lecturer in Agronomy (Dr. J. J. Yates) is the ecology of pastures. The faculty is particularly interested in applications from people with training and research experience in applied Genetics, including both plant and animal breeding. The appointee would also be required to lecture in some aspects of general Agronomy and it would be of great assistance if he could also assist in lectures on biometry in the fourth year compulsory unit, Agricultural Research and Extension Methods. The appointee would be expected to participate in the supervision of honours and postgraduate students and to undertake a personal research programme.

Further particulars and application forms obtainable from Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF.

Applications close on **May 24, 1974.** (1672)

MONASH UNIVERSITY

DEPARTMENT OF GENETICS

SENIOR TEACHING FELLOW

Applicants should normally have completed a M.Sc. or Ph.D. degree and will be responsible for the preparation and presentation of practical classes in Genetics in various years of the science course. The successful applicant will be expected to start late in 1974 or early in 1975. Facilities will be available for the successful applicant to undertake research in collaboration with the staff members of the Department.

Salary range: $A7,270 to $A8,698 per annum with superannuation based on an endowment assurance scheme, the employee and employer contributing 5 per cent and 10 per cent respectively.

Benefits: Travelling expenses for appointee and family, removal allowance, temporary housing for an initial period, repatriation after three years' service if desired.

Further general information and details of application procedure are available from the Academic Registrar, Monash University, Wellington Road, Clayton, Victoria 3168, Australia. Enquiries may be addressed to Professor B. W. Holloway, Chairman of the Department, from whom a detailed statement of current departmental research interests is available. Applicants should quote reference No. 30713.

Closing date: **May 31, 1974.**

The University reserves the right to make no appointment or to appoint by invitation. (1659)

THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF NEW BRUNSWICK, will make an appointment at the ASSISTANT PROFESSOR LEVEL, effective July 1, 1974, at the earliest. Duties will involve the teaching of general and physical chemistry courses in the undergraduate programme, and supervision of graduate students in M.Sc. and Ph.D. programmes. Research interests of the applicant should be compatible with the present interests of the Department and might include, for example, the fields of electrochemistry, nmr-esr spectroscopy, mechanisms of reactions in solution.—Applications, together with a research proposal, should be sent to: Chairman, Department of Chemistry, University of New Brunswick, Fredericton, N.B. E3B 5A3, Canada. (1655)

UNIVERSITY OF CALGARY, GRADUATE Teaching and Research Assistantships are available for students proceeding to the Ph.D. or M.Sc. degrees in all branches of Chemistry and Biochemistry. The Assistantships are valued from $4,100 to $5,000 per annum and carry remission of programme fees and travel allowance.

For further information write to: Department of Chemistry, Calgary, Alberta T2N 1N4, Canada. Please indicate field of interest. (1654)

NORTH-HOLLAND PUBLISHING COMPANY

(member of Associated Scientific Publishers, publisher of scientific journals and books).

has an immediate opening in AMSTERDAM for a full-time

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Description:

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We offer responsible and variable work in a congenial atmosphere.

Handwritten applications, giving details of education and previous experience and an indication of salary expected, should be addressed to:

The Personnel Department,
Associated Scientific Publishers
P.O. Box 2400, Amsterdam
The Netherlands.

(1676)

UWIST CARDIFF

University of Wales Institute of Science and Technology

Department of Applied Biology

RESEARCH ASSISTANTS (or RESEARCH STUDENTS)

required for the following appointments:

1. **For experimental study of effects of dust and sulphur dioxide on plants (3 years).**
2. **To study biological recovery of a polluted stream in South Wales (2 or 3 years).**

Applicants for this post should hold a car driving licence.

Salaries: Post 1: £1,318 to £1,499
Post 2: £1,499 to £1,657

Suitably qualified Research Assistants will be permitted to register for PhD with salary adjustments.

Also

3. **RESEARCH STUDENT to study the ecology of aquatic oligochaetes.**

Research Students will be given grants based on the SRC Awards scheme.

Further particulars and application forms (returnable as soon as possible) from Personnel Section, UWIST, Cardiff CF1 3NU. (1636)

NEW ZEALAND
Health Department

Applications are invited for the undermentioned vacancy:

SCIENTIST
National Health Institute
Wellington, New Zealand

A position is available for a Scientist with considerable experience in immunology and in particular the practical aspects of serological tests. The successful applicant will be responsible for the development of a wide range of serological techniques, production of antigens and antisera, research into and appraisal of new techniques, development of serological proficiency testing.

Qualifications desired: Ph.D. or M.Sc. with considerable experience in immunology or a medical graduate with postgraduate qualifications in microbiology.

Salary up to NZ$11,249 p.a. according to qualifications and experience.

Passages: Fares for appointee and his wife and family, if married, will be paid.

Incidental expenses: Up to $120 for a single man and $800 for a married man can be claimed to cover the cost of taking personal effects to New Zealand.

Application forms and general information are available from the **High Commissioner for New Zealand, New Zealand House, Haymarket, London SW1Y 4TQ,** *with whom applications will close on June 13 1974.*

Please quote reference 2/38/14 when enquiring.

(1647)

South Glamorgan Area Health Authority

Biochemists
(Basic, Probationary)

Vacancies exist in the Department of Chemical Pathology, University of Wales, Heath Park, Cardiff, for graduates in Chemistry or Biochemistry or one of the related biological sciences.

The department is closely linked with The Welsh National School of Medicine and carries out service work and research. It occupies new accommodation and has recently been equipped with a complete range of service and research equipment.

Holders of the posts will be expected to register as post-graduate students with The Welsh National School of Medicine and undertake research work with a view to obtaining the Ph.D degree. They will also be expected as part of their training to study for the Mastership in Clinical Biochemistry. Salaries and conditions of service will be those prescribed for Biochemists by the Whitley Council.

Further particulars and an appointment to visit the department may be obtained by writing to Professor C. N. Hales at the Department.

Application forms may be obtained from the Divisional Administrator, University Hospital of Wales, Heath Park, Cardiff.

(1653)

THE EDINBURGH SCHOOL OF AGRICULTURE

RESEARCH ASSISTANT

Applications are invited from Honours graduates in Microbiology or a related discipline for a research assistantship to work in the microbiology and biochemistry departments on silage deterioration studies financed by the A.R.C. The assistant will be expected to register for a higher degree. Salary £830 by £40 to £910, plus F.S.S.U. and fees.

Further particulars from the Secretary, The Edinburgh School of Agriculture, West Mains Road, Edinburgh EH9 3JG. (1681)

UNIVERSITY OF ADELAIDE

Applications are invited for the following appointments:

LECTURER IN GEOGRAPHY (BIOGEOGRAPHY). Preference will be given to candidates interested in plant and animal geography, ecosystem structure and function, and ecology related to resource management. From January 1, 1975. (31.5.74)

LECTURER IN GEOGRAPHY. Preference will be given to applicants qualified to teach geomorphology and soil geography, with particular reference to Australia. From January 1, 1975. (31.5.74)

Salary scale: Lecturer $A8,698 by $470 (1) by $469 (6) to $11,982; with superannuation on the F.S.S.U. basis. Initial salary will be fixed within the scale in accordance with qualifications and experience.

Further particulars about these posts and the conditions of appointment and other information sought will be supplied on request to the Registrar of the University, or to the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF.

Applications should be sent in duplicate and giving the information listed in the Statement that will be supplied, to the Registrar, the University of Adelaide, North Terrace, Adelaide, South Australia 5001. (1702)

UNIVERSITY OF MANCHESTER

DEPARTMENT OF MEDICAL BIOCHEMISTRY

Applications are invited for a POSTDOCTORAL RESEARCH ASSOCIATESHIP, financed by the British Heart Foundation, to work on "The Biosynthesis of Endothelial Basement Membrane Components of Blood Vessels". The appointment, from October 1, 1974, is tenable for two years. Salary range £2,118-£2,412 per annum plus F.S.S.U.

Applications (3 copies) giving the names of two referees should reach Professor D. S. Jackson, Department of Medical Biochemistry, University of Manchester Medical School, Stopford Building, Oxford Road, Manchester M13 9PT, before May 10, 1974. (1638)

UNIVERSITY OF HULL

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for the post of Postdoctoral Research Assistant, funded by the Agricultural Research Council for a period of three years, for work on the effects of low oxygen concentrations on the regulation of lipid and carbohydrate metabolism of Azobacter, under the direction of Professor E. A. Dawes. Experience with chemostats and in enzymology would be an advantage.

Salary, according to experience, within the range £2,037-£2,223 (currently under review) with F.S.S.U. benefits.

Applications (4 copies) should be sent as soon as possible, to the Registrar, The University, Cottingham Road, Hull HU6 7RX, from whom further particulars may be obtained. (1642)

UNIVERSITY OF ESSEX

DEPARTMENT OF CHEMISTRY

Applications are invited for the post of

LECTURER

(£1,929 to £4,548; scale at present under review) in Inorganic Chemistry for appointment from October 1, 1974.

Applicants should have research interests in synthetic inorganic chemistry or in any other field of inorganic chemistry related to the overall research interests of the Department.

Applications (4 copies, quoting ref. PS/51/N) should reach the Registrar, University of Essex, Wivenhoe Park, Colchester CO4 3SQ, Essex, from whom further particulars are obtainable, by Friday, May 17, 1974. (1705)

UNIVERSITY OF NEW SOUTH WALES
WOLLONGONG UNIVERSITY COLLEGE

to become the

UNIVERSITY OF WOLLONGONG

January 1, 1975

LECTURER—DEPARTMENT OF PHYSICS

The appointment is for a solid state physicist either theoretical or experimental. In the latter case, preference will be given to applicants with a background in solid state spectroscopy. Experience in the use of digital computers will be useful in both cases. The appointee will be required to teach at undergraduate and postgraduate levels and to pursue an active research programme in his own particular field.

Commencing salary according to qualifications and experience, within the range $A8,698 to $A11,982.

Further information may be obtained direct from the College Bursar, or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close May 15, 1974. (1677)

UNIVERSITY OF NOTTINGHAM
DEMONSTRATOR IN BOTANY

Demonstrator in Botany is required from October 1, 1974. The person appointed will be required to demonstrate in all aspects of Botany including Microbiology. The appointment is for three years and the person appointed will be required to register for a higher degree, preferably in the general field of plant cell and protoplast culture and the growth and selection of somatic hybrid cells. Salary on a scale rising to £1,569 per annum.

Application forms, obtainable from the Staff Appointments Officer, University of Nottingham, University Park, Nottingham, to be returned not later than May 17. Reference Number -N. (1684)

UNIVERSITY COLLEGE OF NORTH WALES
BANGOR

DEPARTMENT OF ZOOLOGY

Applications are invited for the post of DEMONSTRATOR.

Applicants for this post should have either a Ph.D. or an honours degree in Zoology.

The initial appointment will be for one year commencing on October 1, 1974, with the possibility of a renewal for a further period of two years.

Salary will be £1,683 per annum plus F.S.S.U. benefits.

Applications (two copies), giving full details of age, qualifications and experience, together with the names and addresses of two referees, should be sent not later than May 31, 1974, to the Secretary and Registrar, University College of North Wales, from whom further particulars may be obtained. (1645)

UNIVERSITY OF SOUTHAMPTON
FACULTY OF MEDICINE

Applications are invited from registered medical practitioners or from science graduates with appropriate qualifications for two TEMPORARY LECTURESHIPS IN PHARMACOLOGY on a full- or part-time basis for the Academic Year 1974/75. Experience in teaching medical students an advantage. Salary within range of £3,588 to £5,091 (clinical) or £1,929 to £4,548 (non-clinical). Superannuation. Further particulars may be obtained from the Deputy Secretary, The University, Southampton SO9 5NH, to whom applications (7 copies from U.K. applicants and one from others) should be sent not later than May 24, 1974. Ref. Na. (1713)

ELECTRON MICROSCOPY TECHNICIAN

(Grade 3) required to assist generally in a fully equipped unit serving research and teaching in biological ultrastructure. The post offers opportunities in the development of techniques and instrumentation for applicants of appropriate interests and experience.

Salary in the range £1,825 to £2,095 per annum.

Applications in writing to Mr. R. Adams, Departmental Superintendent, Department of Botany, Imperial College, London SW7 2BB. (1711)

UNIVERSITY OF MANCHESTER
ASSISTANT KEEPER IN GEOLOGY
THE MANCHESTER MUSEUM

Applications invited for this post, vacant from October 1, from candidates with an interest in mineralogy and petrology. Salary range per annum (under review): £1,929-£3,048. F.S.S.U.

Further particulars and application forms (returnable by May 31) from the Registrar, The University, Manchester M13 9PL. (1644)

Council for Scientific and Industrial Research South Africa

The Council for Scientific and Industrial Research consists of 15 national research laboratories and institutes. The CSIR undertakes and promotes scientific and industrial research into matters affecting the utilisation of the natural resources of the Republic; on the development of its industries and on the proper co-ordination and employment of scientific research to these ends.

The following vacancy is available in the NATIONAL FOOD RESEARCH INSTITUTE, PRETORIA.

FOOD TECHNOLOGIST/ SCIENTIST (Ref. 73/89/268).

This vacancy is in the food technology division of the Institute and the work involves research into the processing and preservation of food products. The work involves basic and applied research as well as the handling of problems of the food industry.

QUALIFICATIONS: At least a four-year degree in Food Science and Technology, Chemistry or Chemical Engineering.

SALARY: According to qualifications and experience; between R3150 and R9300 p.a., plus a 15% pensionable allowance (£2415 and £7130 approx.).

CONDITIONS OF EMPLOYMENT: The many benefits include medical benefit scheme, assisted housing loan scheme, housing subsidy scheme, annual leave bonus, pension and group insurance scheme and annual leave of up to 35 days. Successful candidates will receive assistance with travelling expenses and a contribution towards transportation of household effects. They will be required to make use of the Government immigration scheme and to serve the Council for two years in return for financial assistance.

Please apply to the following address,

The Office of the Scientific Counsellor,
Chichester House, 278 High Holborn,
London WC1V 7HE.
Telephone: 01-242 9641.

Further details and application forms may also be obtained from this address. (1660)

UNIVERSITY OF SOUTHAMPTON
FACULTY OF MEDICINE

CHEMICAL PATHOLOGY

SENIOR CHIEF TECHNICIAN required to establish a rapidly developing metabolic and endocrine research laboratory. The successful applicant will be aged between 30-40 and will be responsible for development and running of multiuser analytical facilities. Will also be required to participate in research with the Professor and opportunities will be available for a research degree. Whitley Council conditions of service and salary will apply. Post available immediately. Further information obtainable from Professor K. G. M. M. Alberti, Tel. Southampton 777222 Ext. 3359.

Application forms and job description obtainable from the Personnel Officer, Southampton Health District, Tremona Road, Southampton SO1 6HU. Tel. Southampton 777222 Ext 3651. Closing date: May 24, 1974. (1635)

UNIVERSITY OF MANCHESTER
DEPARTMENT OF PHYSICS

POSTDOCTORAL RESEARCH

Applications invited for a two-year postdoctoral appointment (from October 1) to study molecular dynamics of condensed matter by laser light scattering. The group is currently studying molecular motion and properties of synthetic and biological macromolecules by techniques which include photon correlation spectroscopy. The post is suitable for candidates who have a background in Physics or Chemistry; previous experience in light scattering or macromolecular research an advantage, but not necessary. Salary range £1,929-£2,553 per annum.

Further information may be obtained from Dr T. A. King, Physics Department, The University, Manchester M13 9PL, to whom applications should be sent together with the names of two referees as soon as possible. (1641)

EAST BIRMINGHAM HEALTH DISTRICT

CYTOGENETICS

Cytogeneticist required (Salary Scale in accordance with Whitley Council scale for Top Grade Biochemists at present £5,190 to £5,919, to take charge of a new Regional Cytogenetics Laboratory within the new East Birmingham Hospital Laboratory now being built. Candidates should have a higher degree (Ph.D. or equivalent) and wide experience of cytogenetics.

The work of the department will include elucidation of chromosome abnormalities, antenatal diagnosis of chromosomal defects, postgraduate teaching and research. The director of the laboratory will also have duties in diagnostic cytogenetics in the Department of Human Genetics in the Infant Development Unit at the New Birmingham Maternity Hospital, adjoining the University. The successful candidate may be accorded honorary status in the University of Birmingham.

Candidates are invited to visit the laboratory by direct arrangement with the Regional Virus Laboratory (Tel: 021–772 4311 Ext 675).

Applications (one copy) must include the names of three referees, age and relevant experience, and must be received by the District Administrator, East Birmingham Health District, 45 Bordesley Green East, Birmingham B9 5ST.

Closing date for applications 17th May, 1974.

(1710)

BP

Product Development Technologist

Over the last few years, the research group of BP Proteins Limited, based at Grangemouth in Scotland, has developed a process for the production of high-protein dried yeast (TOPRINA) from petroleum by continuous fermentation and processing techniques.

Toprina is being marketed as an ingredient of compound animal feeding stuffs and, as part of our continuing development programme, the product range is being expanded to meet a variety of needs in the animal feeds market.

We now require a young graduate to fill a new appointment in the team involved in the Product Development programme. His work will include study of the properties of Toprina and the development of a range of products based on it.

The successful applicant must have a good degree in food technology or a related field. Some previous experience in research and development in the food or feeds industry would be highly desirable; indeed the post might suit a physical or natural scientist with such experience.

■ Salary is competitive and conditions of service are excellent. Please write, giving full details, quoting ref. U. 780, to: The Manager, Central Recruitment, The British Petroleum Company Limited, Britannic House, Moor Lane, London EC2Y 9BU.

(1629)

LIVERPOOL POLYTECHNIC

DEPARTMENT OF BIOLOGY

Applications are invited for the post of NERC RESEARCH ASSISTANT to work under the direction of Dr M. K. Hughes on the development of X-ray densitometry of tree-rings as an ecological tool.

Appointment is for 3 years from July 1, 1974. Salary: £1,251, £1,311, £1,357 per annum.

Details available from Staff Office, Dept., Liverpool Polytechnic, Richmond House, 1 Rumford Place, Liverpool L3 1RH. (1667)

ROTHAMSTED EXPERIMENTAL STATION

HARPENDEN, HERTS AL5 2JQ

PLANT PATHOLOGIST to work on fungal diseases of potato tubers, especially gangrene, during growth and storage. Minimum qualifications good honours degree.

Appointment in grade of Scientific Officer £1,435 to £2,329, or Higher Scientific Officer £2,221 to £2,854. Starting salary in accordance with qualifications and experience. At least two years' postgraduate experience is required for appointment as H.S.O. F.S.S.U. Further details on request.

Applications, naming two referees and quoting reference 2232, to the Secretary by May 31, 1974. (1687)

UNIVERSITY OF SYDNEY

ELECTRON MICROSCOPIST

Applications are invited for the position of Electron Microscopist in the Electron Microscope Unit. This Unit provides a service in Electron Microscopy both by way of research facilities and training courses to the various Departments. In addition to the advertised position, the Unit's staff includes a Director and two Assistant Electron Microscopists. Applicants for this vacancy should be experienced in the biological aspects of electron microscopy.

Salary within range: $A8,698 to $11,982 per annum or $A12,268 to $A14,308 per annum according to qualifications and experience.

Applications including curriculum vitae, list of publications and names of referees by **May 15, 1974,** to the Registrar, University of Sydney, N.S.W. 2006, Australia, from whom further information available. (1662)

UNIVERSITY OF SYDNEY

P. N. RUSSELL CHAIR OF MECHANICAL ENGINEERING

Applications are invited for the P. N. Russell Chair of Mechanical Engineering which will become vacant on January 1, 1975, following the appointment of Professor D. W. George to the position of Vice-Chancellor at the University of Newcastle.

Salary will be at the rate of $A19,102 per annum.

A statement of conditions of appointment and information for candidates may be obtained from the Registrar, University of Sydney, N.S.W. 2006, Australia, with whom applications close on June 6, 1974. (1692)

UNIVERSITY OF CONNECTICUT

POST-DOCTORAL RESEARCH ASSISTANT

Protein Crystallography. $9,000 per year for up to 3 years.

Inquiries to: J. R. Knox, University of Connecticut, U-136 Storrs, Connecticut 06268, USA. (1685)

GRANTS & SCHOLARSHIPS

THE ROYAL SOCIETY

SCIENTIFIC PUBLICATIONS GRANT

Applications for grants from the first allotment of the Government Grant for Scientific Publications for the year 1974 should be made as soon as possible. No application can be considered which is received later than June 15, 1974. The grant is provided for the assistance of scientific publications in the United Kingdom, particularly but not exclusively those of scientific societies and institutions of a national character. A small part of the grant is available for the assistance of the libraries of scientific societies. Applications are receivable only from societies and institutions in the United Kingdom and individuals of British nationality.

Forms of application may be obtained from the Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG. (1698)

Trent Regional Health Authority

Regional Administrative Scientific Officer

(Salary Scale £5,409–£6,648 p.a.)

Applications are invited for the newly established post of Regional Administrative Scientific Officer. The successful applicant will be on the staff of the Regional Medical Officer and will be concerned with co-ordinating the development of the scientific services in the Trent Region, as recommended in Department of Health circulars and the Zuckerman Report.

Candidates, who should be qualified scientists with or without a medical qualification, should have a broadly based rather than a specialist background, and should have the ability to plan and organise.

Application forms and further details of the post are available from the Regional Administrator, Trent Regional Health Authority, Fulwood House, Old Fulwood Road, Sheffield S10 3TH, to whom completed applications, naming three referees, should be sent by May 17, 1974.

(1707)

ULSTER:
THE NEW UNIVERSITY

POSTDOCTORAL RESEARCH FELLOWSHIP IN CHEMISTRY

Organic or organometallic chemists are invited to apply for an S.R.C.-sponsored postdoctoral research fellowship to investigate orbital-symmetry-controlled reactions of hydrocarbon-transition metal complexes, in collaboration with Dr W. E. Watts.

The appointment is tenable for two years and is available immediately.

Salary (with F.S.S.U.): £1,929 by £129 to £2,058.

Further particulars may be obtained from the Registrar, The New University of Ulster, Coleraine, Northern Ireland (quoting Ref. 74/46) to whom applications, including the names and addresses of two referees, should be sent as soon as possible. (1650)

Please mention this Journal when answering advertisements

FELLOWSHIPS AND STUDENTSHIPS

APPLICATIONS OF LASERS IN SPECTROSCOPY

Applications are invited for a POSTDOCTORAL FELLOWSHIP in the applications of lasers to the study of high temperature inorganic species in gases.

Applicants are asked to send a curriculum vitae to Professor I. R. Beattie (Department of Chemistry, The University, Southampton SO9 5NH) from whom further details may be obtained. Salary on the range £1,929-£2,223 (under review) plus F.S.S.U. (1640)

University of Wales
University College of Swansea

The Welsh School of Pharmacy

RESEARCH STUDENTSHIP IN NEUROPHARMACOLOGY

Applications are invited from candidates with, or expecting to obtain, first or upper second class Honours degree in Pharmacy, Pharmacology, Physiology or a related discipline for a research studentship rising from a grant awarded to Dr. N. S. Doggett. The project, which is sponsored by an international drug company, concerns a study of the mechanism of action of tremorigenic compounds and may involve spending some time in the company's own laboratories in Switzerland.

The award is tenable for 3 years from September 1974 and the work will be suitable for submission for the degree of Ph.D. The value of the award will be £750 plus fees per annum, with appropriate additional allowances and extra remuneration for demonstrating duties to undergraduates.

Further details (quoting ref. N) and application forms (returnable by May 24, 1974) from the Academic Registrar, UWIST, Cardiff CF1 3NU. (1704)

UNIVERSITY OF BRISTOL
DEPARTMENT OF PHYSICS

RESEARCH STUDENTSHIP

sponsored by the National Physical Laboratory is available for the study of a new method of precision thermometry between 2 and 20K based on the dielectric properties of helium gas. The studentship is available for three years from October 1, 1974, at a salary which will be rather higher than the current Research Council studentship rate.

The work will be suitable for submission for the degree of Ph.D. Applicants should preferably hold a first- or upper second-class honours degree from a United Kingdom university, and must have an interest in and an aptitude for the use of precision measuring apparatus.

Applications should be sent to Dr. D. Gugan, Physics Department, Tyndall Avenue, Bristol BS8 1TL. (1689)

POSTDOCTORAL FELLOWSHIP IN PHOTOELECTRON SPECTROSCOPY

Applications are invited for an S.R.C. sponsored postdoctoral fellowship to investigate the electronic structure of molecular fragments and ions using beam photoelectron spectroscopy. Applicants should hold or expect to hold a Ph.D. in chemistry, physics or a related subject. Ability and genuine interest in small molecule chemistry are more important than previous direct experience. The post will be for one year in the first instance from a mutually agreed starting date. Salary will be on the range £1,929-£2,223 (under review) plus F.S.S.U.

Applications giving the names of two referees should be made as soon as possible to Professor N. Jonathan, Department of Chemistry, The University, Southampton SO9 5NH. (1639)

UNIVERSITY OF EXETER

DEPARTMENT OF BIOLOGICAL SCIENCES

Applications are invited for a NATURAL ENVIRONMENTAL RESEARCH COUNCIL STUDENTSHIP to investigate the interaction of soil animals and micro-organisms in the decomposition of leaf litter.

The value of the Studentship is £695 per annum plus fees (£540 per annum in the case of students living at home). Other allowances are payable in certain circumstances.

Candidates will be expected to obtain a good honours degree in Biological Sciences. A working knowledge of biochemistry will be useful.

Application forms and further particulars can be obtained from the Senior Assistant Registrar, quoting JMA/NERC/Biol. Sci., Northcote House, The Queen's Drive, Exeter EX4 4QJ. Closing date for applications: May 16, 1974. (1655)

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HERIOT-WATT UNIVERSITY

Applications are invited for a

CASE studentship

in the Department of Chemistry. The successful applicant will work in collaboration with Organon Laboratories Ltd. on new amino steroids in a series already established as possessing interesting pharmacological and clinical properties. The project will involve working both in the University and the industrial laboratories.

The University is situated in a parkland campus just outside Edinburgh.

The student, who should have a good Honours degree, will register for the degree of Ph.D. Enquiries should be sent to Dr. M. M. Campbell, Department of Chemistry, Heriot-Watt University, Riccarton, Midlothian EH14 4AS. (1682)

UNIVERSITY OF NEWCASTLE UPON TYNE

ROBERT WOOD FELLOWSHIP

DEPARTMENT OF CHEMICAL ENGINEERING

Applications are invited for the Robert Wood Fellowship tenable in the Department of Chemical Engineering. Applicants must hold a B.Sc. degree (or be prospective B.Sc. graduates) of the University of Newcastle upon Tyne or Kings College in the University of Durham. Applicants for the Fellowship must intend to undertake research in Chemical Engineering topics. The Fellowship will be of the value of £695 per annum plus fees.

Further particulars and application forms may be obtained from the Registrar of the University of Newcastle upon Tyne, 6 Kensington Terrace, Newcastle upon Tyne NE1 7RU. Completed application forms should be submitted to the Head of the Department of Chemical Engineering by June 30, 1974. (1643)

WESTFIELD COLLEGE
(UNIVERSITY OF LONDON)

N.E.R.C. RESEARCH STUDENTSHIP

for work on "Water and ion movement in live Sphagnum and peat" is available from October 1974. Peat covers about 2 per cent of the Earth's surface. The ecology of one of the major peat forming plants is to be studied.

The basic award is £695 a year and is under the N.E.R.C. regulations.

Applicants who have or hope to have a good honours degree in BIOLOGICAL, CHEMICAL or PHYSICAL SCIENCES will be considered.

Apply by May 29 to Dr. R. S. Clymo, Westfield College, Kidderpore Avenue, Hampstead, London NW3 7ST. (1671)

LANDSCAPE ARCHITECTURE. RESEARCH STUDENTSHIPS available for honours graduates in land use or natural science subjects.—Apply: Department of Landscape Architecture, The University, Sheffield S10 2TN. (1665)

UNIVERSITY OF EDINBURGH

RESEARCH STUDENTSHIPS IN SOCIAL ASPECTS OF SCIENCE

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Further details and application/nomination forms can be obtained from:

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(1703)

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Pleistocene Sediments and Stratigraphy of Forties Field, Northern North Sea (Dr. R. V. Dingle).

Polymorphism and unmixing in alkali feldspars (Dr. I. Parsons).

For further particulars and application forms apply to Professor E. A. Tait, Department of Geology and Mineralogy, Marischal College, Aberdeen AB9 1AS. (1675)

UNIVERSITY OF BRISTOL

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The work is a collaborative project between Dr. D. Woodcock and Mr. D. R. Clifford of Long Ashton Research Station, and Dr. J. N. Gibbs of the Forestry Commission, Alice Holt Lodge, Surrey. The successful applicant may register for a higher degree of the University of Bristol.

Further particulars and application form from the Secretary, Research Station, Long Ashton, Bristol BS18 9AF, to whom they should be returned by May 31, 1974. (1716)

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The Council of Lincoln College invites applications for a Post-doctoral Fellowship, tenable in any of the undermentioned Departments.

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Conditions of appointment giving further details of this position at Lincoln College can be obtained from the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the College in Canterbury, New Zealand.

Applications close on May 31, 1974. (1678)

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Applications with curriculum vitae and names of three referees to Dr Helen Muir, Kennedy Institute of Rheumatology, Bute Gardens, London W6 7DW. (1605)

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Applications are invited for RESEARCH FELLOWSHIPS in each of the following fields:

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2. Chemi-ionization studies by electron spin resonance (experience in spectroscopy would be of value).
3. Shock tube studies of combustion-generated pollution.
4. Shock tube studies of initiation in fuel-air mixtures.

Candidates are expected to hold an appropriate qualification such as an Honours Degree in Chemistry or Physics and have three or more years research experience, although candidates with less experience may be considered.

Further details are available from Professor J. N. Bradley, Department of Chemistry, University of Essex, Wivenhoe Park, Colchester CO4 3SQ. Telephone 0206-44144, Ext. 2030. (1706)

UNIVERSITY OF ABERDEEN

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Applications are invited for above post in collaboration with Dr. A. R. Forrester for work on the detection of reactive intermediates in organic reactions. Appointment is for one year from October 1, 1974, but may be continued for a second year. Post is covered by an S.R.C. grant and salary is in range £1,929 to £2,223.

Further particulars from the Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by May 25, 1974. (1714)

LECTURES AND COURSES

UNIVERSITY OF BRISTOL

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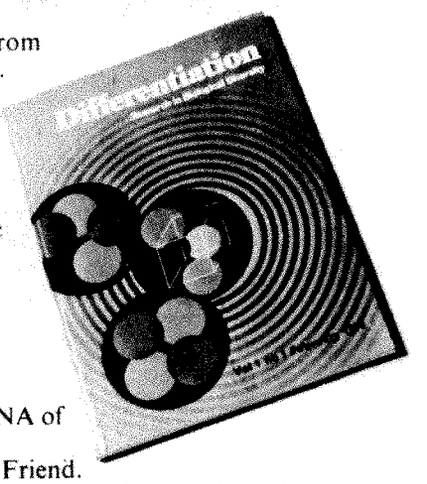
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Degree of differentiation in nonproliferating cells of mammary carcinoma. C. V. Wylie, P. K. Nakane and G. B. Pierce.
Polyribosome profiles and polyribosome-associated RNA of Friend leukaemia cells following DMSO-induced differentiation. H. D. Preisler, W. Scher and Charlotte Friend.
Studies on cytology and differentiation in Sciaridae. 5. Electron microscopic studies on the salivary glands of *Bradysia elegans* Morgante (Diptera, Sciaridae). A. B. Da Cunha, R. W. Reiss, C. Pavan, J. J. Biesele, J. S. Morgante and M. C. Garrido.
The principle of sequential dependence in cellular differentiation. P. A. Riley.
Time lapse filming of cellular interactions in organ culture. 2. The behaviour of malignant cells. E. J. Ambrose and Dorothy M. Easty.
Intercellular levels of cyclic nucleotides during cell enlargement and cell division in excised tobacco pith tissues. C. V. Lundeen, H. N. Wood and A. C. Braun.
Pattern formation in *Dictyostelium discoideum*: temporal and spatial distribution of prespore vacuoles. Ursula Muller and R. Hohl.
The enrichment of egg-type RNA in cleavage stage embryos of the sand dollar *Dendraster excentricus*. Shigeki Mizuno, H. R. Whiteley and A. H. Whiteley.
DNA synthesis and the production of antibodies by lymphoid tissues. G. Harris.
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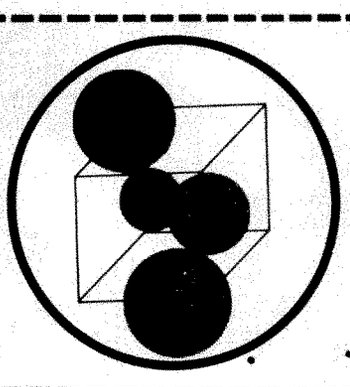
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Contributions for publication are invited by The Editor.

Editor: Dimitri Viza. Faculté de Médecine Pitié-Salpétrière, Laboratoire d'Immunobiologie, 105 Boulevard de l'Hôpital, 75634 Paris Cédex 13, France.

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Recent papers include:
Degree of differentiation in non-proliferating cells of mammary carcinoma, C. V. Wylie, P. K. Nakane and G. B. Pierce. **DNA synthesis and the production of antibodies by lymphoid tissues,** G. Harris. **The principle of sequential dependence in cellular differentiation,** P. A. Riley. Edited by: Dimitri Viza. Faculté de Médecine Pitié Salpétriere, Paris.

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Distribution of Caesium-137 in British Coastal Waters, D. F. Jefferies, A. Preston and A. K. Steele. **Export of Lead from Salt Marshes,** M. Banus, I. Valiela and J. M. Teal. **International Scope of Marine Pollution Damage,** D. P. Tihansky. **Effects of Red Mud on Marine Animals,** R. A. A. Blackman and K. N. Wilson. **Pollution Problem of the Golden Horn,** M. Karpuzcu.

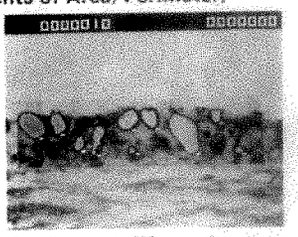
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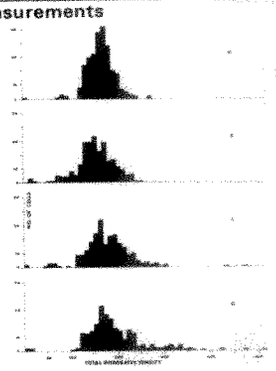
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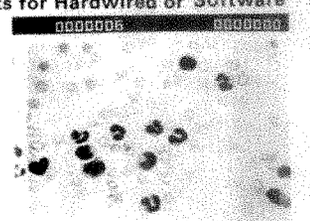
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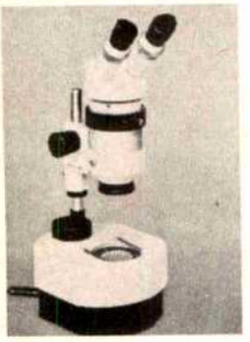
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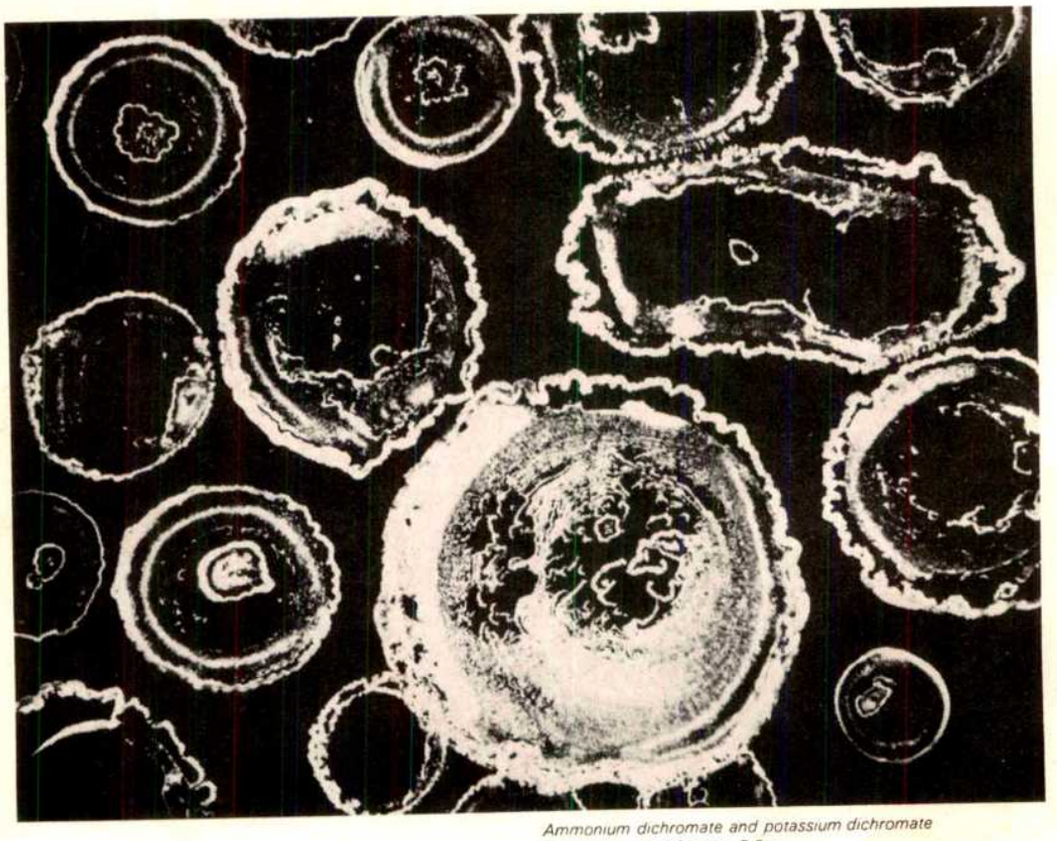


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Cell.

It's not just another new journal.

The first issue of **Cell**, a new monthly journal of cell and viral biology from The MIT Press, was published in January, and already its astonishing growth, both in circulation and reputation, seems destined to make it one of the most important journals in its field. Edited by Benjamin Lewin, formerly the editor of *Nature New Biology*, **Cell** provides a wide-range journal publishing articles of general interest in molecular biology, cell biology, tumor virology, immunology, genetic development, and other related areas. Papers are refereed as rapidly as possible and those accepted are published within twelve weeks.

Cell also includes an extensive review section. In addition to those reviews with the traditional role of discussing a field in some depth, short reviews give conceptual accounts of recent advances in areas of cellular biology, for the benefit of researchers in other areas. Book reviews and discussions of meetings are also included.

The May issue of Cell will include the following articles and reviews:

Interaction of Regulator Proteins with Recognition Sequences of DNA: Benjamin Lewin

A Measurement of the Sequence Complexity of Polysomal Messenger RNA in Sea Urchin Embryos: Glenn A. Galau, Roy J. Britten, and Eric H. Davidson

Studies on Nascent RNA in Mouse Myeloma: Henry Berger, Jr., and Ru Chih C. Huang

Regulation of Intracellular Protein Breakdown in Stringent and Relaxed Strains of E. coli: Devorah Rafaeli-Eshkol and Avram Hershko

Mutations Affecting Adenine Phosphoribosyl Transferase Activity in Chinese Hamster Cells: Lawrence A. Chasin

Mutants of Cultured Chinese Hamster Cells Deficient in Adenine Phosphoribosyl Transferase: G. E. Jones and P. A. Sargent

Type C Viruses of Baboons: Isolation From Normal Cell Cultures: G. J. Todaro, C. J. Sherr, R. B. Benveniste, and M. M. Lieber

Vesicular Stomatitis Virus Envelope Glycoprotein Alterations Induced By Host Cell Transformations: Sue A. Moyer and Donald F. Summers

Inhibition of Transport Systems In Cultured Rat Hepatoma Cells By Colcemid and Ethanol: Peter G. W. Plagemann and John Erbe

Development of hCG-Sensitivity in The Testis of the Immature Rat: Wolfgang Engel and Joachim Frowein

The June issue of Cell will include the following articles, reviews, and book reviews:

The Gene Numbers Game: J. O. Bishop

Evidence for Genetic Recombination Between Endogenous and Exogenous Mouse RNA Type C Viruses: J. R. Stephenson, G. R. Anderson, S. R. Tronick, and S. A. Aaronson

Increased Protease Levels in Transformed Cells: A Casein Overlay Assay for the Detection of Plasminogen Activator Production: A. R. Goldberg

Changes in Adenylate Cyclase, Cyclic AMP, and Protein Kinase Levels in Chick Myoblasts, and Their Relationship to Differentiation: R. J. Zalin and W. Montague

Induction of a Deoxycytidineless State in Cultured Mammalian Cells by Bromodeoxyuridine: M. Meuth and H. Green

Expression of Thymidine Kinase Variants is a Function of the Replicative State of Cells: R. Adler and B. R. McAuslan

Book Reviews

Among the Protozoologists: S. H. Hutner
Cell Culture—the State of the Art: J. Bard
Ribonucleic Acids in Review: E. M. Scolnick

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Cover picture
The green turtle marches into the Atlantic Ocean, to emerge 60 days later on Ascension Island, 2000 km away. How does it manage this precise navigation feat? Carr and Coleman (page 128) suggest an explanation based on the evolution of the Atlantic in the last 100 million years.

nature

Volume 249 May 10, 1974

UNTIL the end of 1973 *Nature* was set in England and printed in both England and the United States. The three-day working week imposed such severe constraints on us, however, that setting, printing and much of the despatch had to be removed to the United States; mailing from Richmond, Virginia, necessarily delayed delivery of *Nature* for Europe, Africa and much of Asia. We thank subscribers for bearing with us in this difficult time. This is the first issue for which we are again printing and despatching in England as well as the United States.

The paper for the English printing is Fletcher's 'Thinprint', of higher quality and much lighter than the coated papers we have used in the recent past. It has been a matter of concern to us that surface mail copies of *Nature* take so long to arrive, and yet that air mail subscriptions are too costly for many subscribers. The production of a lighter edition is the first step towards introducing cheaper air-mail subscription rates for 1975, of which details will soon be available.

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nature

Volume 249 May 10, 1974

Ban the bomb—properly

AT long last there is a sign of life in the test ban business. For so long a sleeping partner among arms control proposals, some form of treaty to control underground nuclear weapons tests now looks like becoming a reality.

Underground weapons tests do little or no damage to people or property, but they do cause some offence on the international scene, particularly to countries such as India and Sweden who are exercising self-restraint in not going nuclear themselves. It must be surmised that the Soviet Union can see a diplomatic gain in negotiating now. Certainly Mr Nixon must see the advantage of being seen again as an international peace maker.

There are many issues which need discussion before the wheels of diplomacy turn too far. One such is the treaty that will be negotiated. There are three types:

- Thou shalt not (comprehensive).
- Thou shalt not be found out (technical).
- Thou shalt keep it in moderation (threshold).

Politicians would say that these three types represent increasing realism on the scientific aspects of monitoring. They also represent increasing cynicism and expedience of the negotiating parties.

There are many possible weapons so small that their testing could proceed unchecked except by espionage. A comprehensive treaty would rule such tests out; violation of the treaty would be a dangerous pursuit if only because the whole machinery of espionage could be brought to bear, with some justification, on exposing infringements. It may thus be politically unacceptable, not only as signing away nuclear rights but also as a treaty too easily abrogated.

At the other end of the spectrum, a threshold treaty would lay down a seismic magnitude above which a test may not be. This is a catastrophic procedure. For a start it ties matters of international security to a hopelessly complex scientific art. In 1968, the occasion of the last east-west scientific discussions on this matter, Russian and American delegates eventually had to agree that they always came up with a different number for the same event and an arbitrary correction had to be applied to prevent the discussions from breaking down altogether. When scientists sit down to write the language of any future threshold treaty they may again be seriously at odds—perhaps to the jeopardy of the treaty. Further, a threshold is a challenge to any nuclear country to improve its ability to decouple explosions from the geological surroundings, by firing in loosely compacted rocks or ultimately in large holes. With technological ingenuity explosions as large as 50 kilotons could be muffled to have a seismic magnitude of only 4.75, a widely discussed threshold value. Thus a threshold treaty would be gradually eroded by a determined nuclear power, and in time could become almost meaningless. And if the purpose of a treaty is to convince potential proliferating countries that the super-powers have a highly moral approach to nuclear weaponry, leaving open the door to tests of tens of kilotons will impress nobody. Finally, a threshold treaty will put back even further prospects for a comprehensive treaty.

If a comprehensive treaty is politically difficult, and a threshold treaty is meaningless, it is desirable to consider seriously a technical treaty. The onus is on a nuclear country not to be caught in activities it regards as vital. There is a continuing pressure on monitoring systems to improve, so the treaty is not so easily eroded. The treaty is not as vulnerable to violations as a comprehensive one would be, for the occasional detected test would be the subject of a discreet hint, not a diplomatic incident. One can hardly claim that a technical treaty is ideal—it too encourages an evasive approach by nuclear countries and it is not entirely inoffensive to non-nuclear countries but it represents a realistic goal for negotiation in the immediate future.

Negotiation on a treaty must not be impaled on the inspection issue. The concept of an annual ration of inspections must surely go; if inspections are needed they must be rare and subject to flexible arrangements.

Finally, explosions for peaceful purposes. In the United States these have made little headway, but the Soviet Union has a vigorous programme involving many explosions every year. Those within the United States who do not wish any treaty—and they include much of the atomic energy establishment—will point out that peaceful shots can be the guise for military tests (at least in the Soviet Union). If peaceful tests are seen as latter-day Trojan horses, then it is better that national programmes be stopped and resumed later under international auspices.

100 years ago



Col. Strange says :—

"I think it is an absolute necessity on the ground of my second postulate, in which I say that all science should be cultivated, even branches of Science which do not appear to promise immediate advantage. It is one of the most important parts of Science, and cannot be omitted without detriment to all the other parts. . . . . Investigations connected with almost the whole of our material economy are required. There is no question connected with sanitary improvement, with water supply, or sewage, or telegraphy, or the enormous number of the requirements of the army and navy, which would not derive advantage more or less from investigations of a physical nature such as would be conducted in a physical laboratory. I think that the whole of our naval and military and social economy is dependent upon investigations such as would be carried on in a physical laboratory."

From *Nature*, **10**, 21, May 14, 1874.

Nature Vol. 249 May 10 1974

INSTITUTE OF ... Date ...

Future of forestry in Britain

A government decision about the future of British forestry is expected shortly. John Gribbin reviews the possible development of forestry in Britain in the light of the recently published annual report of the Forestry Commission.

THE future organisation of forestry activities in Britain has been under consideration now for several years.

The Committee of Public Accounts of Session 1967–68 expressed concern over the substantial losses of the Forestry Commission and the poor prospects for the future. It was at that time that there was a call for a proper consideration of whether the commission's noncommercial activities produced sufficient social benefits to justify the heavy expenditure. So the feeling at government level of a need for a complete overhaul of the Forestry Commission can certainly be traced back six years, and the overall review of forestry in general stems from this careful look at the Forestry Commission.

A White Paper published in June 1972 outlined the direction in which the government saw forestry developing, and recognised the need for landscape improvement and maintaining the character of traditional hardwood areas. But in itself that review had little practical effect, although at about the same time smallwood planting grants were suspended and replaced by an interim grant scheme to bridge the gap until a new structure had been worked out.

By October 1973 Mr Anthony Stodart, the then Minister of State at the Ministry of Agriculture, Fisheries and Food (MAFF), was able to report to the Commons that a general framework for the future of forestry policy had been worked out, and details were then expected to be announced early in 1974. But the General Election and resulting change of government has delayed announcement of a detailed policy; in the House of Commons on April 11 Mr Stodart asked Mr Roland Boyle, the Secretary of State at MAFF who now has responsibility for forestry, whether a decision had now been reached, and was told that "consultations are still in progress". Pressed by a Member concerned that woodland owners had no idea what to do about the 1974–75 planting programme because the decision had not been taken, Mr Boyle replied "we have the matter very much in mind and hope to be able to announce a decision as soon as possible". Since then, the Forestry Commission *Annual Report and Accounts 1972–73* has been published (HMSO, 90p); together with previous government statements this makes it possible to foresee the likely development of forestry in Britain once the awaited government decision is announced.

One key change which has already been made is a reconstruction of the commission's accounts. From 1919 to 1972 the accounts have had to bear interest charges at the appropriate government lending rates, and these have averaged about 5% compounded over that period. Under the new structure the aim is to be a rate of return on funds of 3%, which is accepted as the maximum that forestry can be expected to earn in northern Europe on the best sites close to markets. That is an important proviso, for much of the commission's planting has been undertaken for strategic or social reasons, and cannot be expected to provide such a high return. "Where less profitable activities are undertaken for reasons of government policy", says the report, "the commission will be given . . . subsidies each year to bridge the gap between 3% and the expected rate of return".

This policy is not affected by Britain's entry into the European Economic Community (EEC), which has no common forestry policy. Regulations to implement two EEC directives concerning improvement of genetic and other standards of seeds, young plants and so on became effective on July 1, 1973, and the EEC Commission is considering a draft directive which, although chiefly devoted to improving the structure of agricultural holdings, has some implications for forestry. But otherwise the British government has essentially a free hand in forestry matters.

It must be remembered, of course, that only half of the total productive forest area in Britain is owned by the commission; but in practice the interests of private and state forestry are complementary and cooperative, rather than competitive. A major difference

Table 1 Land use by Forestry Commission at March 31, 1973 (Thousands of hectares*)

	Great Britain	England	Scotland	Wales
Total area	1,212.0	306.7	744.6	160.7
Forest Land: Total	882.1	261.7	481.9	138.5
Under plantations	768.3	239.5	399.5	129.3
To be planted	113.8	22.2	82.4	9.2
Other Land: Total	329.9	45.0	262.7	22.2
Nurseries	0.3	0.1	0.2	—
Agricultural and grazing	151.5	14.2	123.2	14.1
Forest Workers Holdings	5.1	1.1	2.9	1.1
Unplantable and miscellaneous	173.0	29.6	136.4	7.0

* 1 hectare = 2.471 acres

Table 2 Planting in the year ended March 31, 1973 (hectares)

	Total	New planting	Restocking
Great Britain	23,158	19,378	3,780
Conifer	22,865	19,224	3,641
Broadleaved	293	154	139
England	3,154	1,735	1,419
Conifer	2,890	1,608	1,282
Broadleaved	264	127	137
Scotland	17,764	16,174	1,590
Conifer	17,739	16,151	1,588
Broadleaved	25	23	2
Wales	2,240	1,469	771
Conifer	2,236	1,465	771
Broadleaved	4	4	—

Conifers continue to dominate planting by the Forestry Commission, but that pattern is likely to be changed when details of the new government policy are announced.



Peeled woodwool billets stacked for air-seasoning in a clearing of 47-year-old Scots Pine at Bramshill Forest, Camberley.

is that private forests are concentrated on 'traditional' areas whereas the commission has afforested wide areas formerly in marginal agricultural use. The Forestry Committee of Great Britain, which represents private forestry in discussions with the government, generally sees eye to eye with the Forestry Commission, although it is in a better position to speak out against government proposals even to the extent of commissioning its own investigation of the accuracy of government studies relating to forestry. Of course, the two sectors of the forestry industry are in a competitive situation in the 'market place', but that market is so hungry for timber that both sectors are assured of selling their product.

Where the private sector differs from the Forestry Commission, however, is that small planters are in a much more vulnerable position when there is an air of uncertainty about the future direction the industry is expected to take. This is highlighted by the fact that the interim scheme for smallwood planting grants has now run its course, and that since the beginning of this year there has been nothing to replace it. A spokesman for the Forestry Committee said last week that small owners are already being affected, and that "some nurseries are finding it very tough" as a result.

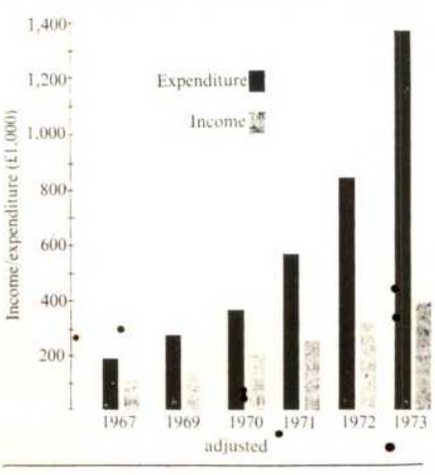
This is a matter of more immediate urgency than the question of what should be planted in the 1974–75 season. For the private sector, the 1973–74 planting season is essentially over, and although there is a clear need for plans for the next season to be put in hand as soon as possible, there is probably less urgency about this at present than at any other time of the year. Of course, there can be little doubt that the detailed government plans will include a hardwood planting supplement—but it is up to the individual owner or planter to decide whether this is attractive enough for him to change his pattern of planting. All this takes time, and the present situation of uncertainty is clearly undesirable.

But the most important fact which must be taken into account when the government announces its new detailed plans is that in forestry terms Britain is very nearly saturated, with about 8% of the country covered by forest. Most of the figures in Table 1 are slightly down on last year; on March 31, 1972, for example the corresponding figures for forest land and total area were 876.2 and 1,215.0 thousand hectares, respectively. The total area of forest land acquired in the year ended March 31, 1973 was 6,000 hectares—more than 10,000 hectares less than in the previous year, and some 15,000 hectares less than in 1971–72. One reason for this is improvements in the sheep farming industry which make less land available for forestry; by and large, there is no longer any suitable land on the market.

In the light of this, and the removal of forestry's strategic role, the future emphasis is likely to be on forestry for amenity use. More hardwoods are likely to be planted than conifers, and existing forests will be promoted as recreational facilities. Indeed, the latter is already happening in a dramatic way.

The commission has also been involved in "plant a tree in '73" activities, including reclamation of 2 hectares of a derelict iron ore site in Cumberland, 6 hectares of coal tip at Kilsyth in Stirlingshire, and 12 hectares of derelict mining valley at Aberdare, Glamorgan.

It is perhaps ironic that these changes should be coming about at a time when world market conditions have caused a dramatic increase in softwood prices; demand for home grown sawlogs almost doubled in the last quarter of the year covered by the report and there is, according to the report, a "strong desire" among British wood-using industries to use the dependable home grown product wherever possible. But with Recreation Planning Officers appointed in most conservancies, a feasibility study underway investigating development of low cost tourist accommodation on commission land, and the general restructuring of the commission's activities the future of forestry in Britain is clearly going to be markedly different from its immediate past. It seems appropriate that the commission is making a move to a new headquarters, near Edinburgh, at this time: it will be installed there by the middle of 1975.



international news

More criticism of nuclear safeguards

Colin Norman, Washington

FOR the second time in less than a month, the Atomic Energy Commission has been hit hard with a critique of its safeguards against the theft of weapons-grade nuclear materials. The latest attack is particularly damaging to the AEC's public defence of its safeguards because it comes not from acknowledged critics of nuclear power but from an internal AEC study by four AEC consultants and one AEC employee.

Released last week by Senator Abraham Ribicoff, in spite of strenuous objections from the AEC, the study concludes that the commission's safeguards are "entirely inadequate" to prevent ingredients of nuclear weapons being stolen from the United States nuclear power programme.

A central criticism is that diversion of weapons-grade material from the power programme poses a greater threat to the public than the risk of a nuclear accident, yet "the relevant regulations are far less stringent". The study goes on to note that "It is our strong feeling that the point of view adopted, the amount of effort expended, and the level of safety achieved in keeping special nuclear material out of the hands of unauthorised people is entirely out of proportion to the danger to the public involved".

The AEC's reaction to these charges, outlined in a statement hastily distributed by its press office, is that there is not much danger of diversion of weapons-grade material at present because very little highly enriched uranium or plutonium is used to fuel the light water reactors which now form the basis of the nuclear power programme. But the commission added that it is "taking a hard look at the study to determine what additional measures should be taken to further strengthen the requirements to safeguard nuclear materials from theft".

The point is, however, that towards the end of the 1970s light water reactors will probably be operating on a plutonium cycle, which means that large quantities of weapons-grade nuclear material will be shipped around the country as reactor fuel. And if the fast breeder reactor lives up to the AEC's expectations, by the end of the century the amount of nuclear material flowing through the power programme could be sufficient to manufacture a quarter of a million bombs a year.

The study first finds fault with the system for accounting for nuclear materials in the fuel cycle. "The uncertainties in the accumulated material balances of the atomic energy operation of the country already make it impossible to say that an explosive mass has not been diverted, and if reliance is placed solely on material balance methods that statement will have to be expanded many, many-fold in the near future," the report says. Furthermore, the present system will not pick up the diversion of nuclear material quickly enough: "A skilled diverter [could] have constructed an explosive device before the system can reach a conclusion that diversion has taken place".

A major recommendation is that the federal government should establish a special security force to guard nuclear materials in transit and for "all protection functions which could require the use of force". The study also recommends that the commission should forge strong relationships with the Central Intelligence Agency and the Federal Bureau of Investigation to make sure that those agencies are devoting sufficient resources to the problem of nuclear theft.

The whole issue of nuclear safeguards is rapidly building into a potent weapon in the hands of nuclear critics in the United States, particularly in respect of plans to build a string of plutonium - producing fast breeder reactors around the country. In response to such criticisms, Representative Melvin Price, chairman of the powerful Joint Committee on Atomic Energy, announced last week that his committee has launched a special review of the mater and will be holding some hearings later this year.

Israel's nuclear plan

Nechemia Meyers, Rehovot

AFTER years of debating the construction of nuclear power stations, a proposition always vetoed by officials in the Finance Ministry because overseas experts assured them that power generated by conventional plants would be far less expensive in the foreseeable future, the Israeli Government has decided to put all its eggs in the nuclear basket.

Minister of Commerce and Industry Haim Bar-Lev has announced that from 1980 onwards, every power station built will be nuclear. The 1970s will see the construction of Israel's last new conventional power plant and her first nuclear one with a power-generating capacity of 600 MW, expected to be completed by 1982, when total national capacity is expected to be 4,200 MW.

An international tender for construction of the nuclear power station will be issued later this year by the Israel Electric Corporation, which has been charged with construction of the plant; the Israel Atomic Energy Commission, which operates the country's two existing research reactors at Sorek and Dimona, will be responsible for ensuring stringent safety standards.

While some locally-made equipment will be used, reactors will almost certainly be bought from the United States, and a representative of General Electric, Asher Engler, has stated that his company is willing to provide the required enriched uranium for the first few years of operation but that future supplies of the strategically important material would depend on an agreement between the governments of Israel and the United States. Uranium could be extracted from phosphates from the Negev Desert, using locally developed methods, but it would still have to be enriched abroad.

Israeli environmentalists say that if Israel must have atomic power stations, they should be located in the furthest depths of the Negev, but the parched southern half of Israel lacks the large quantities of water required by nuclear power facilities for cooling purposes.

Whatever the validity of their arguments, the environmentalists have little chance of influencing government policy on this project. The first nuclear power station will soon be going up, probably near the new Mediterranean port town of Ashdod. If all goes well, there will be seven more by the turn of the century.

Soviet Scientists adopt new ploy

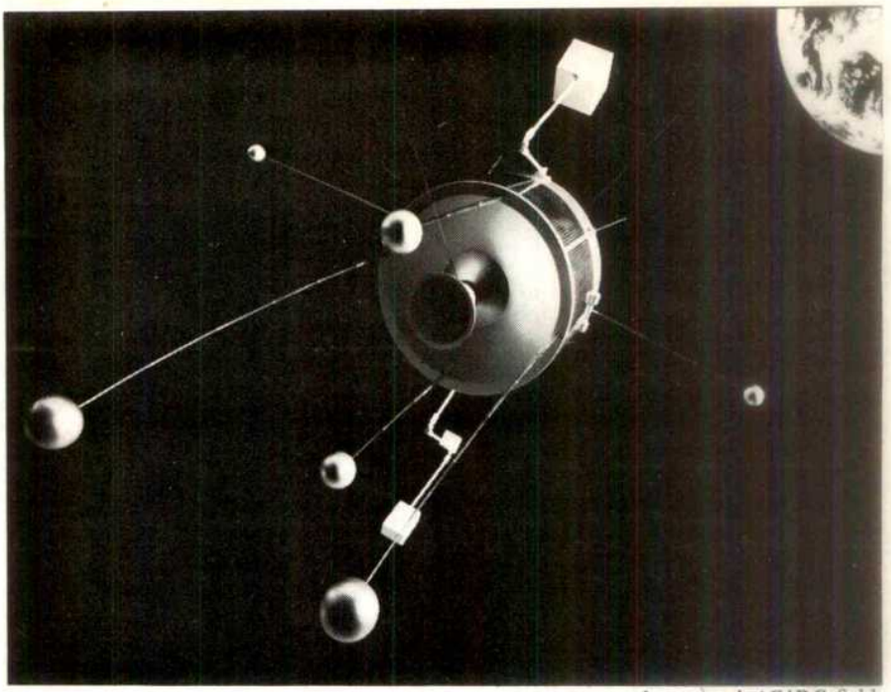
A UNIQUE international seminar will take place on July 1–5 in the Moscow apartment of Dr Alexander Voronel, a Soviet physicist who lost his job last year when he applied for a visa to emigrate to Israel. Organised by 19 Soviet Jewish scientists who have similarly been deprived of their jobs, and sponsored by an international board of advisors, the seminar is an attempt to keep the Soviet scientists in touch with developments in their own disciplines. It will undoubtedly pose something of a challenge to Soviet authorities.

Bearing the rather vague title of 'Collective phenomena and the applications of physics to other fields of science', the seminar has already met with considerable support in the international scientific community. A call for papers went out some seven weeks ago, and according to Dr Norman A. Chigier, professor of chemical engineering at the University of Sheffield, who is one of three international secretaries for the seminar, about 70 abstracts have so far been sent in.

Those who have submitted abstracts have been advised to apply for visas to enter the Soviet Union, and they have been told to make no secret of the fact that will be attending the seminar when they fill out their applications. Asked whether the Soviet authorities are expected to approve the visa applications, Chigier said that there is no way of knowing in advance, but that in the past they have been "unpredictable in their visa policies".

Chigier emphasised that the seminar should not be construed as "a confrontation with Soviet authorities". The Soviet Jewish scientists who are organising the event have been deprived of their right to travel abroad to attend scientific meetings and many have also been denied requests to attend meetings within the Soviet Union. According to Dr Edward Stern, professor of physics at the University of Washington, Seattle, who is also an international secretary for the seminar, the meeting has been organised to help Soviet Jewish scientists "break out of this scientific isolation which smothers and destroys scientific creativity." Chigier added that the event is "a gesture of solidarity".

All formal scientific meetings in the Soviet Union must be approved and organised by the USSR Academy of Sciences, but by labelling this event a seminar and holding it in a private apartment the organisers hope to circumvent the official regulations governing international scientific conferences.



GEOS: launching in 1976 to measure magnetospheric plasma and AC/DC fields.

The French in space

from the staff of La Recherche

THE relaunching of a European space policy, which should be confirmed by the creation as of mid-June of the European Space Agency (ESA), will give a second wind to French space efforts. The third space power, France has in fact launched but twelve satellites—only nine successfully—in twelve years of rather irregular activity. The enthusiasm of the first years was followed by an eclipse of three years (1965-67), then a stabilisation.

For the first time this year the French space budget will reach a thousand million francs. This is to be accompanied by a reorientation of the activities of CNES (the National Centre for Space Studies) towards greater European co-operation. The national programme becomes a support activity but bilateral operations, principally with the United States and the Soviet Union, will continue in order to permit participation in original programmes which are beyond French means. Thus scientific experiments will be put aboard the American probes OSO-1, Pioneer–Venus, IMP-L and Mariner–Jupiter–Saturne, while the Soviet Union will launch two small French technological satellites (Sret) and prepare the experiment EOS (release of a French balloon in the atmosphere of Venus). Co-operation with Germany will come about within a year with the launching of two telecommunications satellites (Symphony).

Thus France is putting its efforts into activities within the European community, in order especially to keep ahead of Germany. While continuing to participate in the programmes of scientific satellites and applications devised by ESRO (European Space Research Organisation), such as the inhabited orbital laboratory Spacelab, Paris is in fact concentrating more on the heavy launcher Ariane. True to its policy of European independence, France considers that the independent exploitation of future European telecommunication satellites depends on having a launcher available, and has therefore decided to finance nearly 65% of the development cost of this launcher (2,472 million francs with 20% for contingencies) and to take control of the project. Technical and financial management has been assigned to CNES, under the control of ESA.

This is clearly an ambitious project and in taking over his post at the beginning of the year the new president of CNES, Mr Maurice Levy, recognised that it is "both a heavy responsibility and a technical challenge to which every resource will have to be devoted".

A three-stage launcher of 200 tons capable of placing 750 kg into fixed orbit. "Ariane" will be the largest rocket constructed in Europe and the first to use cryogenic ergols (liquid hydrogen and oxygen), which assure high performance but of which the Europeans do not yet have much experience.

The success of this project requires a combination of efficient management, thorough pilot studies and the maintenance in active service of teams and resources for French launchings. The launching range in Guiana will have an important part to play; up to 1977 it will have only six Diamant BP4 rockets to launch to put into orbit the

future French satellites Starlette (October 1974), Castor and Pollux (January 1975), D2B (mid-1975), D2B-Gamma (mid-1976) and Dialogue (end of 1977).

Thus the career of Diamant BP4 is practically finished and if a new 'dead period' is to be avoided before the arrival of the launcher Ariane, scheduled for 1980, a replacement must be found immediately.

Several projects are envisaged by CNES, for example a "Z launcher" which would use the two upper stages of Ariane and which could place 575 kg into low terrestrial orbit (400 km); the capability of Diamant BP4 is only 170 kg. But in fact the choice of the future national light launcher depends in part on an eventual programme of French military satellites and until now it has been uncertain whether this would make an entrance before 1980.

The electronic newspaper in view?

Ian Ridpath

WITH newsprint scarcities the world over, this could be an opportune time to turn into reality the science-fiction dream of an electronic newspaper that flashes information into each home at the touch of a button. And, as a result of a test programme currently under way, that is exactly what could be happening in the United Kingdom within two years.

These exciting plans stem from the realisation that almost every home has a data terminal for receiving visual transmissions—a television set. The broadcasting companies have decided to use this exciting facility to provide an extra service.

By coding the electronic newspaper transmissions on normal television broadcasts, and incorporating a decoder in domestic television receivers, the system can be run without using any extra band space on the already crowded airwaves. According to equipment manufacturers, the extra circuits needed in a new receiver would cost a thoroughly reasonable £20, although adding a converter to an existing set might cost between £50 and £100.

The trick in sending out the data is to use two television scan lines that are normally redundant. These are lines 17 and 18, which remain out of sight at the top of the picture. Each of these lines now contains pulsed information for one 'row' of the electronic newspaper's 'page'. A page is a screenful of data; there are 24 rows on each page and each row has 40 characters. Prototype receivers to pick up this year's forthcoming test transmissions are now being produced by industry.

A newspaper of around 60 pages will be produced at first, although up to 99 pages are possible. The complete set of pages will take less than 30 seconds to build up inside the receiver's store and continual changes will be added, second by second, as the news comes in. An editorial team at the transmitting centre will feed news into a computer, which will encode it for transmission.

This service would be particularly valuable at times such as elections, but for normal use the electronic newspaper is likely to carry sports results, stock market prices, weather reports, and news bulletins. Simple line illustrations will be possible on the system, and the independent channels may well include advertising.

It is difficult to foresee how sales of printed newspapers will be affected by electronic data transmission to the home. Existing radio and television coverage has not destroyed the newspaper market and the summarised information of the electronic newspaper may even increase demand for longer printed reports. But the potential of the system may have more subtle long term effects and there is clearly considerable room for lobbying by interested parties.

At home, the viewer with a suitable receiver will be able to watch television programmes normally until he decides on a change. His set will have a push-button console on which he can punch up the page number of the electronic newspaper he wants. Page 1 of the paper will probably be given over to an index of the full contents. The pages of the paper, which have been stored in the receiver, will then be displayed with a header line showing the time to the nearest second, thus increasing the effect of 'instant information'.

Both the BBC and the IBA (Independent Broadcasting Authority) independently developed their own electronic newspaper transimssion systems, which they have been experimenting with since last year. But they have just agreed on an improved common system, engineering details of which are about to be published.

This standard system, which the prototype sets are designed to receive, enables viewers to pick up transmissions on all channels. Test transmissions to prove the new system's potential are to begin in the autumn, although it is not clear whether members of the public will be able to join in the fun of watching the editorial teams iron out their snags.

What is certain is that, after the trial period, a decision will be needed by the broadcasting companies, the receiver manufacturers and the Ministry of Posts and Telecommunications to begin full scale public services. It will be a historic decision, for this will be the world's first commercially available system of its kind.

Drug research in the UK

Peter Newmark

A SERIOUS failing of the drug companies was recently emphasised by Dr J. W. Black in his inaugural lecture as professor of pharmacology at University College, London. Returning to academic life after 15 distinguished years with ICI and Smith, Kline and French, he pointed out that industry was primarily interested in research and development of those drugs that might cure the most common diseases. This makes economic sense to the companies but means that those who suffer from rarer complaints are being cheated of their chance of a cure. The victims of, for example, multiple sclerosis and muscular dystrophy are included in this deprived category.

Professor Black offers a two-pronged approach to the alleviation of this imbalance. First, he is instigating a novel degree course aimed at overcoming the deficiences and hence the paucity of academic, pharmacological research. Second, he suggests a National Drug Research Centre to concentrate on those areas neglected by industry. Where the vast funds for this would come from is anybody's guess. It is a sobering thought that of many thousand folic acid antagonists synthesised and investigated to date, only one, methotrexate, offers much to the treatment of cancer. And for those with the rarer disease it is only an inapt comfort that can be sought in the vast array of money-making antidepressants and tranquillisers that pour from the laboratories of the drug companies.

Latest figures compiled by the Office of Health conomics in London show that expenditure on medical research in the United Kingdom topped the £100 million mark for the first time in the financial year 1972–73. This represented an increase of about one-fifth over the previous year, though must be considerably less in real spending terms. Of the total expenditure, the government contributed 61%, charities and trusts a notable 13% and the pharmaceutical industry a dwindling 26%.

These statistics give no indication of which areas of medical research benefited most from the funds, let alone what returns were received by the investors, be they the public or shareholders. One thing that is certain is that most pharmaceutical research was done by the relevant industry. This is probably as it should be. To what extent it is in the public interest is less certain and the present government is not averse to nationalising parts of the industry if it feels that this is the best action for the nation.

Business report: profit and tax

Roger Woodham

THE more one enquires precisely how much meaning can be attached to a company's 'profit' as presented in its annual report, the more the answer seems to be 'relatively little'. This was highlighted in a paper by Parker and Gibbs presented recently to the Institute of Actuaries in London.

The drift of their paper is that companies of most kinds, except those in service industries, would come up with considerably smaller profits if inflation were not taken into account in some way or another. For example, it is conventional accounting practice not to take into account inflation when amounts are set aside for depreciation of plant and machinery, so the profit seems to be that much larger and has to be dipped into to replace that plant or machinery at the inflated prices. Likewise, "profits are overstated by the inclusion of profits on stock which arise solely from a general increase in price levels".

Parker and Gibbs produce some interesting findings using the CPP (current purchasing power) method of accounting. Here allowance is made for inflation by using a single index, for example the retail price index, to set up the company's accounts in terms of end-of-accounting-year pounds. This is a rather crude method, however, for it does not take into account the rise in price of a particular commodity, which may be much more than the increase in a broadly based index.

Parker and Gibbs show that the chemicals sector would have earnings 15% down on average, oil 25% down, and general engineering and eletricals 50% down according to CPP accounting. More specifically, ICI's earnings for 1972 would have been 45% less and BP's 65% less. In 1973 BP earnt £310 million (compared with £70 million in 1972) but on huge total sales of £4,500 million. Only detailed analysis will reveal what inflation accounting would have made of that.

As J. M. Keynes is thought to have said: "It is better to vaguely right than precisely wrong".

●If the United States Internal Revenue Service (IRS) gets its way about the taxing of multinational companies based in that country, some companies may find it financially worthwhile to shift more of their research and development effort abroad.

Even the IRS admits that the situation is complex, but basically what happens now is that tax calculations for multinationals in the United States do not take into account the fact that some earnings outside the United States are generated by money spent in the United States, for example on research and development.

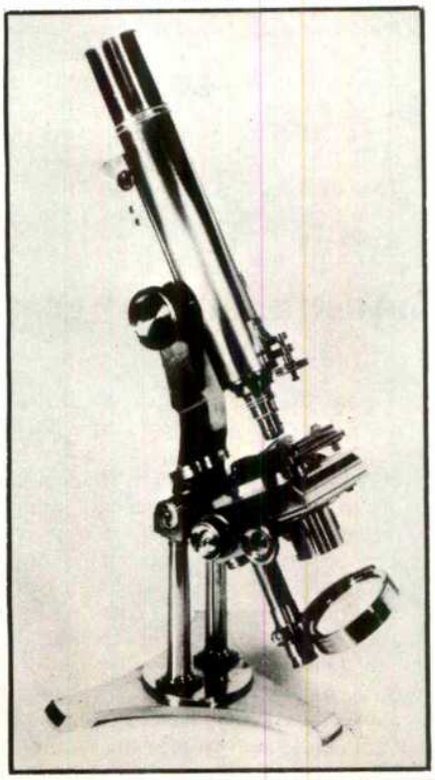
A company can obtain 'foreign tax credits' (which can be offset against United States tax) in respect of taxes paid to foreign governments. The IRS wants a proportion of 'home' expenditure on research and development to be shifted on paper to foreign operations, which means less foreign income and less foreign tax credits in the United States. The IRS has a complex way of calculating these credits and, in the final analysis, the pre-tax profits of multinationals based in the United States would be reduced—some say by 3%, typically. Certain companies, notably in the pharmaceutical industry, stand to lose more because their research and development programmes are particularly centralised; they would face a great pressure to do more research abroad.

The matter is still under consideration at the IRS and a decision is not expected for some months. The interested companies are, however, putting up a formidable legal fight.

Microscopic appeal

David Davies

A microscope as old as the society



THE Royal Microscopical Society has launched an appeal for £50,000 to expand its operations. In particular, it plans to increase its educational activities and publish its *Journal of Microscopy* more frequently. The appeal was launched by retiring President, Professor A. G. E. Pearse on April 30.

The society has had a chequered career. It was founded in 1839 when the microscope was as much as anything the preserve of amateurs but when exciting times were just around the corner for microscopists. Leeuwenhoek's seventeenth century resolving power of 1.25 μm had barely been improved on but the work of J. J. Lister on achromatic objective lens systems was just beginning to open up new horizons. By 1880 the resolution of the best microscopes was down to 0.2 μm and the society's aim to introduce and improve the microscope 'as a scientific instrument' began to be achieved.

Communication amongst microscopists depends very much on quality reproduction but the society was hardly in the forefront of publishing photomicrographs. It had published its first in 1853 (the ubiquitous proboscis of the fly) but it was not until 1906 that the *Journal's* lithographs began to be supplanted by photographs.

After 1890 there was little fundamental technical development possible for the optical microscope and the society went into a long period of decline. "Hibernation" one of its officers says, "is too mild a word for it". The question is whether the society has now been able to catch up with the lost opportunities of the dormant period and whether it will be able in the future to exert influence in the development of microscopy—now, of course, including electron microscopy. Many of the educational activities such as teacher training, provision of a technician's qualification and the publishing of teaching manuals, the bread and butter of many professional societies, are only now being planned. Another field that one fellow described as needing urgent attention is the "abysmally low" general standard of photomicrograph reproduction in books. Yet another problem is that a certain introversion and inscopists seem to possess, perhaps feeling their profession is merely a service industry.

The society has a hard pull ahead of it with much unglamorous work to do in establishing educational programmes. If it fails in its appeal, it will revert to a journal-producing and symposium - organising society, and science will suffer thereby.

Computers and social options

from the staff of La Recherche

FOR the first time at the international level, information scientists and computer architects, sociologists and trade union representatives met earlier this month in Vienna to try to cope with the nagging issue of "Computers and Human Choice". Organised under the auspices of the 35-nation International Federation for Information Processing (IFIP) and the Austrian Cybernetic Society, the conference nicely complemented a symposium held two months earlier in Amsterdam, when the International Federation for Automatic Control pondered the problem of "Productivity and Man". Both meetings are clear evidence of the concern shown by professional societies for how today's machines are amplifying human mind and muscle and where the mechanical surrogate leaves man.

The job which the IFIP has set for itself is a big one: in the words of its president, Heinz Zemanek, it is "to stress the social aspects of the computer —to re-establish the human being as the centre of our existence". Large scale information systems have been motivated, to date, largely by industrial and commercial needs. (The military compulsions were not treated by the conferees at Vienna.) Harold Sackman (RAND Corporation) estimated, for example, that the public treasury of the United States has spent about $100 thousand million thus far "on the development of computer services of all kinds, and the rest of the world has spent probably the same amount. But the individual citizen is the last to see a direct, useful return on his investment." Or, as expressed more effectively by John M. Carroll (University of Western Ontario), "the computer may be regarded by some as a repressive instrument which serves the causes of governments, corporations and large institutions. The common man too often sees himself as billed by them, taxed, enumerated, administered, manipulated, controlled—creased, folded, spindled and mutilated."

There is hope, nonetheless, that the social options open to the wizards of computing will prove to be more humane in the years to come. Citing a recent report by the Japan Computer Usage Analysis Institute, Alfred Dallinger of the Austrian trade union of white collar workers reminded the meeting of the following time spread noted by the Japanese in the application of large scale information handling systems: 1945–70, 'big science,' 1955–80, the management base, and 1980–2000, the individual base.

With this projection in mind Ulrich Briefs (West German Trade Union Federation at Dusseldorf) noted that one of the key problems in the long range strategy of organised labour is "whether it is better to fight for an improvement of the situation of employees . . . with no involvement in management decisions or . . . it is more advantageous to participate actively and cooperatively." In other words, does the noninvolvement of the employee in the management process lead to worker alienation? Kristen Nygaard, a computer scientist who is also an executive of Norway's labour union federation, could foresee that trade unions would help improve system design as labour becomes better informed, "as it acquires more information and, therefore, more power in the management–worker relationship. I could not see the unions in my country," he added, "approving the installation of visual terminals on



the shopfloor unless these were two-way links."

That the computer is not devoted principally to inventory and marketing problems or to payroll and insurance calculations was evidenced by some of the extensive (and impressive) social applications found for the computer in different geographical and ideological settings. G. R. Waddell, computer comptroller of the Edinburgh Corporation, explained the enormously complex base of accounting, personnel and multiple urban data comprising the municipal management information system (MIS) required to run the city of Edinburgh. Carroll described the experience in London (Ontario) of installing a "people's computer as an aid to democracy", of providing "the common man with easy access to computer facilities to even up the social, economic and political balance of power" in the form of a nongovernmental urban information service in that community. And Burt Nanus, director of the Center for Futures Research at the University of South California, recalled that a Delphi study made for the American Federation of Information Processing Societies showed how MIS could "strengthen multinational public enterprises in such areas as [education] public health, criminal justice, pollution control, weather forecasting and disaster control, with major impacts before 1985".

An area of principal concern to the IFIP colloquium was that of fair information practices. Bo Hedberg (International Institute of Management, Berlin) emphasised that "we are approaching an information-rich society". System design of the means to process these data usefully, Hedberg continued, "is concerned with the distribution of power, implicitly or explicitly". Willis Ware, an engineer at RAND, proposed the creation by legislative action of a "code of fair information practice or perhaps an Informational Bill of Rights" in an effort to define, nationally then internationally, what is fair behaviour in this realm, "establishing safeguards and deterring deviation by judicial process and appropriate penalties". The IFIP agreed that it will examine the optimal methods, through an *ad hoc* computers-and-society committee, in order to deal with this and similar issues raised during the week-long deliberations.

Although some expressed the apprehension that "the electronic world is passing us by as we sit here debating—Nero fiddling while Rome burns," the last and far from pessimistic word on the subject went to the venerable, Russian-born physicist Lew Kowarski (CERN). He underlined his trust in man's use of the computer when he declared that the latter, "while it will relieve us of burdens, could also relieve us of all that makes life worth living. Before I count on the unforeseen—and therein lies our hope, albeit a pious one. The danger in computers comes not from the experts, but from the profiteers."

Australian answer

Peter Pockley, Sydney

AFTER studies by various Australian government departments, the Russian request to set up a joint facility "for photographing space objects and studies of the upper atmosphere," reported recently in *Nature*, has been rejected. The announcement was made on the same day that Prime Minister Whitlam called a snap general election. In the election atmosphere, the Labour Party could not afford the predictable attacks on grounds of favouring communists above capitalists, even if the scientific arguments in favour of the facility had been sound.

news and views

Maternal transmission of hepatitis

INTEREST in the transmission of viral hepatitis from the mother to her offspring is not new. The effects of hepatitis on the course of pregnancy and on the foetus have been studied since the report by Saint-Vel in 1862 on an epidemic of severe hepatitis in pregnant women in Martinique (*Gaz. Hôp. civ. milit. Paris*, **35**, 538; 1862). The world literature contains scanty and incomplete data and numerous divergent views. Stokes and associates (*J. Amer. med. Ass.*, **514**, 1059; 1954) presented evidence of 'silent carriage' of hepatitis B virus, and it is relevant to examine this paper in detail now that specific laboratory tests are available for hepatitis B and the problem of maternal transmission of this infection is being actively investigated.

One carrier described by Stokes was known to have transmitted hepatitis to at least four out of twelve recipients of his blood over a period of 3 years. His blood was also administered to four male volunteers, one of whom developed hepatitis. The second carrier is of particular interest. She was the mother of an infant who developed hepatitis at the age of 2 months. Volunteers given serum from her and her infant developed hepatitis. The third carrier was known to have transmitted hepatitis to four recipients of his blood, including one volunteer. The impact of apparently healthy carriers of hepatitis B virus on blood transfusion services and indeed on every aspect of medical practice is generally recognised. The problem of maternal transmission is just beginning to emerge.

Turner and colleagues (*Archs Dis. Childh.*, **46**, 616; 1971) reported the case of a young man who contracted hepatitis B after injection with an unsterile syringe. His wife, who was pregnant, developed the infection some 9 weeks later and her baby was born prematurely. After 59 days hepatitis B antigen was detected in the child's serum and antigenaemia had persisted so far through the first 2 years of life. The child remained clinically well but with some biochemical evidence of liver damage. Wright and associates (*Brit. med. J.*, **4**, 719; 1970) previously described the clinical history of a young dental assistant who developed jaundice late during her pregancy. She was delivered of a normal female infant who, in turn, became jaundiced at the age of 5 months. Hepatitis B antigen was detected repeatedly in the infant's serum and in the mother's serum tested when the infant was admitted to hospital. Liver biopsy of the infant at the age of a year revealed chronic active hepatitis and cirrhosis. The mother, on the other hand, had remained symptom-free and her liver function tests were normal. Ohbayashi and colleagues (*Gastroenterology*, **62**, 618; 1972) tested sera collected in Japan from fifty-four members of three families with chronic liver disease or primary liver cancer. Hepatitis B antigen was detected in fourteen of fifteen affected members of the families and their siblings and in twenty of the twenty-four children of the female siblings. By contrast, the antigen was detected in only one of the eight children of the male siblings, and the antigen was found in the serum of his mother.

The finding of the antigen in these three families coincided remarkably well with the distribution of severe chronic liver damage or primary liver cancer in the family tree. Familial clustering of cirrhosis has been reported in ten families in Japan since 1963. Of the thirty-nine affected members of these families, eleven died of cirrhosis and nine died of cirrhosis and liver cancer. It is notable that successive occurrence of liver disease for two generations was recognised in seven of the ten families, and the mother or her siblings were affected in each family. The next report by Merrill and her colleagues (*New Engl. J. Med.*, **287**, 1280; 1972) illustrates the other end of the spectrum. Of five children born to mothers who had hepatitis B infection at the time of delivery, four acquired the antigen 6–12 weeks after birth, but they remained clinically well and fulfilled the criteria for symptomless carriers. The fifth mother–child case is of interest in that the infant had hepatitis B antibody in his serum at birth (as did his mother) and he did not acquire the antigen.

Schweitzer and colleagues (*Gastroenterology*, **65**, 227; 1973) investigated some of the factors influencing neonatal infection by hepatitis B virus. A striking difference in the frequency of infection was noted between infants of mothers who had acute hepatitis B and those who were symptomless carriers of the antigen. Thirteen babies of twenty-seven mothers with the acute disease acquired persistent antigenaemia, especially if the maternal illness occurred near the time of delivery. By contrast, only one of twenty-one symptomless maternal carriers had a child who became antigen positive. The antigen was detected by radioimmunoassay in nearly half of twenty-one umbilical cord sera tested. The placental barrier to the antigen did not seem to differ in the acutely infected mother from that in the healthy carrier, and the presence of antigen in the cord blood was not necessarily related to subsequent acquisition of the antigen by the infant. In another report, Schweitzer *et al.* (*Amer. J. Med.*, 55, 762; 1973) noted that the frequency of transmission of hepatitis B from mother to infant was high (76%) when acute hepatitis B occurred during the third trimester of pregnancy or early in the postpartum period, and low (10%) when hepatitis occurred during the first 6 months of pregnancy. Acute hepatitis developed in two of seventeen infants and the illness in both was mild, antigenaemia was transient and the homologous antibody developed during convalescence. No clinical illness was observed in thirteen infants (two infants were lost to the survey), but the antigen persisted in their serum accompanied by prolonged elevation of an enzyme frequently associated with liver damage. Liver biopsy performed on ten infants revealed this histological features of unresolved hepatitis and increased portal fibrosis was present in one infant. On electron microscopy, virus-like particles were found in the nuclei of six out of nine antigen-positive infants examined. Dunn and colleagues (*Exp. molec. Path.*, **19**, 113; 1973) found virus-like particles in explant cultures prepared from the remaining portions of some of these biopsies and they concluded that the particles proliferated within the nuclei of the liver sells.

In a more recent study on maternal transmission of hepatitis, Mazzur *et al.*, (*Nature*, **274**, 41; 1974) determined the subtypes of hepatitis B antigen in 174 positive sera collected together with pedigrees in eighteen villages in south central Bougainville, an island in Oceania. Two different combinations of antigens were segregating in eleven out of thirty-two families tested, indicating that

these carriers had not been infected by each other nor from the same source. In families where the mother was a carrier, however, the twenty-two children who had antigenaemia had, with a single exception, the same subtype as their mother. Among the twelve families where the mother was positive only one had mixed subtypes; while ten out of twenty families in which the mother did not carry the antigen had antigen of different subtype. Among six families in which the father was positive, mixed subtypes were found in four of the families.

The data thus suggest that vertical transmission from mothers to their children occurs, and also that horizontal transmission clearly takes place. Although vertical transmission and perinatal transmission from mother to child occur, the relative importance of each mode of transmission is not yet determined.

The significance of hepatitis B infection in early life lies not only in its relation to liver disease but also in its importance in the genesis of prolonged carriage of hepatitis B viris. Zuckerman and Taylor (*Nature*, **223**, 81; 1969) described a well-documented healthy former blood donor carrying hepatitis B antigen for at least 20 years. That a reservoir of chronic carriers may become established among children is, therefore, a cause for the utmost concern to the community. Methods for passive immunisation of susceptible newborn infants are already under evaluation, but the need for safe and effective vaccines is pressing. As a corollary the serum of pregnant women should now be routinely examined for hepatitis B antigen.

ARIE J. ZUCKERMAN

More to muscle than myosin

IN ways not anticipated, myogenesis has yoked in an uneasy alliance muscle biochemistry, molecular genetics and cell differentiation. The finding of Ishikawa and colleagues (*J. Cell Biol.*, **43**, 312; 1969) that arrow-head complexes—the *sine qua non* of actin—can be induced by H-meromyosin in the cortical regions of cartilage, nerve and intestinal epithelial cells, as well as in many kinds of embryonic cells, has been confirmed in many laboratories. Adelstein and colleagues (*Proc. natn. Acad. Sci. U.S.A.*, **69**, 3693; 1972) and others have found myosin in platelets, fibroblasts and nerve cells. Tropomyosin, the third of the contractile protein triumverate, has also been found in several cell types (Cohen and Cohen, *J. molec. Biol.*, **68**, 383; 1972). Clearly many kinds of cell synthesise molecules identical or similar to actin, myosin and tropomyosin—molecules not long ago thought to be unique to terminally differentiated muscle cells.

Such results might satisfy the egos of muscle biochemists, but perplex those attempting to understand cell differentiation. Current models assume that the differentiated state is based on the activity of different sets of genes in different kinds of cells. A possible resolution of the dilemma of ubiquitous contractile proteins, and one with considerable theoretical implications for other aspects of differentiation, is the notion that there are several structural genes for different myosins and different tropomyosins, and that in different kinds of cell different myosin and tropomyosin genes are transcribed. Consistent with this scheme is the finding that the myosin light chains and the LMM portion of the myosin rod are products of different structural genes in fast, slow, smooth and cardiac muscles. Possibly during the differentiation of fast muscle, only those structural genes for fast myosin and fast tropomyosin become available for transcription and translation, whereas during the differentiation of platelets or cartilage cells two other sets of genes for myosin and tropomyosin become available. This means that the genes for platelet or cartilage myosins are no more available in skeletal muscle cells than are the genes for albumin or pituitary hormone.

This ready solution, regrettably, is not as likely to explain the role of actin in the developmental programmes of virtually all cells. The issue with this strikingly conservative protein is whether the products of a single structural gene can be coupled to different kinds of myosins and tropomyosins, or whether there are several identical actin genes possibly even in different chromosomes participating in different developmental programmes. Circumstantial evidence for the latter stems from experiments showing that bromodeoxyuridine (BUdR)-suppressed myogenic cells

If at first you don't succeed . . .

RADIOASTRONOMERS are about to begin another programme of 'listening' for signals from intelligent life within our Galaxy. Since the days of the original Project Ozma a decade and a half ago, radio observing equipment and techniques have improved and the rationale behind such a search has become more sophisticated. Previous efforts chiefly concentrated on hydrogen frequencies, since hydrogen is the most abundant element in the Universe. But recently there has been a growing feeling that any really intelligent race would probably preserve just these frequencies as free from artificial noise, because of their importance to radioastronomers. So the new investigation, which begins on May 8, will be using frequencies appropriate to emission from water.

The rationale behind this is partly the importance of water to life as human beings know it. In the wild, creatures meet at the water hole, so why not try the same sort of thing for contact between ourselves and extraterrestrial creatures? The frequencies are also ones which have been found in natural astronomical sources, notably clouds of gas and dust believed to be associated with young, forming stars. They are not expected to occur naturally in the region of older stars, which improves the likelihood that any such 'signal' from near such a star is a sign of life.

The astronomers responsible for this particular investigation are P. Feldman, of York University, Ontario, and A. Bridle of Queen's University, Kingston, Ontario; their programme involves the 150-foot Algonquin telescope, and is a "passive" one—Bridle is reported as saying that "we would not be capable of sending a signal our own equipment could detect over interstellar distances". Some six stars will be studied intensively from time to time (the first observing period devoted to the programme is 5 days) and 300 to 500 stars will be looked at more briefly over a year or two. The targets have been chosen on the basis that they are non-variable, slowly rotating (and so likely to have planets) and middle-aged (so that intelligence has had time to evolve).

News of this project arouses mixed feelings, since the 150-foot is a valuable instrument that might arguably be used for 'better things'—in the sense of studies which would definitely produce results. But 5 days here and there need be no great loss, and there will, of course, be no quibbling about the project if the North American team hit the jackpot.

JOHN GRIBBIN

synthesise actin destined for the cell surface, whereas actin for thin filaments does not accumulate.

These considerations require re-evaluation of reports of the time during myogenesis when the messenger RNAs for myosin are transcribed and translated. If the presence of a molecule with the molecular weight of 200,000 is taken as evidence for the synthesis of myosin (Yaffe and Dym, *Cold Spring Harb. Symp. quant. Biol.*, **37**, 543; 1972) then all cells, including replicating presumptive myoblasts, must be transcribing and translating mRNAs for some kind of myosin. Accordingly it is not surprising that Rubinstein and colleagues (*Biochem. biophys. Res. Commun.*, **57**, 438; 1974) now report that myosin and actin are indeed coordinately synthesised and turned over, not only in myotubes but also in mononucleated presumptive myoblasts, cartilage cells, fibroblasts and BUdR-suppressed myogenic cells.

Lastly, a smouldering controversy of considerable interest to molecular geneticists exists between those who claim that myoblasts withdraw from the cell cycle as a precondition to fusion, and those who claim that cessation of DNA synthesis results from fusion. The most emphatic statement of the second position is that of Doering and Fischman (*Devl Biol.*, **36**, 225; 1974). Based on what is termed a "% fusion" index they conclude that myogenic cells whose DNA synthesis is blocked by Ara-C fuse; according to these authors replicating myogenic cells have the option to replicate, or to fuse, and, as a consequence of fusing, cease replicating. Before these important conclusions can be accepted, however, it should be noted that: (1) Ara-C is routinely used to kill replicating cells, so that the design of the Doering and Fischman experiment must yield a spuriously high "% fusion" index; (2) early fusion cannot be measured with the light microscope; and (3) it is impossible to distinguish 'fibroblasts' from presumptive myoblasts. Given these uncertainties it will be interesting to see if those who speculate that myoblasts withdrew from the cell cycle as a precondition to fusion can devise more cogent experiments.

H. HOLTZER

Slaved disk model for Hercules X-1

from James Pringle

A POSSIBLE solution to some of the more puzzling aspects of the X-ray star Hercules X-1 has been proposed in a recent article by Roberts (*Astrophys. J.*, **187**, 575; 1974). The X rays from Her X-1 are regularly pulsed with a period of 1.24 s and the source of X rays is one member of a binary star system which has an orbital period of 1.7 d. The X-ray source is thought to be a neutron star with a strong dipole magnetic field and the X rays are produced by material transferred from the 'normal' star of the binary system accreting on to the compact neutron star. Because of the strong magnetic field, the accreting material is channelled down on to the magnetic polar caps of the neutron star dissipating its kinetic energy of infall when it strikes the surface. So much energy is liberated in such a small region that the temperature rises to more than 10^7 K. Thus most of the radiation emerges as X rays. The two magnetic polar caps are then two X-ray emitting hotspots: if the magnetic axis is offset from the rotation axis, rotation of the star with a 1.24 s period provides a natural explanation of why the observed X rays are regularly pulsed.

Furthermore, the X-ray source displays a third periodicity. The X rays are visible for only about 10 to 12 consecutive days, reappearing regularly at 35-d intervals. Explanations of this phenomenon—for example, variable mass transfer or precession of the pulsing neutron star—have difficulty in accounting for another aspect of the problem. The 'normal' component of the binary system was identified in 1972 as the previously known variable star HZ Herculis. This star is so close to the luminous X-ray source that X-ray heating causes one side of it to be about four times brighter than the other. The brightened face follows the neutron star around its orbit, and thus HZ Her can be observed to vary regularly in brightness with a period of 1.7 d. The puzzling feature of this 1.7-d optical variation is that, although the X rays can be seen for only 10 out of every 35 d, the heating of the side of HZ Her nearest to the X-ray source continues throughout the cycle, even when no X rays are observed.

Roberts' explanation of this is quite simple. He suggests that HZ Her has its rotation axis at an angle to the orbital rotation axis. In this case, the rotation axis of HZ Her precesses about the orbital rotation axis with a period of about 35 d. The material transferred by HZ Her towards its companion has too much angular momentum to be able to fall on to it directly and, instead, forms an accretion disk around the neutron star. In the disk, viscosity transfers angular momentum outwards, allowing the material to spiral slowly inwards. Roberts notes that the disk, which is opaque to X rays, will lie in the plane perpendicular to HZ Her's rotation axis, and thus in general not in the orbital plane of the system. Roberts names this the Slaved Oriented Disk, or SOD for short. Thus the angle between our line of sight to the X-ray source and the plane of the SOD varies with the 35-d precession period of HZ Her. Since the X-ray source can only be seen when this angle is sufficiently large, one expects the visibility of the X rays to vary with the 35-d period. On the other hand, HZ Her is so close to the X-ray source that the (X-ray) shadow cast on its surface by the disk is quite small. Thus one expects the heating of HZ Her to continue, even when the disk temporarily obscures the X-ray source from observation.

This is by no means the whole story. Roberts also explains why the rotation axes might be expected to be misaligned and shows how his model can account for several further observational details. There are now a number of models which seem to account for the 35-d period of Her X-1 and its associated problems, but nobody has yet performed any detailed calculations. Roberts, however, excuses himself by quoting J. A. Wheeler's First Moral Principle: "Never do a calculation until you already know the answer".

Asia as a composite continent

from Peter J. Smith
Geomagnetism Correspondent

WHETHER or not it is realistic to suppose that any of the present continents could have formed by the accretion of separate crustal blocks, the important question of continental fusion continues to receive considerable attention. Most recently the debate has centred particularly on Africa (see, for example, Piper *et al.*, *Nature*, **245**, 244; 1973) with new evidence suggesting that the orogenic belts between the major cratonic areas of that continent may not be sutural but more probably formed *in situ*, although the idea of what Kropotkin (*Eos*, **53**, 180; 1972) has termed the "composite continent" was actually first applied to Asia by Argand (*Coll. Rep. 13th Int. Geol. Congr. 1922*, **1**, 171; 1924) just 50 years ago and long before the new global tectonics had been thought of.

Since then, evidence supporting the composite nature of Asia has been adduced from palaeomagnetism, palaeontology, geology and petrotectonics. In a useful and commendably concise article Burrett (*Earth planet. Sci. Lett.*, **21**, 181; 1974) has now brought all these data together to delineate the Asian block boundaries and to estimate the timings of the various collisions thought to be involved. Although the field information is not entirely clear in all cases, there seem to be about nine blocks present; and Burrett sum-

marises the evidence for each.

The general conclusion that Burrett comes to is that the coincidence of orogenic belts containing zones of high pressure metamorphism, ophiolites and deep water sediments with faunal province boundaries only makes sense in plate tectonic terms, the belts being the sutures along which the crustal fragments collided and coalesced. He then goes on to discuss when these collisions took place, beginning with the assumption that in the Lower Palaeozoic the various blocks were separated by considerable widths of oceanic crust. According to this analysis the first collision took place in the Upper Carboniferous, although the fusion of all blocks into what is now Asia was not complete until well into the Mesozoic.

Burrett admits that his model is based on "very incomplete data" but nevertheless suggests that "there does not appear to be much room in which to alter the boundaries of the blocks here identified nor the timing of their collisions". It remains to be seen whether, as in the case of the African orogenic belts, a static model can be made to explain the data even more convincingly.

Sources of natural radiation

from J. R. A. Lakey

THE largest source of ionising radiation exposure to the United Kingdom population originates from terrestial and cosmic radiation. This natural radiation was the subject of a meeting of the Society for Radiological Protection held on April 2. C. R. Hill (Institute of Cancer Research, Sutton) stressed that the exposure is by no means uniform. The living habits of human beings have a marked effect on the dose because of the natural radionuclides in the diet and the radioactivity of building materials. These differences have been exploited—in attempts to deduce a dose/effect relationship—but with little success because control populations are hard to find. Nevertheless there has been some pressure to use the apparently solid ground of natural radiation exposures as a basis for setting radiation protection standards. The International Commission on Radiological Protection has not made its recommendations on this basis but J. Vennart (Medical Research Council, Radiobiology Unit, Harwell) reminded the society that natural radiation is deliberately neglected and controls are applied only to artificial radiation exposure.

An interesting dilemma arises from the use of building materials which are naturally radioactive. G. A. M. Webb (National Radiological Protection Board, Harwell) revealed that the Soviet Union has limits designed to avoid 'abnormal' radiation exposure and prohibits building materials containing more than 10 pico curies of radium-226 per gram. Most building materials are not significantly radioactive so that the house walls and foundation act as a shield against natural radiation exposure.

Several speakers referred to the more insiduous radon-222 which is present in air at around 0.1 pico curies per litre and in some cases appears in drinking water at concentration as high as one million pico curies per litre. E. I. Hamilton (Institute of Marine Environmental Research, Plymouth) said that the apparently inexplicable variations in the activity of radon in drinking water occur because the gas can move through the crack system from rocks as deep as 3,000 m.

The dose from natural radiation has been reviewed extensively by the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR). F. W. Spiers University of Leeds) discussed the dose due to internally deposited radioactivity, and said that doses reported by UNSCEAR for cortical and trabecular bone should be revised. The fat cells, which account for 53% of bone marrow, concentrate carbon-14 and the dose becomes four times higher than UNSCEAR reported. Surface-seeking alpha emitters also increase the dose to osteoblasts lining the cavities in bone. The genetic effect of natural radiation has been of most concern to human populations and it must account for some of the births subject to genetic disease. R. J. Berry (Royal Free Hospital, London) said that the radiation dose to double the mutation rate is thought to be between 30 to 80 roentgens. Nobody has been able to correlate natural radiation dose with genetic diseases and it is not necessarily correct to assume that all mutations are harmful. Indeed there is some evidence to suggest that some radiation-induced effects are repaired.

Sendai virus receptors in a model membrane

from a Correspondent

VERY little is known about the first step in replication of animal viruses—the absorption of the virus to receptors of the host cell. It is surprising, for instance, that the question of whether the small oncogenic DNA viruses bind to specific cell receptors is still unresolved. By contrast, the complementarity involved in the reaction of myxoviruses and paramyxoviruses, such as Sendai virus, with specific receptors, at least in the non-permissive erythrocyte, is well known. Haywood (*J. molec. Biol.*, **83**, 427-436; 1974) has extended this system to a study of the interaction of Sendai virus with a model membrane incorporating the specific receptors.



Electron micrograph of Sendai virus adsorbed to a ganglioside-containing liposome. The virus can be distinguished by the spikes on the outer surface. The length of the attachment of the two viruses is 2,100 and 2,500 Å. The bar represents 1,000 Å. (From Haywood, *J. molec. Biol.*, **83**, 427; 1974.)

Sendai virus has a spiked appearance, the spikes being surface glycoproteins which absorb to sialic acid groups of the receptors of erythrocytes and presumably also of permissive host cells. Haywood now reports that sialic acid groups of gangliosides work well as receptors for Sendai virus if they are incorporated into synthetic lipid membranes at relatively high surface density. Electron micrographs show virus particles absorbed to the liposomes containing ganglioside along long stretches (210 nm–250 nm) of the virion. Assuming the area of attachment to be a circle of this diameter, then it seems that the virion is absorbed to a considerable patch of membrane and makes multiple attachments between spike glycoproteins and clusters of gangliosides, perhaps as many as 3,000. It would be interesting to know if the clustering of receptor molecules is actually induced by binding to the virus. If so, then this process might be related to cell fusion induced by viruses such as Sendai, since high local concentrations of gangliosides destabilise lipid bilayers.

The absorption of Sendai virus to

liposomes is specific for structures containing gangliosides or a positive lipid such as stearylamine. The types of binding to these two sorts of liposomes are clearly differentiated, however, since only the membranes which incorporate the sialic groups can compete effectively for virus in mixture with erythrocytes. The weak nonspecific binding of Sendai virus to liposomes containing stearylamine is probably electrostactic since the virions have a net negative charge.

The way in which sialic acid groups are presented to the virus is of prime importance for high affinity binding. For example, very small liposomes containing gangliosides obtained by sonication are less potent inhibitors of viral haemagluttination than are liposomes with a greater total surface area. Second, ganglioside micelles do not compete with erythrocytes for virus. Probably both a threshold surface area and ganglioside density are needed in order that the virus can make a critical number of contracts with the absorbing surface. A somewhat similar interpretation probably explains the finding, made earlier by several groups, that the tightness of binding of myxoviruses to sialoglycoproteins depends critically on the molecular size and is drastically decreased by proteolysis of the receptor glycoproteins. The reason that a large number of contacts are required for firm attachment to receptors containing sialic acid might be explained if the bond energy between receptor groups and viral spike glycoproteins is low.

Whether gangliosides or sialoglycoproteins or both are the naturally important receptors for Sendai virus in cells which support a lytic infection is unknown. Haywood suggests that the probable topography of the cell surface is such that the first viral contacts may be with glycoproteins, after which virions are handed over to gangliosides situated nearer to the bilayer and in a better position to initiate events leading to penetration. Perhaps this process involves the viral neuraminidase in localised reactions which break the initial binding of virions to receptor glycoproteins and allow attachment to internal gangliosides to take place.

New advances in SEM

from a Correspondent

AT the seventh Annual IIT Research Institute Symposium on Scanning Electron Microscopy in Chicago from April 7 to 11, probably the most significant advance in instrumentation was that reported by O. Wells and A. Broers (IBM Research Laboratories). These workers immersed the specimen in the high field region of the final lens and collected the low energy loss electrons coming from the edge of a highly tilted specimen. They were then able to demonstrate centre/centre resolution of 3 nm and an edge resolution of 1.5 nm. The instrument used was the 100 Kv LaB_6 gun designed by Broers, who thought that the resolution could now only be further improved by using a single crystal of LaB_6 to replace the finely pointed sintred rod.

M. Isaacson (University of Chicago) presented a frank discussion of the problems associated with specimen preparation for the high resolution scanning transmission electron microscope presently under development in several laboratories. Although the instrumental potential now exists for atomic resolution, specimen contamination and beam damage represent real problems which must be solved before a wide range of biological macromolecules are going to be imaged in these new microscopes. Isaacson and his colleagues considered the answer to these problems lay in the use of LHe II stages, low atomic weight negative stains and computer image processing to remove background noise.

Several speakers discussed real-time stereo viewing and dimensional reconstruction of stereo pairs. A. Boyde (University College, London) outlined the problems associated with such reconstructions and demonstrated the usefulness of the method in analyses of complex tri-dimensional objects and in interpreting dynamic experiments in the microscope. But the most convincing demonstration of real-time stereo viewing was given by E. Chatfield and colleagues (Ontario Research Foundation). By using a single post lens deflection coil they took a dual signal which was displayed on a colour TV set utilising the red and green guns. The image could then be viewed using red and green separation filters in order to reconstitute the 3D image.

N. Barbi and colleagues (Prairie View, Illinois) gave some data on a windowless energy dispersive X-ray detector in which it was claimed that they are able to get significant signals for C, O and N though problems of contamination and beam collimation still remain. There were several comments on some of the ancillary techniques associated with SEM, including advances in cathodoluminescence, autoradiography and histochemistry. None, however, demonstrated the necessary breakthrough which would make these techniques of any significant use in scanning microscopy. In the biological sciences, J. Nowell (University of California at Davis) gave more details of the injection replication technique for internal cavities of animal tissue and W. Humphreys and colleagues (University of Georgia) outlined a useful variation on the critical point drying technique which enables cryofractured specimens to be visualised in the SEM.

Scanning electron microscopy seems to have come of age and there are now more than 25 different models of microscope from which to choose. The IITRI SEM symposia have played no small part in the growth of the subject and it will be of interest to see whether future meetings can continue to contribute to the requirements of scanning electron microscopists and not degenerate into a dreary catalogue of photographs of what can be seen using this instrument.

Activation energies under scrutiny

from our Chemical Physics Correspondent

FOR many years discussions of thermal reaction rates have centred on the activation energies, E, of reactions whose rates have followed the Arrhenius expression, $k = A \exp(-E/RT)$ and its more modern variants. Its justification requires reagents to possess the energy E in order to surmount an energy barrier and proceed to reaction. The degrees of freedom holding the energy were considered irrelevant except that their number might influence the factor A. It has long been recognised that this assumption was not fully correct but experiments to show the specific influence of particular categories of initial energy have not been easy to devise.

In recent years a number of cases have been studied where the excess energy of the products of an exothermic reaction has resided in specific vibrational excitation. The principle of microscopic reversibility then requires that analagous reactions in the reverse direction should proceed preferentially with the reagents in excited vibrational states. It is just such a process which has been elegantly demonstrated by Douglas, Polanyi and Sloan at Toronto (*J. chem. Phys.*, **59**, 6679; 1973).

The reaction chosen was

$$HCl + Br \rightarrow HBr + Cl$$

for which the HCl reagent could be prepared in excited vibrational states from the reaction

$$HI + Cl \rightarrow HCl\ (v = 1 \text{ to } 4) + I$$

Such a stream of vibrationally excited states was crossed with a stream of Br atoms which was pulsed on and off 15 times a second. The occupancy of the vibrational levels of the HCl was monitored through the infrared emission spectrum and the change between the on and off periods for the Br was simultaneously obtained by means of a phase sensitive detector set to 15 Hz. The frequency setting of the spectro-

meter monitoring the emission was swept and its resolution was such that each separate vibration–rotation state characterised by v and J could be monitored. As expected it was found that whereas the levels $v = 1, 2, 3$ and 4 were easily seen in emission, the depletion of these levels by reaction with Br for $v=$ was greater than for $v = 3$, but changes in the populations of the levels $v = 2$ and 1 by reaction were scarcely detectable.

Although this result in itself is not surprising and leaves the principle of microscopic reversibility intact, the elegance, comparative simplicity and excellent sensitivity of the technique imply that its future sophistication will lead to considerable detailed experimental evidence about reaction energy surfaces. This should prove valuable in a field where theory has tended to outrun experiment.

Double repression of galatose genes

from Benjamin Lewin
Molecular Genetics Correspondent

THE interactions which control repressible and inducible operons in bacteria are now well understood in principle: in the lactose operon, for example, repressor protein, cyclic AMP receptor protein (activated by cyclic AMP) and RNA polymerase act at sites on DNA close to the structural genes under control. The galactose operon, however, is a more puzzling system than usual, and it has been difficult to envisage molecular mechanisms for its control. By characterising a new regulator mutation, Hua and Markovitz (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 507; 1974) have now identified a new control site in the operon, analysis of which may help define this system.

The galactose operon is controlled by three systems. A repressor protein (specified by the regulator gene gal^R) inhibits transcription in the absence of galactose by binding to an operator (defined by mutation) located at one end of the structural gene cluster. The cyclic AMP receptor protein and RNA polymerase interact at a site (presumed to be close to this operator) to initiate transcription. The third system of control establishes repression in response to the cap^R gene, which is part of a regulator system responsible for controlling synthesis of products required for formation of the capsular polysaccharide. Because mutations in the gal^R and cap^R genes each relieve only some of the repression of the operon, and appear to act independently, it has seemed likely that the galactose operon might possess two operators.

This hypothesis has now been confirmed by the results reported by Hua and Markovitz. Operator mutants lacking response to an active gal^R locus have in the past been isolated; and these identify the operator at which galactose repressor protein binds. By selecting cells which do not respond to an active cap^R gene, they obtained a mutant with the characteristics expected of an operator unable to respond to the cap^R product. The mutation is closely linked to the first structural gene of the operon.

Since the cyclic AMP system stimulates transcription *in vitro*, it seems very likely that, analogous to the lactose operon, it acts at a promotor adjacent to the galactose operator responsive to gal^R product. That the second operator, on which the cap^R product acts, may also be accompanied by a promotor, this one unresponsive to the cyclic AMP system, is suggested by the observation that bacteria deficient in cyclic AMP can grow on galactose. From the characteristic of mutants affecting expression of the galactose operon, Hua and Markovitz suggest that the gal^R responsive sites lie immediately adjacent to the structural genes, with the cap^R responsive sites lying just beyond them.

Obvious topological problems are raised by the presence of two operators in one operon. How does repression at one influence transcription from the other? Given present models for repressor–operator interactions, it is not immediately obvious how expression from the further control sites takes place when the inner sites are repressed. And when both sites are supporting transcription, one might expect changes in the state of DNA to occur as polymerases readthrough the inner sites—do these influence its activity?

How may these control systems be better characterised? One obvious experiment is to map the two operators more precisely to determine their order and distance apart. Also important is the necessity of characterising the promotor(s) by mutation, since their postulated properties have been inferred almost exclusively from biochemical experiments. It will be interesting to see whether the two operator and promotor sites are indeed each independent or instead represent overlapping sequences in one region of DNA. Isolation of the cap^R product is another urgent necessity, since this may permit experiments *in vitro* to characterise the mechanism of transcription. Even with such critical points yet to be elucidated, however, one important conclusion is clear: an operon may be controlled by more than one repressor protein and may have more than one operator sequence. This has obvious implications for control of gene action in eukaryotic cells.

Interplanetary dust on Earth

from Peter J. Smith
Geomagnetism Correspondent

EXTRATERRESTRIAL material captured by the Earth is generally believed to comprise mainly interplanetary dust, the more spectacular contribution from meteorites being relatively small. Unfortunately, however, not only is interplanetary dust much more difficult to identify than meteoritic debris, there are also likely to be problems in separating it from the terrestrial material in which it is highly diluted. The result is that the nature of most of the material reaching the Earth from outside remains largely unknown. On the other hand, there is no reason in principle why the products of interplanetary dust on Earth, if they exist, should not be identified. Wasson (*Icarus*, **2**, 54; 1963), for example, showed that the presence of extraterrestrial dust would be proved if radioactive nuclides produced by the action of high energy (5-100 MeV) solar protons on the dust in interplanetary space could be detected in terrestial materials. It is true, of course, that most of these radionuclides may also be produced from spallation reactions induced by galactic cosmic rays; but although such processes are predominant in meteorites they are fortunately negligible in bodies smaller than about 1 cm.

The detection of extraterrestial dust by radionuclides thus seems promising, although even here there is a practical problem in that some of the expected nuclides are also produced on Earth by other natural nuclear processes. ^{26}Al, for example, seems particularly attractive because of its high rate of production, its long half life (0.7×10^6 yr) and its β^+ radiation which is relatively easy to detect. But unfortunately it is also formed by spallation of atmospheric argon at a rate apparently comparable to that expected from processes involving interplanetary dust. The precise relative importance of terrestrial and extra terrestrial ^{26}Al is actually a matter of dispute; but as long as the dispute exists, convincing evidence for the presence of extraterrestrial dust is unlikely to be obtained from this particular nuclide.

The only two nuclides suitable from the points of view of production rate and half life and thought to have no significant natural production on Earth are ^{53}Mn and ^{59}Ni. Accordingly, Bibron *et al.* (*Earth planet. Sci. Lett.*, **21**, 109; 1974) have attempted to detect and measure ^{53}Mn in samples of snow from the central area of the Eastern Antarctic Plateau. This site was particularly suitable for the purpose because it is remote from marine, continental and

industrial sources of terrestrial contaminants. Moreover, the average annual temperature is −60° C and the maximum temperature never exceeds 0° C, so that materials precipitated are perfectly preserved and do not diffuse. There is also another important advantage. Although there are no known natural processes on Earth likely to give rise to ^{53}Mn, this nuclide could be produced as a result of thermonuclear bomb tests in the atmosphere. There is apparently no published data which can either confirm or refute this possibility; and so in order to avoid the problem altogether it is necessary to investigate only snow which fell prior to 1952. At the site in question the rate of snow accumulation has been extremely low, making it possible without too much difficulty to collect firn layers deposited well before nuclear testing began.

^{53}Mn was detected in all three samples of snow analysed (corresponding to the period 1935-1950), the average activity being 0.82 ± 0.17 disintegrations per minute per 10^3 tons of snow. This is apparently the first experimental evidence for the presence on Earth of the natural nuclide. But did it really originate from the supposed interplanetary dust? There is, in fact, another possibility—that the ^{53}Mn was produced not from the dust but from the material volatised or dispersed from meteorites during their flight through the atmosphere. To account for the observed ^{53}Mn activity, however, the volatilisation of over 3×10^6 tons of chondritic material would have been required between 1935 and 1950 whereas, in spite of uncertainties in the estimates, it appears unlikely that the actual meteoritic influx exceeded 6×10^4 tons (or perhaps twice that, taking into account objects with pre-atmospheric masses of less than 10^3 g). In short, the meteoritic material available would have been insufficient by a factor of at least 25. Bibron and his colleagues thus conclude that the only possible source of the ^{53}Mn is indeed the interplanetary dust.

Accepting this conclusion they go on to estimate the total global influx of extraterrestrial dust. During the period 1935-1950 the rate of deposition of ^{53}Mn in Eastern Antarctica was $(2.2 \pm 0.5) \times 10^{-5}$ disint. min^{-1} m^{-2} yr^{-1}, although this is not a value which can be extrapolated directly to the whole Earth. It is probable that a significant proportion of the extraterrestrial material volatilises when it enters the atmosphere, becoming fixed on the stratospheric aerosols and thus ultimately being deposited in such a way that the concentration is maximum in temperate latitudes and minimum at the poles and the equator. The deposition in Antarctica should thus represent a lower limit of the average over the Earth's surface. Assuming (in the absence of any more definite information) that the ^{53}Mn distributes itself in a way similar to that of the ^{90}Sr injected into the stratosphere by thermonuclear explosions, Bibron *et al.* estimate the average global deposition of ^{53}Mn to be $(6.6 \pm 1.5) \times 10^{-5}$ disint. min^{-1} m^{-2} yr^{-1}. From this figure it is then possible to show that the interplanetary dust (assumed to have a chondritic composition) accumulates on the Earth at a rate of $1\text{-}6 \times 10^5$ tons a year.

In summary, then, Bibron and his colleagues have provided the first inputable (their word) evidence for the presence on Earth of ^{53}Mn, a radionuclide thought to be formed only from interplanetary dust which builds up on the Earth at a rate of about 10^5 tons a year. But this is only a beginning, for most of the questions about interplanetary dust remain unresolved. As Bibron *et al.* point out, a decisive step forward would be the simultaneous determination of ^{53}Mn, ^{59}Nn and ^{26}Al, although there is still plenty of scope in tracing the variations of ^{53}Mn itself during the past hundred thousand years or so by analysing deep ice cores from polar ice sheets.

Vegetational change in an aboriginal environment

from Peter D. Moore
Plant Ecology Correspondent

EVER since Iversen (*Danm. geol. Unders.*, 2R, **66**, 1; 1941) described the influence of Neolithic cultures on the vegetation of Denmark, palaeocologists have been forced to accept the fact that the introduction of agricultural practices in north-west Europe (perhaps 3,500 radiocarbon years BP for the British Isles) profoundly altered the contemporary environment. More recent work has suggested that many of the vegetational changes which were once thought to be climatically determined must now be regarded as anthropogenic, for example the decline in lime (*Tilia*) in Britain (Turner, *New Phytol.*, **61**, 328; 1962) and the initiation of blanket peat formation (Moore, *Nature*, **241**, 350; 1973). Some have even considered it possible that pre-agricultural Mesolithic cultures may have modified vegetation on a large scale by using fire to drive game or to favour selectively plant species which provide a source of food such as hazel, *Corylus avellana* (Rawitscher, *Nature*, **156**, 302; 1945). This view has received some indirect support as the result of the recent work of Martin (*Aust. J. Bot.*, **21**, 283; 1973) on the environment of aborigines in the Nullabor region of South Australia.

Martin's interest has centred on the palynological analysis of three radiocarbon-dated cave deposit profiles and their interpretation in the light of modern pollen deposition in the area. Her modern pollen studies have provided a means of recognising past plant communities which, in this arid region, are fairly simple in composition.

The present vegetation of the Nullabor region consists of mallee scrub in the coastal region, which has a slightly higher precipitation (23-25 cm yr^{-1}) than the inland area (16-18 cm yr^{-1}). Temperatures in the two regions are virtually the same, but the number of rain days in the coastal region is roughly twice that experienced inland (80 rather than 30-50 d inland). In the arid interior there is tall, open scrub dominated by *Acacia* and *Myoporum*.

Palynologically the two vegetation types can be separated on the basis of the ratio of Myrtaceae (largely *Eucalyptus* and *Melaleuca*) pollen to that of Chenopodiaceae (many genera including *Atriplex* and *Kochia*). Surface pollen analyses in the arid scrub region consistently showed Myrtaceae : Chenopodiceae (M : C) ratios of less than 0.2. M : C ratios in samples from the less arid mallee scrub zone, on the other hand, were always in excess of 0.5. This simple characteristic of the pollen fallout from the two vegetation types permitted Martin to recognise these communities in the fossil pollen assemblages of the cave deposits.

At one site (Eucla) the M : C ratio increases from a low level at 20,000 BP to a maximum at the present day. Its rate of increase mirrors effectively the postglacial eustatic rise in sea level resulting from the gradual melting of the icecaps. The vegetational change in which arid scrub gives way to mallee can thus be explained by a rise in climatic wetness resulting from the increasing proximity of the coastline.

Two other sites tell a rather different story. They show an increasing abundance of mallee scrub (that is, a higher M : C ratio) until about 4,000 BP when it apparently declined. The date of this decline coincides with an increasing abundance of aboriginal artefacts at these sites. The aborigines of recent times are known to have burned scrub extensively, both for ritualistic purposes and to encourage certain food plants, such as the wild gooseberry. Under the climatic stress of aridity it is quite feasible that repeated burning of the scrub could have reversed the trend in vegetational development caused by ameliorating climate and have produced an anthropogenic plagioclimax of arid scrub. This seems to be one of the best documented examples of the influence of a pre-domestication human culture on the course of vegetational development in a wide geographic region.

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European tumour virology at Aviemore

from Elizabeth Rogers

THE momentum of tumour virus research is such that pauses for breath are essential if one is not to be left gasping behind. So it was that an informal meeting of European tumour virologists at Aviemore, Inverness-shire from April 3–5 provided an opportunity for gathering together the threads of recent progress. The meeting was organised by Professor J. Subak-Sharpe (Institute of Virology, Glasgow) and the sixty or so short contributions covered research into all major groups of tumour viruses. Outstanding were the descriptions of the use of restriction enzymes as tools for analysing the genomes of DNA viruses, and the use of pieces of DNA as infective agents in order to define, for instance, the size of the transforming 'gene'.

The small DNA papova viruses have, not surprisingly, yielded most rapidly to analysis with restriction endonucleases. Linear SV40 DNA produced by *Escherichia coli* R_1 endonuclease can transform rat embryo kidney cells (P. J. Abrahams, State University, Leiden), although it can no longer cause productive infection in BSC-1 monkey kidney cells. Transforming activity of restriction fragments smaller than the whole genome is being investigated. A new SV40-specific antigen reported by C. de Vaux St Cyr (Institute of Cancer Research Villejuif) will provide food for thought for genome analysers, already overburdened with gene products to fit into too little virus DNA. This antigen—named 'C'—is cytoplasmic and distinct from T antigen in several other respects. Those who think that polyoma might behave like SV40 will be increasingly disillusioned by reports like that of R. Kamen (Imperial Cancer Research Fund, London), who has found that late in polyoma infection, the major virus-specific RNA is a total transcript of the minus strand—unlike the situation in SV40-infected cells. A transcription map of the polyoma genome is now available, derived from analysis of eight fragments formed by *Haemophilus parainfluenzae* endonuclease II (Hpa_{II}). The origin of replication has been localised in fragment 5 by M. Fried and L. Crawford (ICRF, London). There is a small region of homology with SV40 DNA.

Larger DNA viruses are more complex substrates for restriction analysis, but already considerable progress with adenoviruses (Ad) 2 and 5 has been made. Using a combination of Eco_{RI} and Hpa_I restriction enzymes, P. H. Gallimore (Medical School, Birmingham) has analysed the parts of the adenovirus genome which are present in Ad2-transformed cells. In some lines as little as 14% of the genome remains. J. F. Williams (Institute of Virology, Glasgow) also reported that two Ad5-transformed cell lines have, respectively, $2\frac{1}{2}$ and $5\frac{1}{2}$ copies of the left end, representing about 30% and 40% of the genome. These findings relate to the question of how much adenovirus DNA is essential for transformation. F. L. Graham (State University, Leiden) has treated rat embryo cells with sheared adeno-DNA. Ad5-DNA will transform these cells even when only about 1×10^6 daltons in size. If half-molecules of Ad5-DNA are made, the transforming activity can be localised in the GC-rich half, and after restriction with Eco_{RI}, it is further localised in the large A fragment, which includes the left end of the molecule. Considering that transformed cell lines exist which only contain the terminal 12% of the left half of the genome, transforming activity must be confined to this site.

Although the problems presented by herpes and Epstein-Barr viruses seem much more immense, these viruses are slowly yielding to the same methods as have been used successfully with small DNA viruses, and their role in human carcinomas is becoming clearer at the same time. Herpes virus (HSV) is a potential candidate for a human tumour-inducing virus. Patients with different carcinomas have been shown to carry HSV-specific non-virion antigens in their sera, and many studies of the transforming potential of HSV or HSV-DNA are now in progress. For example, infection of human embryonic lung cells with HSV is abortive in certain conditions, and cells can become stably transformed (K. Munk, Institute of Virology, Heidelberg). HSV cannot be rescued by cocultivation, so that this may be a good experimental system for studying latency of HSV in human tissue. Another system has been developed by J. C. M. Macnab and N. M. Wilkie (Institute of Virology, Glasgow), who have obtained focal transformation of rat embryo cells by HSV types 1 and 2, as well as by temperature-sensitive (*ts*) mutants and sheared DNA derived either from wild type or from *ts* mutant viruses. As with adenovirus and SV40 DNAs, infectivity of DNA is far more sensitive to shearing than is transforming activity. Sheared HSV-DNA of $5\text{-}7 \times 10^6$ daltons (whole genome is 100×10^6 daltons) will induce transformation. J. K. McDougall (Medical School, Birmingham) demonstrated by *in situ* hybridisation that among a population of human cervical carcinoma cells, only very few contain detectable amounts of HSV-DNA, which may explain why whole virus cannot be isolated from the carcinoma. *In situ* hybridisation, because it allows detection of viral genomes at a cellular level, provides another dimension in the overall analysis of the biochemistry of tumours.

H. Diggelman (Swiss Institute of Cancer Research, Lausanne), introducing a series of papers on RNA viruses, said that a central problem in understanding the function of RNA tumour viruses is still that of determining whether the 70S RNA genome is polyploid. 70S RNA is not found attached to polysomes, nor even in the first budding virus particles, but is apparently assembled later. J. T. Parsons (University of Zurich) has evidence from fingerprint analysis that the genome size is about 3×10^6 daltons—the size of one subunit. Conclusive proof that avian leukaemic myeloblasts contain at least one complete integrated avian myeloblastosis virus (AMV) genome was provided by H. Ogura (Robert Koch Institute, Berlin) and F. Lacour (Institute Gustave Roussy, Villejuif) who have found that AMV production can be induced after infection of helper factor-negative chick cells with leukaemic myeloblast DNA. Similar results were got with wild type or *ts* mutant Rous sarcoma (RSV)-infected cell DNA. The minimum infective size suggests that the information from one RSV subunit may be sufficient. Viral mutants are necessary additional tools in these experiments. R. R. Friis (Robert Koch Institute, Berlin) has isolated representative mutants of the whole replicative cycle in RSV, including one which does not assemble reverse transcriptase into the virion.

Another aspect of RSV transformation was discussed by A. Vaheri (University of Helsinki), who has found that a type-specific glycoprotein, which is normally an integral part of the chick fibroblast cell membrane, disappears on transformation of the cells by RSV. If a *ts* virus mutant is used, the glycoprotein reappears at the non-permissive temperature. W. F. H. Jarrett (University of Glasgow) has studied the horizontal transmission of feline leukaemia virus (FeLV), and the incidence of leukaemia itself as compared with FeLV antibodies in different cat populations. He concludes that one of the primary events in leukaemia may be immunodepression of the response to FeLV antigens themselves. Significantly, the existence of horizontal transmission may allow the development of anti-FeLV vaccines (see *Nature*, **248**, 230; 1974).

Certainly this meeting served to demonstrate again the increasing finesse of the techniques being used to study tumour viruses. One hopes that probing into the mechanism of transformation in these ways will lead to an understanding of the spontaneous events which produce tumours.

Reversible restoration of the birefringence of cold-treated, isolated mitotic apparatus of surf clam eggs with chick brain tubulin

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Mitotic aparatuses have been isolated which can incorporate heterologous tubulin and can assemble this tubulin into birefringent fibres similar to those of mitotic apparatuses of living cells.

Several mechanisms have been proposed to explain the separation of chromosomes by the mitotic apparatus (MA). Suggestions such as generation of a tangential force by the spindle body[1], sliding of microtubules[2,3], reversible polymerisation of spindle fibres[4] or participation of a non-microtubule 'movement factor'[5], possibly actin[6], have arisen from observations on living or fixed intact cells. Such studies suffer from the inability to experiment directly with the MA except as it exists in the complex whole cell. If it were possible to isolate MAs which could separate chromosomes *in vitro* it should be possible to study those processes relevant to the mechanism of chromosome separation and those that are incidental to it. We report here observations on isolated MAs which (a) incorporate added heterologous tubulin and (b) after removal of homologous tubulin, assemble added heterologous tubulin into birefringent fibres similar in distribution to those of MAs in living cells.

Mitotic apparatus isolation

MAs isolated by methods using organic solvents[7,8] differ from MAs in living cells in that their birefringence does not disappear at low temperatures. Recent observations by Weisenberg[9], which demonstrate that calcium ion concentration must be reduced to enable microtubules to be polymerised *in vitro*, suggested that low calcium media be tried in MA isolation procedures. We found that a modification of the microtubule polymerisation mixture described by Weisenberg allows MAs to be isolated from at least eight species of eggs and that the MAs are cold labile, calcium sensitive, stable at room temperature and possess a birefringence approximately equal to that of *in vivo* MAs[10]. The isolation mixture consists of 0.1 M MES[9] or PIPES[11] buffer, *p*H 6.85, 0.25–1 mM $MgCl_2$, 1–5 mM EGTA, 10 mM of a proteolytic enzyme inhibitor, *p*-tosyl arginine methylester HCl (TAME) and Triton X-100 (0.2–1% depending on the species of egg used). Addition of GTP (1 mM), which is essential for the *in vitro* polymerisation of microtubules[9,11], is not required for MA isolation but was included in all reassembly experiments described below. TAME could be eliminated from the isolation mixture but MAs were then obtained in poor yield and were fragmented and irregular in shape. We report experiments using MAs from eggs of the surf clam *Spisula solidissima*.

Eggs of the clam were obtained and handled as previously described[12,13] and were parthenogenetically activated with KCl. Fully formed first meiotic MAs at metaphase were uniformly present by 12–14 min after activation[12]. Vitelline membranes were removed with 1 M glycerol solutions[14] and the eggs were immediately suspended in 2–3 times their volume of isolation medium with Triton X-100 at room temperature. The eggs were then dispersed by agitation with a Vortex mixer and the MAs collected by centrifugation at 400–500*g* for 1.5–2 min at room temperature. The MAs were then resuspended in at least five times their volume of isolation medium without Triton X-100 (Fig. 1*a*). Measurements of the birefringence of the isolated MAs were made with a Zeiss polarisation microscope (Photomicroscope II) equipped with a Brace-Koehler compensator and a potentiometric device for automatic recording of compensator angle.

Chick brain tubulin was routinely isolated by homogenising the brains of 1–3-d-old chicks in a volume of polymerising solution (1 mM EGTA, 1 mM GTP, 1–2 mM, $MgCl_2$, 0.1 M PIPES buffer, *p*H 6.9) equal to 1.5 times the wet weight of the brains. The homogenate was centrifuged for 1 h at 28,000*g* at 4° C. The supernatant was then incubated at 37° C for 45 min until it became very viscous as a result of the forma-

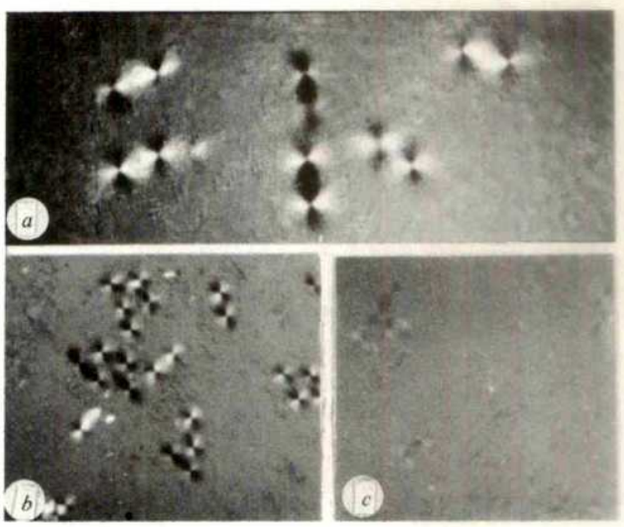


Fig. 1 *a*, Isolated MAs of *Spisula* eggs at metaphase in isolation medium. Distance between astral centres averages 22 μm. ×2,500. *b*, Isolated MAs in isolation medium to be compared with the same preparation cooled to 4° C for 20 min. *c*, Considerable decrease in birefringence is visible and on measurement amounts to one-quarter to one-half the birefringence originally present. ×1,000.

TABLE 1 Spisula mitotic apparatus isolated at metaphase at 22–24° C

Treatment of MAs		Retardation*	% of control retardation
Control	Normal MAs in isolation mix at 25° C	2.70 μm	100%
	Normal MAs in isolation mix at 4° C for 15 min	1.25 μm	46%
Tubulin	MAs in tubulin (9 mg ml^{-1}) at 25° C for 15 min	3.70 μm	137%
	MAs in tubulin (9 mg ml^{-1}) at 4° C for 20 min	1.10 μm	41%
	MAs from above, removed from cold and incubated at 30° C for 15 min	2.40 μm	90%

* Each retardation is based on 10–13 measurements. The tubulin was purified from chick brain

tion of microtubules. The microtubules were collected from the supernatant by centrifugation at 28,000*g* for 15 min at 25° C. The sediment of microtubules was resuspended in an amount of polymerising solution equal to one-third to one-quarter the volume of the original supernatant and placed on ice for 30 min to depolymerise the microtubules. This preparation was centrifuged at 105,000*g* for 1 h to remove any remaining intact microtubules. The supernatant which contained the tubulin subunits was either used immediately for polymerisation with the isolated MAs or, in some experiments, was further purified by one or two additional cycles of polymerisation and depolymerisation. Tubulin from chick brain was used rather than tubulin from *Spisula* eggs because it was much easier to obtain in large amounts, and other work had recently shown that heterologous systems of chick brain tubulin and sea urchin flagellar axonemes could copolymerise[15].

Birefringence

The rapidity with which cold treatment reduced the birefringence of isolated MAs was strongly dependent on the time between MA isolation and immersion in a cold bath.

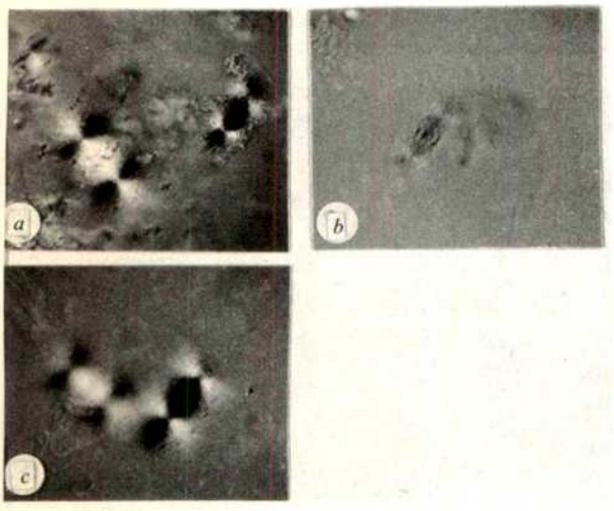


FIG. 2 *a*, MAs were isolated and incubated in concentrated tubulin solutions at room temperature for 30 min. They were then centrifuged out of tubulin, washed in isolation medium and mixed with intact MAs from the original isolation which had remained in isolation medium at room temperature. The difference in size is readily apparent and is uniform. ×2,500. *b*, The MA remnant results from cooling in tubulin and should be compared with the original MAs in tubulin at room temperature. *c* was taken several minutes after suspension of intact MAs in tubulin and significant growth had already occurred. ×2,500.

MAs cooled to 0–4° C within 1 min of isolation completely disappeared and were not visible in the polarisation microscope. Those treated 5–6 min after isolation required only 10–15 min at low temperature to reduce their birefringence to a constant level about one-quarter to one-half that of normal, (Table 1 and Fig. 1*b* and *c*) whereas if greater than 15 min elapsed between isolation and cooling, reduction of birefringence was delayed for several hours. In the latter cases a remnant survived which had the form of an MA with considerably reduced birefringence[10]. Electron microscopic observations showed that microtubules were absent from cold-treated MAs with reduced birefringence but that such MAs contained chains of ribosome-like particles aggregated with some amorphous material[10]: it is this material which presumably gives rise to the residual form birefringence of the MA remnant[16,17] (Fig. 1*c*). The MAs depleted of tubules still contained the chromosomes and the mitotic poles (centrosomes).

Tubulin assembly

Intact MAs to be resuspended in brain tubulin were obtained in isolation medium, centrifuged once at low speed and then resuspended in IM plus tubulin at the desired concentration. This procedure, which diluted the Triton X-100 concentration to less than 0.1% and reduced the concentration of free clam tubulin subunits, took 5–6 min. Such suspensions of intact MAs, in solutions containing up to 10 mg ml^{-1} chick brain tubulin (approximately the concentration of tubulin in intact eggs[18,19]), in contrast to MAs suspended in isolation medium alone, retained their cold lability for at least 1 h. If these intact MAs were kept at room temperature in tubulin they increased their birefringence over 15 min (Table 1) with little observable change in shape. At greater concentrations of tubulin (15–20 mg ml^{-1}) the MAs became considerably wider at the equator, the length of the astral fibres increased and the distance between astral centres increased by up to 40% (Fig. 2*a*). These results show that chick brain tubulin can assemble into clam MAs which still contain clam microtubules.

Perhaps the most interesting observations in these studies concern the return of birefringence in MAs which were depleted of their own microtubules by cold treatment and were then warmed to 30° C in the presence of chick brain tubulin. In these experiments, the isolated MAs were sedimented from isolation medium and then resuspended in isolation medium + tubulin (9 mg ml^{-1}). Control MAs were suspended either in isolation medium alone or in isolation medium with tubulin and 10^{-3} M colchicine. The MAs were then cooled to 4° C for 15 min and the birefringence was reduced by about 60% (Fig. 2*b* and *c* and Table 1). When cold-treated MAs were incubated at 30° C the birefringence in the preparations which contained isolation medium and tubulin partially returned in 15 min, (Table 1) and within 0.5–0.75 h the birefringence had returned to the level seen before cold treatment. Moreover, these MAs continued to increase in size and after 2 h at 30° C many were three to four times as long as the normal MAs (Fig. 3*a* to *d*). Control MAs in the presence of isolation medium alone or which contained colchicine in addition to the tubulin showed no restoration of normal birefringence when warmed from 4° C to 30° C. Reassembled MAs could be cold depolymerised after periods of up to 2 h and on addition of fresh GTP and rewarming to 30° C birefringence returned to the cold-treated remnant although such doubly-restored MAs were generally less birefringent than those restored once. The doubly restored MAs were still cold labile. Restored MAs could be spun out of tubulin solutions and suspended in isolation medium alone, in which they were stable at room temperature. Thus, restored MAs, resembling those isolated directly in isolation medium, are stable to dilution of the tubulin subunits in which they were for-

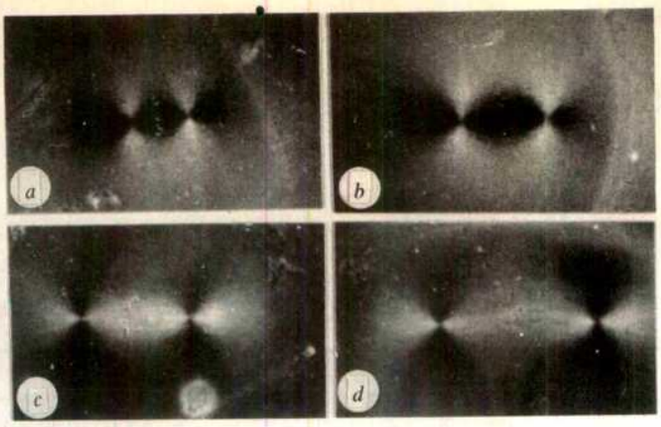


FIG. 3 *a*, Example of a MA depleted of tubules incubated in tubulin at 30° C. for 30 min. Compare with the MAs in Fig. 2*b* and *c*. Note the metaphase chromosomes. ×2,500. *b–d*, MAs from the same preparation as in (*a*) but incubated for 1 h (*b*) or 1.5 h (*c* and *d*). The length of the MA in *d* is approximately 3.5 times normal (compare Fig. 2*c*). At least half of the reconstructed MAs in the preparation attained lengths about that of the MA in *c*. ×2,500.

merly bathed. The birefringence of restored MAs is removed by 10^{-3} M calcium but is stable in 10^{-5} M calcium. This range of calcuim concentration would be expected from *in vitro* observations on neurotubule polymerisation where microtubule assembly can take place and the microtubules remain stable in 10^{-4} M but disappear at 10^{-3} M calcium. These results show that brain tubulin can reversibly assemble into clam MAs which have been depleted of their own microtubules by cold and that such reassembled MAs are cold labile, calcium sensitive, stable to dilution and capable of considerable increase in overall length.

Attempts were made to follow the reformation of birefringent fibres in the MA remnants. Birefringence appeared to return throughout the MA rather than at any focal point. Similarly, during cold treatment, birefringence is lost all along the MA which does not shorten in length. Since *in vivo* MAs decrease in length as well as birefringence on being slowly cooled to low temperature[4] the question of the behaviour of the isolated MAs compared with those *in situ* may be raised. *In vivo Lytechinus* MAs at first cleavage, however, lose birefringence simultaneously along the whole MA when cooled rapidly with large temperature jumps and restore birefringence uniformly along the MA when the eggs are returned to the original temperature[20]. This behaviour more nearly resembles our results of restoration of birefringence in cold-depleted MAs.

It is important to note that MAs isolated by our techniques increase in length but do not shorten and that they are

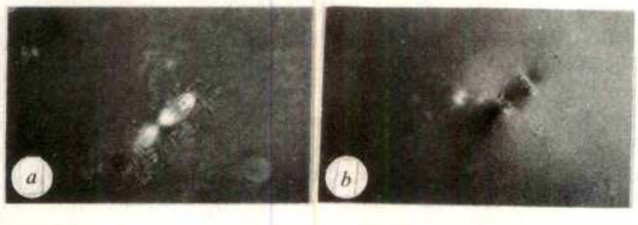


FIG. 4 *a*, MA at anaphase in isolation medium. ×2,500. *b*, Regrown MA from an anaphase preparation after cold treatment. Chromosomes appear to cluster about the astral centres. Although numerous anaphases were present in the original preparation (as well as metaphase MAs) after about 1 h at 30° C only metaphase and 'telophase' configurations could be seen. The birefringence of the region between chromosome clusters in the latter is lower than that in chromosome–pole regions of metaphase MAs in the same preparations. ×2,500.

stable to dilution of tubulin. This indicates that they can incorporate tubulin but that they can not disassemble it easily once it is integrated. Since disassembly as well as assembly is an important part of the process of chromosome separation[4] and it did not seem to occur in the metaphase MAs, we attempted to isolate MAs at anaphase. This proved difficult because of the very short anaphase time in *Spisula* and because such MAs were considerably more fragile than metaphase MAs and tended to fragment. Nevertheless, some anaphase MAs were obtained (Fig. 4*a*) whose birefringence was cold labile and could be restored when incubated in tubulin solutions. In some cases, cold depleted anaphase MAs incubated in tubulin for about 1 h attained a configuration resembling very late anaphase or early telophase, (Fig. 4*b*) with chromosomes at the centres of quite small asters. Details of how this configuration was achieved are now being investigated.

We have described a technique for isolating MAs which can incorporate heterologous tubulin into the existing intact MA and which, after depletion of native microtubules by cold treatment, can utilize added tubulin to reconstruct the birefringent material[15,16,18,21] in approximately normal distribution. Such MAs continue to grow in tubulin solutions at room temperature or above and can grow considerably longer than normal. The ability to incorporate exogenous tubulin is at least one of the functions of the *in vivo* MA and is part of the process of chromosome separation. Whether our results with anaphase MAs are due to disassembly of microtubules from the isolated MAs is not clear but it may now be possible with proper modification of the medium and variation of tubulin concentration to obtain chromosome separation *in vitro* in mass-isolated MAs.

Similar experiments have been carried out recently in other laboratories in which observations have been made on MAs of single cells perfused with tubulin solutions[22,23].

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1 Ostergren, G., Mole-Bajer, J., and Bajer, A., *Ann. N.Y. Acad. Sci.*, **90**, 381 (1960).
2 McIntosh, J. R., Hepler, P. K., and Van Wie, D. E., *Nature*, **224**, 659 (1969).
3 Nicklas, B., *Adv. Cell. Biol.*, **1**, 152 (1972).
4 Inoue, S., and Sato, H., *J. gen. Physiol.*, **50**, 259 (1967).
5 Forer, A., *Chromosoma*, **19**, 44 (1966).
6 Forer, A., and Behnke, O., *Chromosoma*, **39**, 145 (1972).
7 Mazia, D., Mitchison, J. M., Medina, H., and Harris, P., *J. biophys. biochem, Cytol.*, **10**, 467 (1960).
8 Kane, R. E., *J. Cell Biol.*, **12**, 47 (1962).
9 Weisenberg, R. C., *Science*, **177**, 1104 (1972).
10 Smith G., thesis, Univ. of Virginia (1973).
11 Borisy, G., and Olmstead, J. B., *Science*, **177**, 1196 (1972).
12 Rebhun, L. I., and Sharpless, T., *J.Cell Biol.*, **22**, 488 (1964).
13 Allen, R. D., *Biol. Bull.*, **105**, 213 (1953).
14 Rebhun, L. I., *Biol. Bull.*, **117**, 518 (1959).
15 Binder, L. I., and Rosenbaum, J.,*Biol. Bull.* (in the press).
16 Goldman, R. D., and Rebhun, L. I., *J. Cell Sci.*, **1**, 179 (1969).
17 Rebhun, L. I., and Sander, G., *J. Cell Biol.*, **34**, 859 (1967).
18 Cohen, W. D., and Rebhun, L. I., *J. Cell Sci.*, **6**, 159 (1970).
19 Raff, R. A., Greenhouse, G., Gross, K. W., and Gross, P. R., *J. Cell Biol.*, **50**, 516 (1971).
20 Inoue, S., and Fuselor, J. W., *J. gen. Physiol.*, **57**, 255 (1971).
21 Sato, H., *Am. Zool.*, **9**, 592 (1969).
22 Inoue, S., Borisy, G., and Kiehart, D. P., *J. Cell Biol.* (in the press).
23 Cande, W. Z., Snyder, J., Smith, D., Summers, K., and McIntosh, J. R., *Proc. natn. Acad. Sci., U.S.A.* (in the press).

Roles for *E. coli* DNA polymerases I, II, and III in DNA replication

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Chain elongation during E. coli *DNA replication proceeds in three distinct stages. Participatory roles of the three* E. coli *DNA polymerases in each of these stages has been determined.*

ALTHOUGH the overall process of DNA chain elongation during DNA replication is semiconservative[1], the biochemical events involved are unknown[2]. *E. coli* DNA polymerase I (PolI) cannot catalyse *in vitro* DNA synthesis in a semiconservative manner using isolated DNA as a template[3]. *E. coli polA* mutants[4], deficient in PolI, grow normally, suggesting that PolI is not an essential enzyme. *PolA* mutants have been used to purify further DNA polymerising enzymes including DNA polymerase II (PolII) and DNA polymerase III (PolIII)[5–8]. *E. coli polB* mutants[9,10] deficient in PolII, and *dnaE* mutants[11,12], deficient in PolIII, have been isolated and studied. *PolB* mutants are similar phenotypically to their *polB*$^+$ parental strains, although differences in DNA repair ability have been detected[13,14]. In contrast, only thermosensitive *dnaE* mutants have been isolated, suggesting that PolIII is an essential enzyme.

PolI preferentially catalyses a 'repair' polymerisation reaction *in vitro*: conversion of partially single-stranded DNA (ssDNA) into the double-helical species. Although optimal reaction conditions are different, the polymerisation reactions catalysed by purified PolII and PolIII *in vitro* are also substantially 'repair' reactions[7,15].

In the work reported here, the physiological roles of the three known *E. coli* DNA polymerases in DNA replication were examined. The temporal fate of newly replicated DNA obtained from *E. coli* mutants deficient in one or more of these DNA polymerases was analysed using alkaline sucrose gradients. Results indicate that PolIII is essential for one elongation step and involved, if not essential, in a second, that PolI is involved but not essential for a third elongation step, and that PolII is needed and involved only in the absence of one or both of the other two enzymes (Fig. 4).

Pol I

SsDNA, labelled for 1 min in W3110 *polA*$^+$ cells, sedimented in a broad band when synthesised at both 30° C (Fig. 1*a*) and 43° C (Fig. 1*c*). The sedimentation values (30S to 110S) corresponded with molecular weights intermediate to parental sized 120S to 130S ssDNA and the short newly synthesised 15S to 20S ssDNA fragments[16]. In contrast, 1-min-labelled ssDNA from p3478 *polA1* cells[4], deficient in polI (0.5%–2% W3110 activity[19]), sedimented more slowly (Fig. 1*b* and *d*) when synthesised either at 30° C (10S to 40S) or at 43° C (15S to 50S). During subsequent DNA synthesis in both strains (10 min and 40 min cold chase profiles), the short DNA pieces were converted first into intermediate sized pieces and then into large pieces, at a rate somewhat slower at 30° C than at 43° C. The rate of conversion was slower in p3478 than in W3110, both at 30° C and at 43° C, in agreement with earlier observations[17,18]. Thus, PolI is required for rapid conversion of newly synthesised DNA into intermediate sized pieces. The residual slow rate observed in p3478 may be due to residual PolI activity[19] or to attempts by another DNA polymerase to substitute for PolI.

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Pol III

E. coli E486 *dnaE*486 is a temperature-sensitive *E. coli* mutant[11] with normal PolI and PolII activities and no detectable PolIII activity *in vitro* at either 30° C or 43° C[20]. The sedimentation profiles (Fig. 2*a*) of ssDNA synthesised by E486 at 30° C are similar to those of ssDNA from W3110 cells (Fig. 1*a*). The increased relative amount of slowly sedimenting DNA synthesised for 1 min in E486 suggests that PolIII is partially defective *in vivo* in E486 at 30° C, as *in vitro*[20].

The sedimentation profiles of DNA synthesised at 43° C by E486, however, are strikingly different (Fig. 2*b*). DNA synthesis in E486 was greatly reduced at 43° C and occurred mainly within the first minute after shift to 43° C. Most of the DNA synthesised for 1 min or 3 min sedimented slowly (20S or less). During the subsequent 7 min and 37 min cold chase, about half of the labelled DNA was converted slowly into intermediate sized pieces, with some DNA degradation. Even after 40 min of synthesis at 43° C, however, about half of the initially synthesised DNA continued to sediment slowly. Much slowly sedimenting ssDNA remained even after 80 min synthesis at 43° C (not shown). Thus, PolIII is required for the complete conversion of short DNA pieces into intermediate sized pieces, and, when PolIII is defective, the rate of synthesis, or processing, of these short pieces is greatly reduced. The slow rate of the observed partial conversion may have been due to residual *in vivo* PolIII activity at 43° C or to an attempt by another DNA polymerase to catalyse this synthesis. Further, nearly all the ssDNA sedimenting more rapidly than about 20S was found as intermediate sized 70S to 120S pieces, even after 80 min of synthesised and/or processed, they were converted rapidly version of intermediate sized into parental sized pieces.

Very little DNA was found at any time in the region 30S to 70S, suggesting that once the 20S short pieces were fully synthesized and/or processed, they were converted rapidly into 70S to 120S intermediate sized pieces. Figure 1 shows that PolI, when present, was responsible for the rapid conversion. This role of PolI in the synthesis of the intermediate sized pieces was further examined using *E. coli* E4862, a *polA1* derivative[20] of E486. Sedimentation profiles of DNA synthesised by E4862 at either 30° C or at 43° C (Fig. 2*c*) are identical to those from E486, except that a substantial fraction of the 10 min and 40 min ssDNA from E4862 sedimented as 30S–70S material. Thus, when PolI activity was greatly reduced, processed short DNA pieces were converted slowly into 70S to 120S pieces, whereas in the presence of wild-type PolI activity, this conversion occurred sufficiently rapidly that very little 30S to 70S DNA was observed.

The slow conversion of short DNA pieces into intermediate

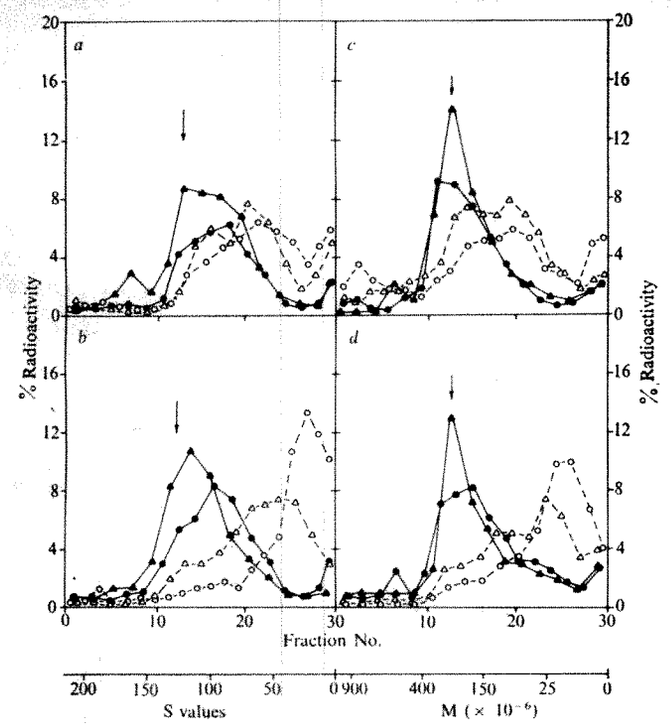


FIG. 1 Alkaline sucrose gradients of DNA from W3110 and p3478. *E. coli* W3110 *thy*⁻ and p3478 *thy*⁻ *polA1* were grown in a Tris–glucose–minimal salts (TG_0) medium containing 0.1% casamino acids and ^{14}C-thymine (2 μCi ml^{-1}, 2 μg ml^{-1}) at 30° C to 2 × 10^8 cells ml^{-1}. Half of each culture was collected by centrifugation (4° C, 5 min, 10,000 *g*), resuspended in an equal volume of TG_0 minus glucose, and added to a supplement containing ^{3}H-thymidine, to give 6 μCi ml^{-1}, and glucose, casamino acids and thymine in amounts to yield TG_0 plus 0.1% casamino acids. The cultures were incubated at 30° C with aeration. After 1 min and 3 min, quarter volume samples were frozen immediately in dry ice–acetone. At 3 min, non-radioactive thymidine to 50 μg ml^{-1} was added. At 10 min and 40 min, two further quarter volume samples were frozen immediately. The remaining halves of each of the two original cultures were shifted to 43° C for 15 min, collected by centrifugation (4° C, 5 min, 10,000 *g*), resuspended in prewarmed TG_0 minus glucose, added to supplement, and incubated at 43° C with aeration. Samples were frozen at the four time points, and the cultures were chased with non-radioactive thymidine, as described above. 43° C was attained within 30 s after cultures were placed at 43° C. A 1 ml sample of each frozen cell suspension was thawed, the cells were converted into lysozyme–EDTA spheroplasts, and ≤5 × 10^7 cells (50 μl of each sample) were lysed by layering gently into 0.1 ml 0.5 N NaOH over a 4.7 ml alkaline sucrose gradient (5%–20% linear gradient in 0.1 N NaOH), using the procedures of Smith and Meun[35]. Following centrifugation (30,000 r.p.m., 15° C, 105 min, Spinco SW50.1 rotor), fractions were collected directly onto Whatman 3 MM paper squares. These were dried, washed twice in cold 5% Cl_3 CCOOH, twice in 95% ethanol, once in acetone, dried, and assayed for radioactivity. The total radioactivity layered on each gradient was 7,000–35,000 c.p.m. for ^{3}H and 500–1000 c.p.m. for ^{14}C. Sedimentation coefficients and molecular weights were determined using known relations [36,37] and phage T4 DNA as a standard. ^{14}C-DNA sedimentation profiles were similar in each gradient and nearly identical to the 40 min ^{3}H-DNA profiles; the peak of the ^{14}C-DNA is indicated by the arrow. *a*, W3110 *polA*$^+$, 30° C; *b*, p3478 *polA1*, 30° C; *c*, W3110 *polA*$^+$, 30° C; *d*, p3478 *polA1*, 43° C. ○, 1 min ^{3}H-DNA; △, 3 min ^{3}H-DNA; ●, 10 min ^{3}H-DNA; ▲, 40 min ^{3}H-DNA.

sized pieces observed in E4862 at 43° C (Fig. 2*c*) may involve participation of PolII, as E4862 is deficient in PolI and PolIII at 43° C, although residual PolI and/or PolIII activity *in vivo* at 43° C is possible. We examined these possibilities further using an independent *dnaE* mutant, *E. coli* BT1026 *polA1 dnaE*1026. BT1026 possesses greatly reduced PolI activity, normal PolII activity, little detectable PolIII activity (<1% wild type) at 42° C, and reduced PolIII activity (<10% wild type) at 30° C *in vitro*[19,20,21]. Although sedimentation profiles of DNA synthesised by

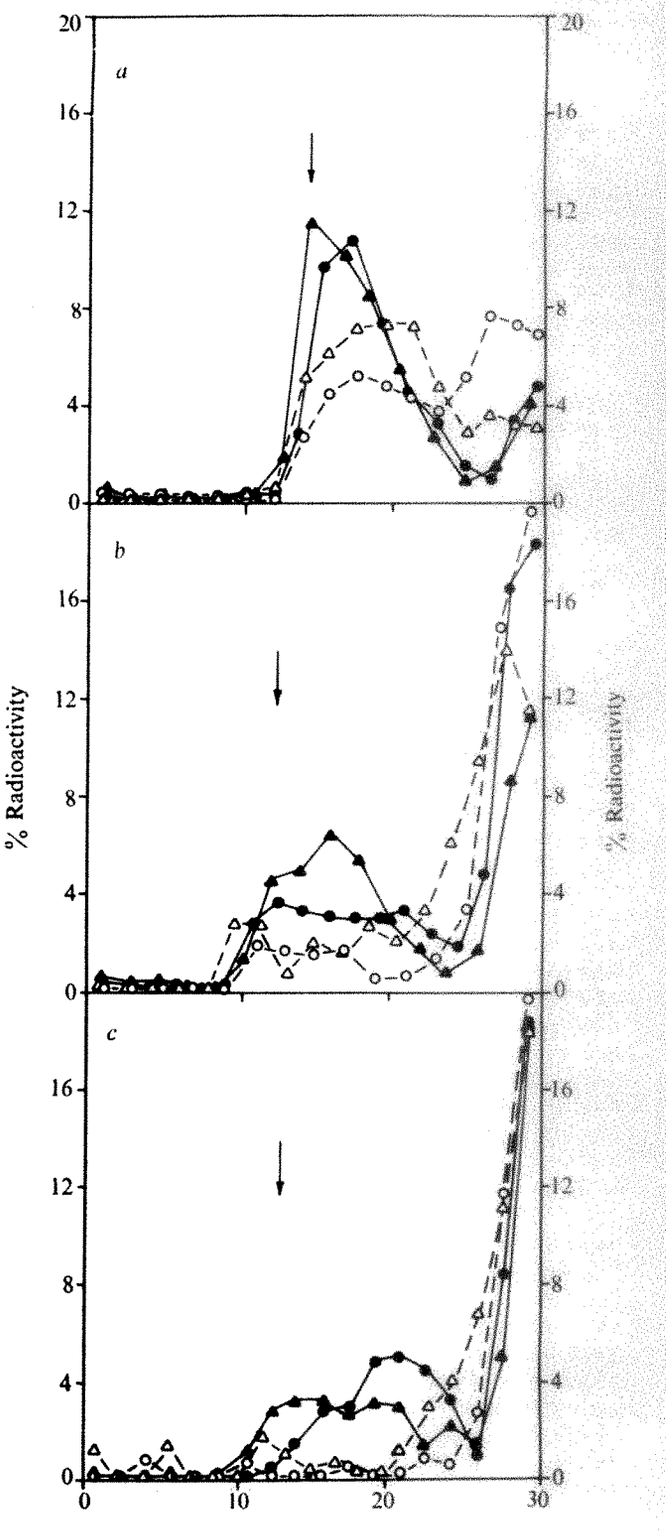


FIG. 2 Alkaline sucrose gradients of DNA from E486 and E4862. *E. coli* E486 *thr–leu–thi–thy–lac–met–dnaE*486 and E4862 *arg–his–thy–polA1 dnaE*486 were separately cultured and the DNA was labelled and analysed as described in the legend to Fig. 1. E4862 *thy*⁻ was obtained from E4862 *thy*$^+$ using trimethoprim selection[38]. The types of curves are as in Fig 1; the ^{14}C-DNA profiles were similar to the 40 min ^{3}H-DNA profile in (*a*) in each case, with the peak indicated by the arrow. The total radioactivity was 900–13,000 c.p.m. for ^{3}H, and 1,000–5,000 c.p.m. for ^{14}C. *a*, E486 *polA*$^+$ *dnaE*486, 30° C; *b*, E486 *polA*$^+$ *dnaE*486, 43° C; *c*, E4862 *polA1* *dnaE*486, 43° C.

BT1026 at 30° C (Fig. 3*a*) are similar to those of p3478 (Fig. 1*b*) as expected, much of the ssDNA from BT1026 sedimented slowly even after the 37 min chase. These features suggest that PolIII in BT1026 is also partially defective at 30° C *in vivo*, as *in vitro*[20], but to an even greater extent than in E4862. The above sedimentation properties of the DNA synthesised by BT1026 at 43° C were considerably accentuated (Fig. 3*b*). The amount of DNA synthesised was reduced greatly, and some degradation occurred in time. Most of the DNA sedimented slowly even after a 37 min chase and the larger pieces sedimented as intermediate sized pieces. Since these sedimentation properties are similar to those of ssDNA from the independent *dnaE* mutant E4862 (Fig. 2*c*), residual PolIII activity at 43° C probably was not involved in the slow conversion of short DNA pieces into intermediate sized pieces.

A less complete conversion by BT1026 of short DNA pieces into intermediate sized pieces occurred at 43° C than at 30° C (compare Fig. 3*a* and *b*), suggesting that PolIII, when PolI activity is reduced, participates in this conversion process. This possible role for PolIII is obscured, however, by the requirement for PolIII in the maturation of the short DNA pieces.

Pol II

A role for PolII in this slow conversion was examined using a mutant of BT1026 deficient in PolII. This mutant, H10265, has no detectable PolII activity at 43° C and very little (<0.02% wild type) PolII activity at 30° C[10]. The amount of DNA synthesised by H10265 is similar to that by BT1026 at 30° C. However, two differences in the ssDNA sedimentation profiles are apparent. First, the rate at which short ssDNA pieces were converted into intermediate sized pieces was reduced in H10265 (3 min DNA distributions, Fig. 3*a* and *c*). This suggests that, when PolI is deficient, PolII participates in the conversion of short 20S ssDNA fragments into larger 70S to 120S pieces. Second, the 40 min DNA from H10265 sedimented as rapidly as did the parental DNA. Since PolIII seemed to be partially defective *in vivo* in BT1026 at 30° C (Fig. 3*a*), the presence of PolII, when PolIII is partially defective, apparently prevents conversion of 70-120S DNA into the size and/or shape typical of parental ssDNA. Very little DNA synthesis was observed in H10265 at 43° C; the resulting sedimentation profiles were similar to those of BT1026 at 43° C (Fig. 3*b*).

Further evidence supporting these proposed roles for PolII in DNA replication was obtained in similar studies using *E. coli* HMS83, PolB derivative of p3478 containing no detectable PolI activity *in vitro*[9]. First, the rate of conversion of short ssDNA pieces in HMS83 to intermediate sized pieces was reduced when compared with p3478 (data not shown), again suggesting that PolII, when PolI activity is reduced, participates in this conversion. The slow residual conversion observed in HMS83 was probably due either to residual PolI activity[19] or to PolIII activity. Second, intermediate sized pieces were converted into parental sized ssDNA within 40 min at 30° C, indicating that neither PolI nor PolII were required for this conversion when PolIII was present at wild type levels.

Three stages in chain elongation

Our results can be summarised in the schematic model shown in Fig. 4. Three stages in DNA chain elongation are visualised. In the first stage, synthesis of a short DNA fragment is initiated, the fragment is elongated to a 20S piece, and processed into a form ready for further elongation. These pieces are the short DNA pieces discovered by Okazaki *et al.*[16] When PolIII is deficient, synthesis of these pieces and/or their maturation proceeds very slowly and incompletely. In the second stage, the 20S pieces are converted into intermediate sized 70S to 120S pieces. This conversion proceeds very rapidly when PolI is present in the cell compared with the first stage of chain elongation. When PolI is deficient, both PolII and PolIII can participate in this conversion. In

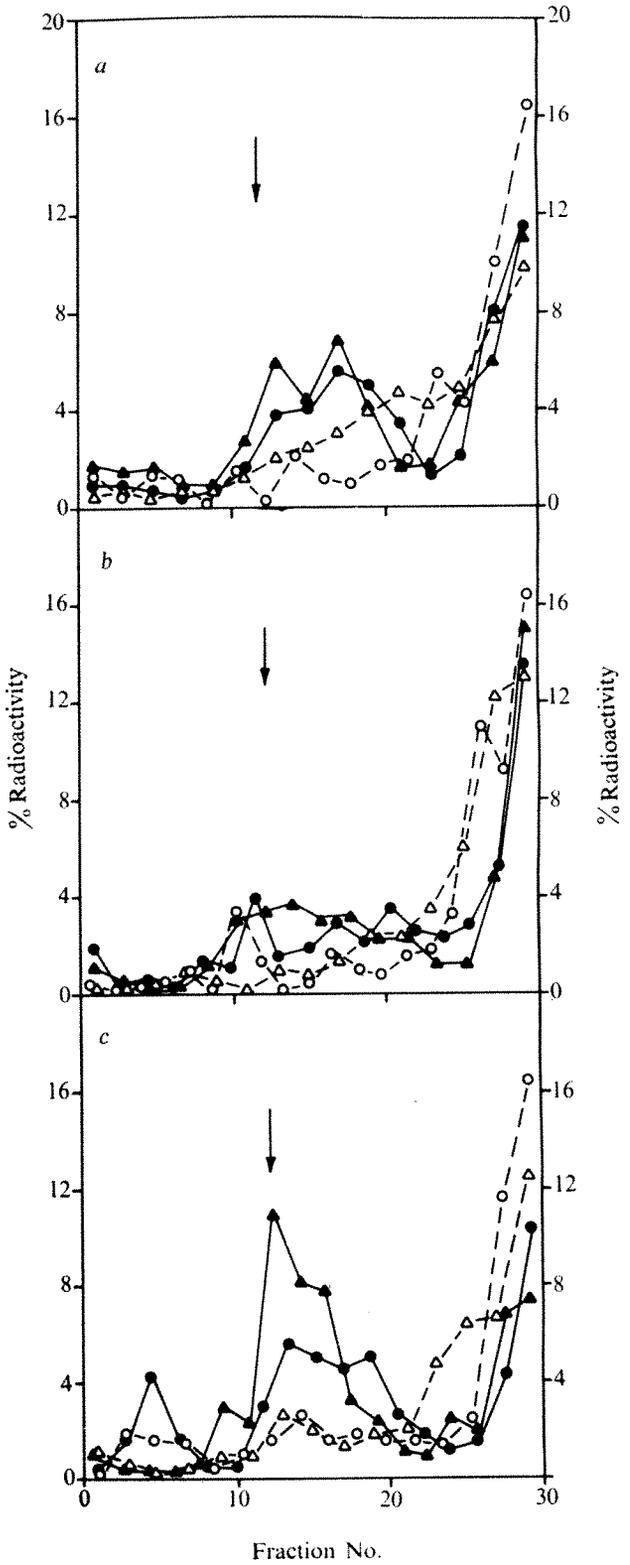


FIG. 3 Alkaline sucrose gradients of DNA from BT1026 and H10265. *E. coli* BT1026 *thy endA polA1*, *dnaE*1026 and H10265 *thy endA polA1 polB*⁻ *dnaE*1026 were cultured separately and the DNA was labelled and analysed as described in the legend to Fig. 1. The types of curves are as in Fig. 1; the ^{14}C-DNA profiles were similar to the 40 min ^{3}H-DNA profile in (*c*) in each case, with the peak indicated by the arrow. The total radioactivity was 500–2,000 c.p.m. for ^{3}H, and 900–5,000 c.p.m. for ^{14}C. *a*, BT1026 *polA1 dnaE*1026, 30° C; *b*, BT1026 *polA1 dnaE*1926, 43° C; *c*, H10265 *polA1 polB*⁻ *dnaE*1026, 30° C.

the third stage, intermediate sized pieces are converted into parental sized pieces. In cells containing PolII, PolIII is required for this process. However, when PolII is deficient in a cell containing a defective PolIII, the conversion seems to take place.

Although biochemical reactions could not be determined in our experiments, the following possibilities seem worth mentioning. PolIII is required for elongation and complete synthesis of the most newly synthesised Okazaki DNA pieces. The RNA found covalently attached to these pieces[22] may serve as a primer for initiation of synthesis of these short pieces, permitting a subsequent elongation reaction similar to the preferred gap-filling reaction catalysed *in vitro* by PolIII[15,23]. This elongation could continue until a termination signal were reached, such as running into the adjacent short DNA piece, terminating stage I.

To begin stage II, the products of stage I may need to be processed. Attainment of a fixed size may suffice. Alternatively, removal of the putative RNA primer may be involved. A subsequent rapid repair of the gap left by the excised RNA, coupled with a ligase joining of the individual short pieces, could result in the 70S to 120S pieces. Since this conversion occurs very rapidly in cells containing PolI, PolI may serve as an RNase H[24], catalysing the exonucleolytic removal of the RNA from the RNA–DNA duplex. Although *E. coli* contains a distinct RNase H activity[25], PolI possesses RNase H activity *in vitro*[26] and seems to have an RNase H role *in vivo* during colE1 DNA replication[27]. When PolI activity is substantially reduced, either PolII or PolIII, or both, may possess RNase H activity and remove the RNA primer. Alternatively, the *E. coli* RNase H may remove the RNA, and PolII and/or PolIII may fill in the gap left by the excised ribonucleotides. Joining of these matured short DNA pieces by a ligase would yield the 70S to 120S pieces.

In stage III, the intermediate sized pieces (50–250 × 10^6 daltons) are converted into parental sized pieces. For a chromosome of 2,500 × 10^6 daltons, 5 to 25 such pieces would exist per chromosome. As an appealing hypothesis, the products of stage II may correspond to the newly synthesized DNA strands of the 12 to 80 loops found in the *E. coli* folded chromosome[28] by Worcel and Burgi[29]. Stage III would then comprise the biochemical events joining these loops together to yield the supercoiled folded bacterial chromosome[29]. This hypothesis also provides a rationale for involvement of PolIII in stages I and III. Considerable indirect evidence for a replication site on the bacterial cell surface has accumulated[30–34], and PolIII seems to prefer a hydrophobic environment[15,21]. Worcel and Burgi[29] propose that the 12 to 80 loops may emanate from an RNA core, since RNA is essential for the integrity of the folded chromosome[28]. Such an RNA core could be part of the DNA replication site at the cell surface. Stage I could then take place in the hydrophobic environment of the DNA replication site, with PolIII as the primary DNA replicase, whereas stage II might not require this environment and/or site and could occur in the cytoplasm. Stage III could then again occur at the replication site, as the Worcell–Burgi loops projecting into the cytoplasm from the replication site were joined together. This hypothesis would then partially compartmentalise the *E. coli* DNA polymerases, localising PolIII to the hydrophobic environment of the cell surface, thus in part explaining the need for different DNA polymerase enzymes to catalyse similar gap-filling polymerisation reactions.

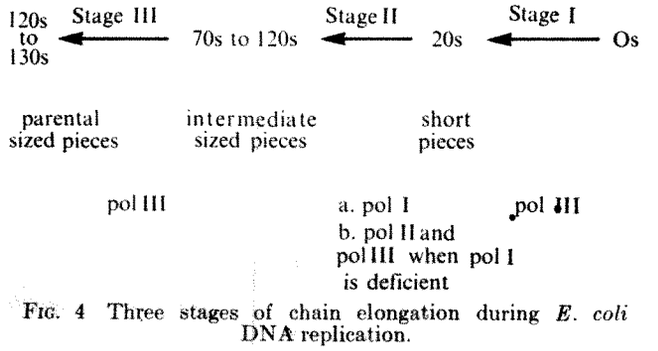


FIG. 4 Three stages of chain elongation during *E. coli* DNA replication.

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1 Meselson, M., and Stahl, F. W., *Proc. natn. Acad. Sci. U.S.A.*, **44**, 671–682 (1958).
2 Smith, D. W., *Prog. Biophys. molec. Biol.*, **26**, 321–408 (1973).
3 Goulian, M., *A. Rev. Biochem.*, **40**, 855–898 (1971).
4 DeLucia, P., and Cairns, J., *Nature*, **224**, 1164–1166 (1969).
5 Kornberg, T., and Gefter, M. L., *Biochem. biophys. Res. Commun.*, **40**, 1348–1355 (1970).
6 Moses, R. E., and Richardson, C. C., *Biochem. biophys. Res. Commun.*, **41**, 1557–1564 (1970).
7 Wickner, R. B., Ginsberg, B., Berkower, I., and Hurwitz, J., *J. biol. Chem.*, **247**, 489–497 (1972).
8 Kornberg, T., and Gefter, M. L., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 761–764 (1971).
9 Campbell, J. L., Soll, L., and Richardson, C. C., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2090–2094 (1972).
10 Hirota, Y., Gefter, M. L., and Mindich, L., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3238–3242 (1972)
11 Wechsler, J. A., and Gross, J. D., *Molec. gen. Genet.*, **113**, 273–284 (1972).
12 Gross, J. D., *Current Topics Microbiol. Immun.*, **57**, 39–74 (1972).
13 Tait, R. C., Harris, A. L., and Smith, D. W., *Proc. natn. Acad. Sci. U.S.A.* (in the press).
14 Masker, W., Hanawalt, P., and Shizuya, H., *Nature new Biol.*, **244**, 242–243 (1973).
15 Kornberg, T., and Gefter, M. L., *J. biol. Chem.*, **247**, 5369–5375 (1972).
16 Sakabe, K., and Okazaki, R., *Biochim. biophys. Acta*, **129**, 651–654 (1966).
17 Kuempel, P. L., and Veomett, G. E., *Biochem. biophys. Res. Commun.*, **41**, 973–980 (1970).
18 Okazaki, R., Arisawa, M., and Sugino, A., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2954–2957 (1971).
19 Lehman, I. R., and Chien, J. R., in *DNA Synthesis in vitro* (edit. by Wells, R. D., and Inman, R. B.) 3–12 (University Park Press, Baltimore, 1973).
20 Gefter, M. L., Hirota, Y., Kornberg, T., Wechsler, J. A., and Barnoux, C., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 3150–3153 (1971).
21 Nusslein, V., Otto, B., Bonhoeffer, F., and Schaller, H., *Nature new Biol.*, **234**, 285–286 (1971).
22 Sugino, A., Hirose, S., and Okazaki, R., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1863–1867 (1972).
23 Wickner, R. B., Ginsberg, B., and Hurwitz, J., *J. biol. Chem.*, **247**, 498–504 (1972).
24 Stein, H., and Hausen, P., *Science*, **166**, 393–395 (1969).
25 Miller, H. I., Riggs, A. D., and Gill, G. N., *J. biol. Chem.*, **248**, 2621–2624 (1973).
26 Keller, W., and Crouch, R., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3360–3364 (1972).
27 Goebel, W., and Schrempf, H., *Nature new Biol.*, **245**, 39–41 (1973).
28 Stonington, O. G., and Pettijohn, D. E., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 6–9 (1971).
29 Worcel, A., and Burgi, E., *J. molec. Biol.*, **71**, 127–147 (1972).
30 Jacob, F., Brenner, S., and Cuzin, F., *Cold Spring Harbor Symp. quant. Biol.*, **28**, 329–347 (1963).
31 Ganesan, A. T., and Lederberg, J., *Biochem. biophys. Res. Commun.*, **18**, 824–835 (1965).
32 Smith, D. W., and Hanawalt, P. C., *Biochim. biophys. Acta*, **149**, 519–531 (1967).
33 Earhart, C. F., Tremblay, G. Y., Daniels, M. J., and Schaechter, M., *Cold Spring Harbor Symp. quant. Biol.*, **33**, 707–710 (1968).
34 Ryter, A., *Bact. Rev.*, **32**, 39–54 (1968).
35 Smith, K. C., and Meun, D. H. C., *J. molec. Biol.*, **51**, 459–472 (1970).
36 McGrath, R. A., and Williams, R. W., *Nature*, **212**, 534–535 (1966).
37 Studier, F. W., *J. molec. Biol.*, **11**, 373–390 (1965).
38 Stacey, K. A., and Simson, E., *J. Bact.*, **90**, 554–555 (1965).

Barbed bone points from Central Sudan and the age of the "Early Khartoum" tradition

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Barbed bone points, typical of those from the early Holocene settlement of "Early Khartoum", have been found at three sites along the White Nile, south of Khartoum. The form of the fragments and the stratigraphy of the sites throw light on the environment and technology of the early settlements along this part of the Nile.

During archaeological and geomorphological fieldwork along the White Nile south of Khartoum in February, 1973 (see Fig. 1), we found at three sites, fragments of barbed bone points typical of those from the early Holocene settlement of "Early Khartoum"[1]. No radiocarbon dates exist for the "Early Khartoum" settlement, but at El Qoz[2] the characteristic pottery is found stratified below that associated with the Neolithic settlement at Esh Shaheinab, the average date of which is 5,253 ± 415 yr BP (ref. 2, page 107). One of our discoveries, from near the village of Tagra, is of particular interest because it came from a depth of 1.3 m in a soil pit for which two radiocarbon dates are already available. A second discovery was made on an ancient point bar of the White Nile, in swamps opposite Esh Shawal near Guli. The third site, which we studied in some detail, was located at Shabona and yielded an industry identical to that from "Early Khartoum". Shabona is a settlement site and only the second with this cultural pattern to have been found and excavated. A similar site was located about 3 km distant from Shabona so that it seems likely that other similar riverside settlements would be found in this part of the White Nile between Khartoum and Kosti.

Tagra site

Williams[3] in 1963 collected subfossil freshwater and amphibious Mollusca from 1.6 to 1.9 m depth in a soil pit near Tagra, the shell giving a radiocarbon date of 8,370 ± 350 yr BP (Isotopes Inc. 1485, dated by courtesy of Professor A. J. Whiteman). This pit was resampled by Williams and Adamson in 1972 and a shell radiocarbon date of 8,130 ± 225 yr BP (Sydney University Radiocarbon Laboratory SUA-68) was obtained from a depth of between 1.2 to 1.4 m. In February 1973, Adamson extended and sampled this pit yet again for more shell material and found, at a depth of 1.3 m, two fragments of a barbed bone point, fragments of small bones of mammal, fish bones including cat fish spines, and shells of *Pila wernei* (Philippi) and *Lanistes carinatus* (Oliver). The presence of large complete *Pila* up to 9 cm long suggests deposition of the shells and bone *in situ* and lack of disturbance by soil cracking and mixing.

The location and stratigraphy of the Tagra pit are shown in Fig. 1. The pit is located 230 m on a 42° bearing from the steel beacon marking the surveyed position of 13°56′N, 32°22′E and it lies about 0.5 km east of the present 377.8 m high level of the White Nile as controlled by the Jebel Aulia dam. The pit surface is between 378.2 and 378.6 m which is somewhat lower than the elevation first reported[4]. Before the dam was built, local mean low water level was 372.2 m and local mean flood level was 375.4 m at the nearby Ed Dueim gauge[5]. The pit surface is, therefore, about 6 m above the former low level, about 3 m above the former high level and about 0.4 to 0.9 m above the present high water level caused by the Jebel Aulia dam.

The barbed point fragments, other bones and shells just mentioned, occurred near the base of a dark-grey clay 1.5 m thick, which overlies a calcic layer 10 cm thick (1.5 to 1.6 m) containing abundant hard calcium carbonate nodules and softer calcareous aggregates embedded in fine sand and silt. Beneath the calcic layer is 15 cm of olive-grey finely laminated silts (1.6 to 1.75 m) with thin bands of reddish-yellow limonite accentuating the bedding planes. From 1.75 to 1.9 m, very pale greyish-brown clayey sand contains occasional shells of *Pila wernei* and *Melanoides tuberculata* (Müller). From 1.9 to 2.05 m there are alternating bands of rippled brown clay and grey or yellow sand, each band being about 0.5 to 1.0 cm thick. Occasional strongly calcareous lenses occur in this horizon. The shells for the 8,370 ± 350 yr BP date came from between 1.6 and 1.9 m and were *in situ*. Light olive-grey clayey sand from 2.05 to 2.20 m formed a transitional layer above light grey fine quartz sand at least 60 cm thick. The total depth examined was 2.85 m.

Sequence of events at Tagra

A tentative interpretation of the sequence of events at this site is offered. Fluviatile fine sands and clayey sands were laid down in the White Nile up to at least 376 m, that is about 4 m above the normal minimum water level before the Jebel Aulia dam was built. A further 30 cm of fluviatile aggradation occurred and both aquatic and semi-aquatic molluscan shells were incorporated in the silts, clays and sands some 8,000 to 8,500 yr ago at an elevation at least 0.5 to 1.0 m above the pre-dam flood level. The presence of *Melanoides tuberculata* indicates permanent water and *Pila wernei* and *Lanistes carinatus*, being amphibious species, could indicate seasonal inundation. Their presence suggests a mixed assemblage of shells such as could occur at the boundary between permanent water and seasonally inundated swamps. The close alternation of rippled sands and clays in the horizon immediately below laminated grey silts is consistent with seasonal deposition between high and low water

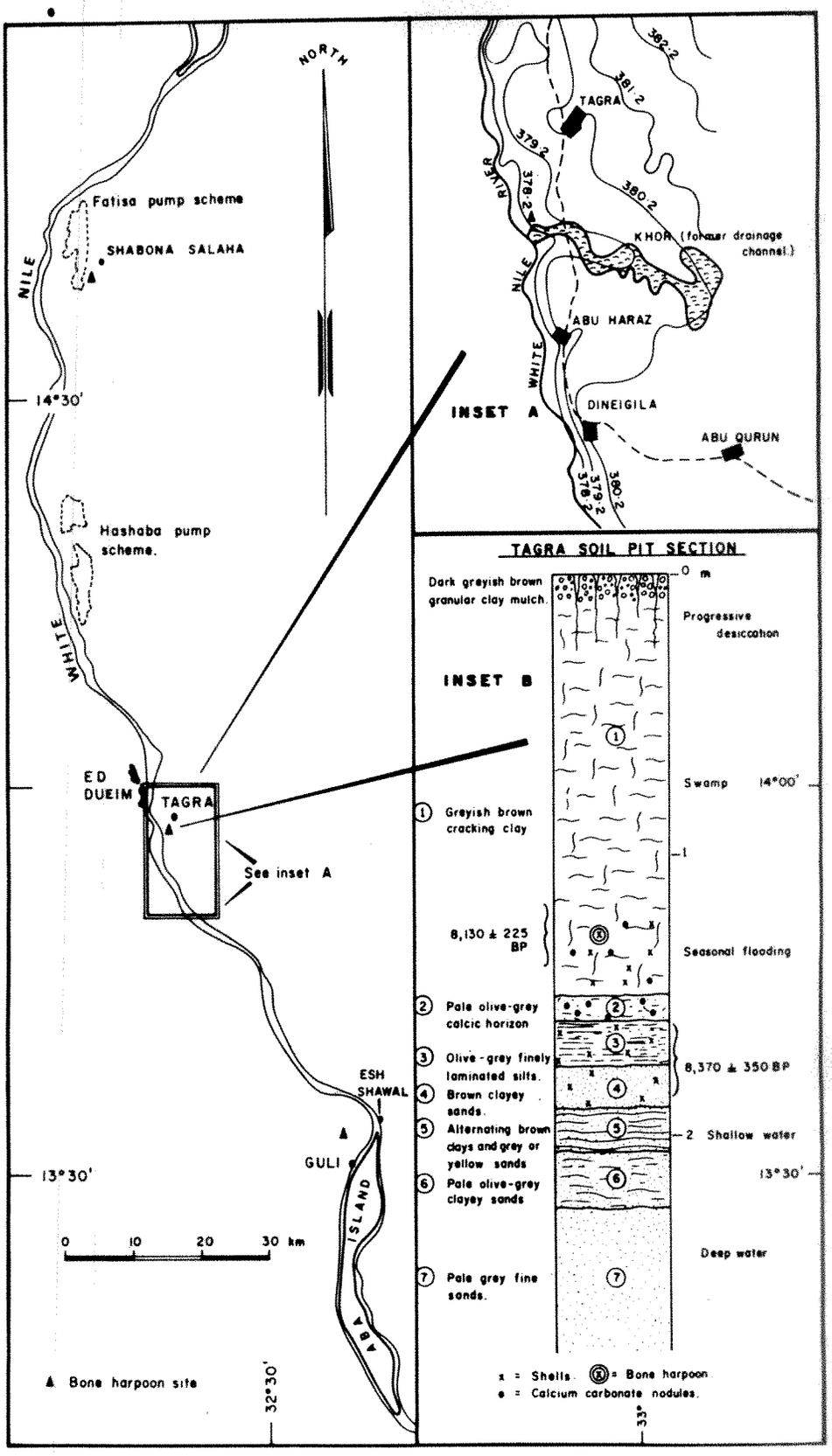


FIG. 1 Location of the three sites in Central Sudan, with inset showing section of soil pit at Tagra.

level. The limonite bands between gleyed silt laminae also suggest an alternation of oxidising and reducing conditions associated with seasonal deposition in a shallow littoral environment.

The deposition of clay, the calcic layer, the absence of permanent-water mollusca and the signs of human activity are also consistent with the cessation of permanent water conditions and a change to seasonal inundation. The clay indicates inundation by slow moving water carrying only fine sediment. The site may have been subject to the type of annual flooding and partial drying which now occurs in 'toich' soils which adjoin rivers or 'khors' in the permanent swamps of the Sudd region in the southern Sudan[8]. A zone of carbonate accumulation, which may mark the depth of

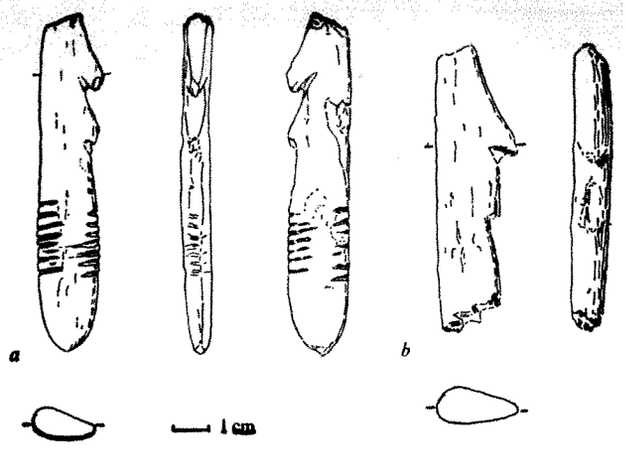


FIG. 2 *a*, Tagra bone point fragments after joining; *b*, Guli specimen (drawn by R. Vermilion).

seasonal drying, is common in 'toich' soils and the horizontal calcic band in the Tagra profile may be analogous. If the permanent, dry season water level of the White Nile was about 376–377 m, the flood season levels would have created 'toich' conditions at this site during the whole period of clay deposition.

The recession of the White Nile and the end of clay deposition by seasonal flooding at this site was governed by the down-cutting of the Blue Nile and consequent lowering of the White Nile[7]. The White Nile fell by about 4 m within the past 6,000–8,000 yr, sufficient to account for a lowering of the level of permanent water, that is dry season level, at this site from about 376–377 m to the present pre-dam level of 372.2 m. The effect of the Jebel Aulia dam when full is to raise the White Nile close to its prehistoric dry-season level of 8,000 yr ago.

Guli site

A second fragment of barbed bone point, heavily encrusted with calcium carbonate, was found on a small sandy island in the White Nile swamps near Guli (Fig. 1) at 13°34′N, 32°34′E at an estimated elevation of about 378.5 to 379 m, that is about 1 m above the present high water level caused by the Jebel Aulia Dam (377.8 m). Before completion of the dam, the maximum White Nile level, estimated by interpolation between the Ed Dueim and Rabak gauges, was about 375.6 m and the minimum about 373.0 m. The island is part of an old point bar in the large area of swamps and point bars, about 7 km wide, on the west side of the river opposite Esh Shawal. The bone tool was found accidentally while sampling a dense deposit of *Pila wernei* shells (0-35 cm deep) for purposes of radiocarbon dating. The spoil from a drainage ditch across one corner of the deposit revealed abundant potsherds with impressed fish-spine decoration, fossilised and subfossil fish bones, and subfossil *Pila* shells, indicating that the shell deposit was associated with an old occupation site. The *Pila* shells were predominantly mature specimens averaging 30–50 mm, with large specimens up to 90 mm long. The whole shell assemblage was evidently not a natural one and this suggests that they were collected and brought to the occupation site as food and/or bait. A sample of shell from a depth of 30–35 cm in the midden gave an age of 5,500 ± 90 yr BP (or 3530 ± 90 b.c.) (SUA-211) thus suggesting that the Guli site is contemporaneous with the "Khartoum Neolithic" tradition as represented at Esh Shaheinab and other sites north of Khartoum.

Bone point fragments

The Tagra bone point fragments (Fig. 2*a*) have been cleaned and the carbonate removed by the Technical Department of the British Museum (Natural History) to the staff of which our thanks are gratefully acknowledged. After the two fragments are joined, the specimen is seen to comprise the butt end and two partially damaged barbs, both on the same lateral edge. The distal end of the specimen is missing but there is no noticeable convergence of the lateral edges so that it is likely to have been furnished with at least one additional barb. The existing length is 91 mm; the maximum breadth at the upper barb is 18 mm and, at a distance of 21 mm from the proximal end, the maximum breadth is 16 mm. In section it is a flattened elipsoid, more or less the same thickness throughout its length — 7.5 to 7.0 mm.

The specimen is made from a longitudinally split fragment of long bone from a large mammal, rubbed smooth and polished on a grindstone. This smoothing and grinding have been insufficient to remove entirely evidence of the cancellous bone on the interior surface. The barbs have been made by working on each face opposed V-shaped grooves; these were set obliquely to the long axis and deepened so as to intersect at the edge. The intersection was then worked back by further transverse rubbing to deepen and enlarge the barb. The backs of the barbs are slightly concave, as in many of the "Early Khartoum" examples, and although the tip ends have been broken away, they may similarly have been given a reverse curve, for the beginnings of such can be detected on the upper of the two surviving barbs.

As in the uniserially barbed bone points from "Early Khartoum", the Tagra specimen has also been provided with seven obliquely cut grooves between 40 mm and 21 mm from the proximal end. These grooves are believed to serve as an anchor for the binding that secured the point to the shaft so that, since the butt carries no perforation, the specimen is technically the non-detachable point of a spear rather than a harpoon.

The Tagra specimen closely resembles the "Early Khartoum" points in general dimensions, nature of barbs and grooving of the rounded butt end. Only a small number, however, out of more than 270 fragments at "Early Khartoum" have a flattened, elipsoidal cross-section, most being circular or elliptical in section. Variability of this kind is not unexpected between independent settlements of this kind over 180 km apart. There is, however, no indication that the Tagra point was associated with any living site debris; it seems to be an isolated find that had, probably, been lost in use away from the immediate settlement area.

The specimen from Guli (Fig. 2*b*) is the central portion of a similar uniserially barbed bone point; the distal and proximal ends are both missing. It is 76 mm long, 22 mm broad across the first barb and the thickness varies between 9.0 mm and 8.0 mm. It is made in a similar way from a segment of longbone rubbed down on a sandstone grinder. The upper of the two barbs is complete but only the basal part of the second, adjacent to the stem, is preserved. The back of the complete barb exhibits the concavo-convex profile characteristic of the bone points and harpoons from both "Early Khartoum" and Esh Shaheinab. The section across the surviving barb is a flattened elipse, similar to that of the point from Tagra. The missing distal portion probably ended in a point as it can hardly have been either long or broad enough to accommodate another barb unless this was of a vestigial nature. Similarly, there is no indication from the remaining length of the proximal end that there were ever more than two barbs. Although rare, forms with only two barbs are known from both "Early Khartoum" and Esh Shaheinab. The specimen is coated with a carbonate skin which has not yet been removed, but there is nothing to lead one to suspect that the butt end bears groovings characteristic of points of "Early Khartoum" type. Neither is the surviving portion lugged and perforated as were the Neolithic harpoons, however, since the butt itself is missing, it is not possible to be certain that it may not have been pierced for

the attachment of a line, though this seems unlikely.

The Guli example resembles more closely, therefore, the "Early Khartoum" type of bone point than it does the perforated and lugged type of harpoon from Esh Shaheinab, although the radiocarbon determination suggests that it is more closely related in time to the Neolithic than the "pre-Neolithic". Stylistic preferences and time differences between river bank settlements at this time are surely to be expected, as also is variability due to differences in the local availability of specific resources at the different sites and so in the equipment with which these were exploited. The Neolithic bone harpoon may well be related to hippo-hunting from dugouts or reed boats, as Arkell has previously hinted (ref. 2, page 31). The barbed bone point, on the other hand, is more probably a spear for Nile perch and mud fish as well as for land mammals; barbed spears and harpoons have a very long history in the Nile basin[8] and in the west African Sahel[9].

The pottery from Guli shows affinities with the Khartoum Neolithic impressed wares though there are no sherds of "Burnished Dotted Wavy-Line" or opposed triangle impressed pottery in the collection. The majority of sherds seem to come from well-fired, deepish spherical bowls with inturned rims, the upper half at least of the exterior of the pot, and sometimes the lip, being decorated with horizontal rows of impressed dots, some executed with a comb (cat fish spine or stamp) and others again with a rolled cord or grass roulette (compare ref. 2, plate 29:2). Rocker stamp decoration occurs on the interior of one sherd and the rim of a second. Another shows two rows of stamping on the rim and parallel grooving below; the sherd is also perforated. Another rim sherd shows a stamped chevron design such as occurs rarely also at Esh Shaheinab. These sherds are comparable also to the Khartoum Variant pottery from the 2nd Cataract and that from the Khashm el Girba region on the Upper Atbara which is similarly unburnished[10]. The pottery confirms in general, therefore, the age determination of the Guli midden and bone point fragment.

Age determinations on bone, shell and charcoal from the pre-Neolithic settlement at Shabona have not yet been received, but the similarity of the barbed bone points from this site (where the flattened section is more common than the circular form) and those from "Early Khartoum" to the Tagra specimen, suggest that this particular industrial tradition most probably belongs in the seventh and sixth millennia bc. The "Early Khartoum" tradition had been replaced by that of the Khartoum Neolithic, which grew directly out of it, by the middle of the fourth millennium bc on the evidence of the dates from Esh Shaheinab and Guli, but its lower limit has yet to be determined. There is every indication that it was a tradition of long duration evolving out of the local late Palaeolithic and early epi-Palaeolithic industries, through the gradual development of a specialised waterside technology and the acquisition at different times in its history of pottery and other improved cultural traits.

The only other early site on the Nile yielding barbed bone points is the fishing camp of Cat Fish Cave in Upper Egypt (22°43′N, 32°07′E) dating to before 6400 b.c.[11]. No pottery was found here but the points bear only general resemblance to those of the "Early Khartoum" tradition. There is no pottery either at the epi-Palaeolithic site of El Kab (c. 25°15′N, 32°53′E) dating to 6400± b.c., neither, however, are there bone points[12]. Pottery is again absent from the epi-Palaeolithic sites found by Wendorf[13] in the Fayum depression and dating between 5190 ± 120 and 6150 ± 130 b.c. A specialised form of barbed point made from a cat fish spine is associated with these small fishing camps. The absence of pottery from these seventh millennium sites in Egypt suggests, therefore, that the Tagra barbed bone point might belong to a similar early Holocene local Sudanese tradition that had not yet developed the art of pottery making. On the other hand, if the dates from Shabona prove to be coeval with that from Tagra, the possibility that the characteristic "Wavy-Line" pottery and other wares of "Early Khartoum" type were an early and independent development on the Upper Nile or in the Sahara, as Arkell has suggested[14], becomes much more likely.

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1 Arkell, A. J., *Early Khartoum* (Oxford University Press, London, 1949).
2 Arkell, A. J., *Shaheinab*, 101 (Oxford University Press, London, 1953).
3 Williams, M. A. J., *Nature*, **211**, 270–1 (1966).
4 Williams, M. A. J., *Nature*, **211**, 270–1 (1966).
5 Hurst, H. E. and Phillips, P., *The Nile Basin*, 3 (second suppl.) (Physical Department Paper No. 37, Schindler's Press, Cairo, 1939).
6 Jonglei Investigation Team, *Equatorial Nile Project; the Jonglei Report* (Sudan Government, Khartoum, 1955).
7 Williams, M. A. J., and Adamson, D., *Georgr. J.*, **139**, 498–508 (1974).
8 Evans-Pritchard, E. E., *The Nuer* (Clarendon Press, Oxford, 1940).
9 Ligers, Z., *Les Sorko (Bozo) Maîtres du Niger* (CNRS, Paris, 1964).
10 Hays, T. R., thesis (Southern Methodist Univ., Dallas, Texas, 1971).
11 Wendt, W. E., *Postilla*, **102**, 1–46 (1966).
12 Vermeersch, P., *L'Elkabien. Chronique d'Egypte*, **45** (89), 45–68 (1970).
13 Wendorf, F., Said, R. and Schild, R., *Science*, **169**, 161–71 (1970).
14 Arkell, A. J., *Actes du IVe Congres Pan-Africain de Prehistoire et de l'Etude du Quaternaire*, **2**, 283–7 (1962).

Localisation of a fibroblast growth factor and its effect alone and with hydrocortisone on 3T3 cell growth

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FGF, a polypeptide found in brain and pituitary, provokes the initiation of DNA synthesis by resting 3T3 cells. When glucocorticoids are present FGF stimulates DNA synthesis as effectively as serum.

MULTIPLICATION of animal cells *in vitro* requires certain macromolecular factors which are usually found in serum. Since none of these factors, except for somatomedin A[1,2] had been isolated until recently, their mode of action has remained unclear. It is also unclear whether one or more of these factors is required to initiate division *in vivo* or *in vitro*. It has been suggested[3–7] that the pituitary gland is a source of growth factors *in vivo*. Bovine pituitary glands have yielded an ovarian growth factor which controls the division of an ovarian cell line[4]. Other reports describe the presence in impure preparations of pituitary hormones such as thyroid stimulating hormone and luteinising hormone of factor(s)

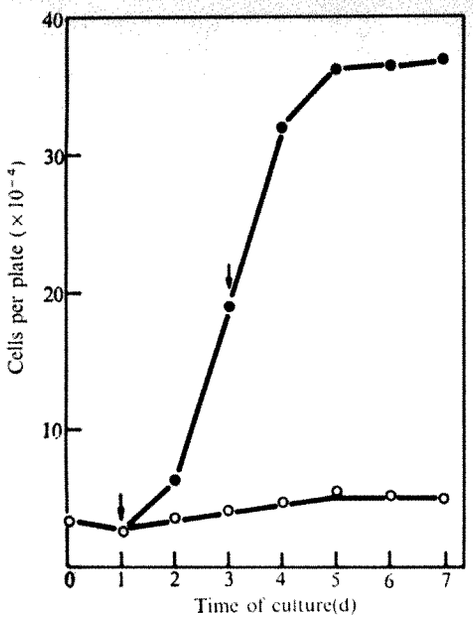


FIG. 1 Stimulation of division of resting 3T3 cells by FGF. 3T3 cells were plated in Dulbecco's modified Eagle's medium (DME) in the presence of 10% calf serum (4×10^4 cells per 60×15 mm plate). Eight hours later the medium was changed to DME with 0.4% calf serum (day 0). On day 1 and day 3 (arrows), the FGF was added (50 ng ml^{-1}). The number of cells per dish was counted every day and plotted against time both in the presence (●) and the absence (○) of FGF.

able to stimulate growth of chondrocytes[5] or 3T3 cells[6,7].

To ascertain whether or not the pituitary is the sole source of growth factors, crude extracts of a variety of bovine organs were tested for their ability to stimulate division of resting 3T3 cells. The highest concentration of fibroblast growth factor (FGF) was found in the brain. Pituitary extract was five to ten times less potent. Although FGF has been purified from the pituitary[8], the effort to purify it from the brain has been impeded by the high concentration of basic polypeptides of low molecular weight present in brain extracts. These polypeptides remain associated with the FGF making its purification from brain more difficult than from pituitary.

Studies of the control by purified FGF of the division of resting 3T3 cells maintained at low serum concentration have shown the stimulation of DNA synthesis by FGF to be less than that induced by a high serum concentration. For maximal DNA synthesis, hydrocortisone (a glucocorticoid) is required. This requirement is similar to that of an ovarian cell line[3], which requires dexamethasone or hydrocortisone for full cell division. In serum-free medium, FGF plus hydrocortisone stimulates DNA synthesis in a manner comparable with serum.

Localisation of activity

Growth factor activities of crude extracts of the following organs from freshly slaughtered cows were compared: heart, liver, muscle, pituitary, brain, placenta (cotyledon), adrenal cortex, adrenal medulla, ovary and lung. Each tissue was homogenised at 4°C in 0.15 M $(NH_4)_2SO4$ in a Virtis homogeniser. The *p*H was adjusted to 4.5 and the homogenate was allowed to stand in the cold. After 2 h the homogenate was centrifuged and the supernatant was dialysed against deionised water overnight. The extract was then centrifuged again; the supernatant was lyophilised and stored for use.

The 3T3 cells used were grown in DME (Dulbecco's modified Eagle's medium) supplemented with 10% calf serum (Colorado Serum Co. Laboratories) and incubated at 37°C in a CO_2 and humidity controlled incubator. The cells were collected routinely using trypsin (0.25% in phosphate-buffered saline). Aliquots of the washed cells were plated onto plastic tissue culture dishes (Falcon). The serum concentration was decreased to 0.4% to make the cells enter a resting stage (Fig. 1). DNA synthesis was assayed quantitatively by measuring ^{3}H-thymidine incorporation into trichloroacetic acid-precipitable material.

When brain or pituitary extracts were added to cultures of resting 3T3 cells, DNA synthesis was enhanced in proportion to concentration (Fig. 2). In contrast, muscle and kidney extracts were inactive except when high concentrations (2.5 μg ml^{-1}) of protein were present. The slight growth factor activity of kidney extracts was consistent with the activity to be expected due to the presence of serum in the kidney at the time of extraction.

The effects of crude extracts of diverse tissues is presented in Table 1. The incorporation of ^{3}H-thymidine by 3T3 cells is clearly much higher in the presence of crude brain or pituitary extract than in the presence of extracts from any other tissue tested. The slight degree of stimulation by extracts from other tissues can, in certain cases, be attributed to their serum content. For example, if liver is perfused with saline before extraction most of the stimulatory effect disappears.

Brain extract was a more potent stimulant of DNA synthesis than pituitary extract (Fig. 2). The minimum concentration of brain extract which stimulated ^{3}H-thymidine

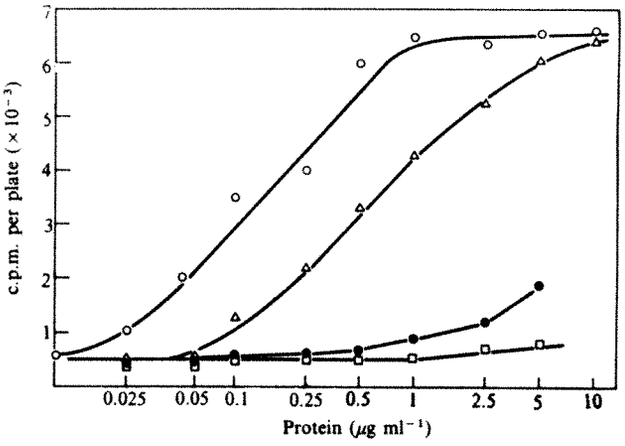


FIG. 2 DNA synthesis in 3T3 cells in response to various concentrations of diverse crude extracts. 3T3 cells were plated in DME with 10% calf serum (4×10^4 cells per 60×15 mm dish with 5 ml of medium per dish). Eight hours later the medium was removed and replaced by DME with 0.4% calf serum. Twenty-four hours later the same washing was repeated after which the cells were maintained for 3 d without changing the medium. On day 3, various concentrations of samples dissolved in the presence of 0.5% crystalline bovine albumin (to minimise nonspecific adsorption) were added to the cultures. Samples were added in fluid volumes ranging from 25 to 100 μl. Sixteen hours later, 50 μl of DME containing 10 μCi of ^{3}H-thymidine (^{3}H-methyl, NEN) and 6 μg of cold thymidine were added to each dish. After 11 h incubation at 37° C the dishes were washed once with a buffered salt solution, after which 2 ml of 0.5 N NaOH was added and the dishes were further incubated for 8 h at 37° C. DNA was then precipitated by adding trichloroacetic acid (TCA) to a final concentration of 10%. The precipitates were collected on Whatman GF/C glass fibre filters, washed twice with 10% TCA and counted (scintillation counter with toluene-PPO-POPOP solution) as radioactivity incorporated in DNA. The incorporation of ^{3}H-thymidine was 650 c.p.m. per dish in controls. All points were done in triplicate. Standard errors did not average more than 10% of the mean. ○, Brain; △, pituitary; ●, kidney; □, muscle.

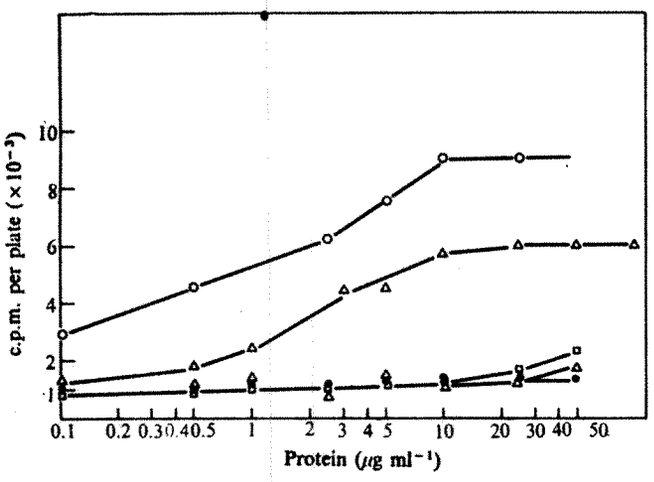


FIG. 3 DNA synthesis in C6 glial cells in the presence of various concentrations of diverse crude extracts. C6 cells were grown as described for 3T3 cells. Cells were plated in DME plus 5% calf serum (6×10^4 cells per 60×15 mm dish with 5 ml of medium per dish). Eight hours later the medium was removed and replaced with DME plus 0.2% calf serum. Twenty-four hours later the same washing was repeated after which the cells were maintained for 3 d without changing the medium. On day 3, various concentrations of samples dissolved in the presence of 0.5% crystalline bovine serum albumin (to minimise nonspecific adsorption) were added to the cultures. Samples were added in fluid volumes ranging from 25 to 100 μl. Determination of ^{3}H-thymidine incorporation was as described in Fig. 2. The incorporation of ^{3}H-thymidine was 850 c.p.m. per dish in controls. All points were done in triplicate. Standard errors did not average more than 10% of the mean. ○, Brain; △, pituitary; □, liver; ●, adrenal cortex; △, ovary.

incorporation was 25 ng ml^{-1} compared with 100 ng ml^{-1} for pituitary extract. This is a comparison of pituitary extract and extract of whole brain. If growth factor activity in the brain can be localised to some small region, the specific activity of that region will be much greater than that of the more 'dilute' whole brain preparation and, accordingly, much greater than that of the pituitary extract.

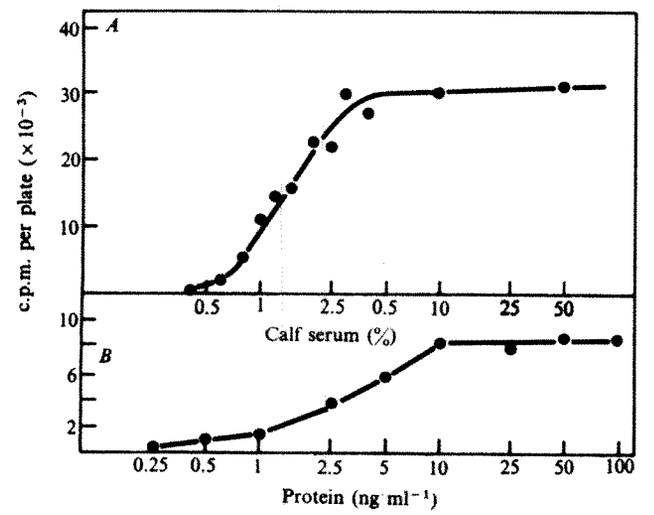


FIG. 4 DNA synthesis in 3T3 cells in the presence of serum compared with FGF. 3T3 cells were plated and maintained as described in Fig. 2 (4×10^4 cells per 60×15 mm dish). The incorporation of ^{3}H-thymidine was 600 c.p.m. per dish in controls. *A*, Effect of increasing concentrations of calf serum on the incorporation of ^{3}H-thymidine into DNA. *B*, Effect of increasing concentrations of FGF on the incorporation of ^{3}H-thymidine into DNA.

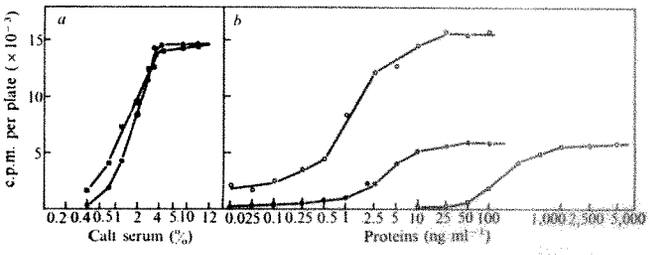


FIG. 5 DNA synthesis in 3T3 cells in the presence of serum, hydrocortisone and FGF. 3T3 cells were plated and maintained as described in Fig. 2 (2.4×10^4 cells per 60×15 mm dish). The experiment was conducted as described in Fig. 2. *a*, 3T3 cells maintained in the presence of increasing concentrations of serum (●) and serum + 0.5 μg ml^{-1} of hydrocortisone (■). The incorporation of H-thymidine was 300 c.p.m. per dish in the controls. *b*, 3T3 cells maintained in the presence of increasing concentrations of FGF in the presence (○) and absence (●) of 0.5 μg ml^{-1} of hydrocortisone. The stimulatory effect of NIH bovine LH-B-8 (bovine luteinising hormone) where the FGF is present as contaminant is shown by the lowest line. The purified FGF is about 200–500 times as potent as that preparation.

To test the ubiquity of the growth stimulating effect of brain and pituitary extracts cell lines other than 3T3 have been used. Figure 3 shows that the growth of C6 glial cells

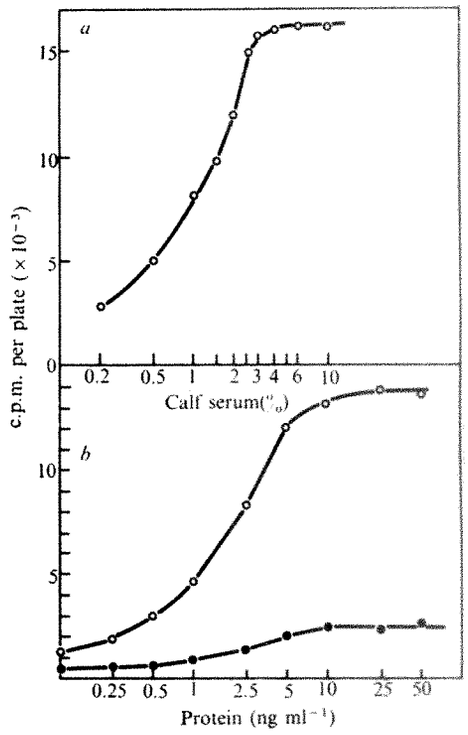


FIG. 6 DNA synthesis in 3T3 cells in the presence of either serum or FGF plus or minus hydrocortisone. 3T3 cells were plated and maintained as described in Fig. 2 (2.4×10^4 cells per 60×15 mm dish). Twenty-four hours before the addition of FGF and hydrocortisone the 0.4% calf serum medium was removed and replaced with DME containing 0.25% crystalline bovine serum albumin and 0.5 μg ml^{-1} of hydrocortisone; in some dishes, hydrocortisone was not added. Twenty-four hours later FGF and serum were added to the dishes in graded concentrations and the experiment was conducted as described in Fig. 2. The incorporation of ^{3}H-thymidine was 300 c.p.m. per dish in the controls. *a*, Effect of increasing concentrations of calf serum on the incorporation of ^{3}H-thymidine into DNA of 3T3 cells maintained in the absence of serum and in the presence of hydrocortisone. *b*, Effect of increasing concentrations of FGF on the incorporation of ^{3}H-thymidine into DNA of 3T3 cells maintained in the absence of serum and in the presence (○) or absence (●) of hydrocortisone.

TABLE 1 DNA synthesis in 3T3 cells maintained in the presence of increasing concentrations of various crude extracts

Crude extracts	Protein concentrations (ng ml^{-1})			
	100	250	1,000	5,000
Brain	4,600	8,500	10,200	9,500
Pituitary	2,500	5,100	7,600	10,200
Liver	680	1,000	1,860	3,600
Perfused liver	720	690	1,250	1,820
Cotyledon	620	650	1,200	1,150
Heart	610	670	750	1,600
Muscle	625	810	925	1,750
Ovary	650	710	1,200	1,560
Adrenal cortex	670	925	970	1,920
Adrenal medulla	580	570	420	750
Kidney	630	825	1,250	1,850

Results are expressed in c.p.m. of ^{3}H-thymidine incorporated into DNA. 3T3 cells were plated as described in Fig. 2 (32,000 cells per 60 × 15 mm dish) and ^{3}H-thymidine incorporated into DNA was assayed as described in Fig. 2. The incorporation of ^{3}H-thymidine was 630 c.p.m. per plate in the controls. All points were done in triplicate and standard errors did not average more than 10% of the mean.

is also stimulated. This cell line was chosen because the cells remain differentiated in culture, as demonstrated by the existence of a functional nor-adrenaline receptor[9] and by their secretion of S_{100} protein, a specific marker for glial cells[10]. As with 3T3 cells, C6 cells respond to crude brain and pituitary extracts by an increase in DNA synthesis. However, C6 cells are less sensitive to the extracts than are 3T3 cells. Furthermore, the maximum level of stimulation produced by brain extract is twice that produced by pituitary extract. Extracts of other tissues had no effect (Fig. 3). I now have evidence that crude extract of brain or pituitary can induce division not only of 3T3 and C6 cells but in primary cultures of glial cells as well. The growth factor activity in pituitary and brain is then not restricted to a single cell type.

Purification and activity

The purification of FGF from pituitary glands (described in full in ref. 8) is briefly as follows. Bovine pituitary glands are extracted at *p*H 4.5 with 0.15 M $(NH_4)_2SO_4$. Addition of HPO_3 to *p*H 3.0 removes an inactive fraction. FGF activity was precipitated between 2.34 and 3.8 M $(NH_4)_2SO_4$ at *p*H 7.0 and further purified by carboxymethycellulose chromatography. The activity was adsorbed from 0.01 M potassium phosphate, *p*H 5.9, and eluted with 1 M NaCl and 0.1 M potassium phosphate, *p*H 5.9. Further purification was achieved by chromatography on Sephadex G-75 and Sephadex SE C-50. The yield of purified FGF averaged 1 mg kg^{-1} of pituitary. Acrylamide gel electrophoresis at *p*H 4.5 (ref. 11) revealed a single band. Contamination by pituitary hormones was negligible.

As mentioned earlier, purification of the FGF from the brain is more difficult and has progressed more slowly. An effort is under way to purify FGF from the brain in order to determine whether or not it is identical to the FGF purified from the pituitary.

When purified FGF was added to resting 3T3 cells maintained at low serum concentration, they entered a division cycle as evidenced by an increase in cell number compared with controls, and as demonstrated by autoradiography (Fig. 1).

The initiation of DNA synthesis by 3T3 cells maintained in the presence of 0.4% calf serum in response to FGF was assayed quantitatively by measuring ^{3}H-thymidine incorporation into trichloroacetic acid-precipitable material. As Fig.

TABLE 2 Degree of stimulation or inhibition by steroids on the incorporation of ^{3}H-thymidine into DNA of 3T3 cells maintained in the presence of FGF

Steroids	Conc. (μg ml^{-1})	^{3}H-thymidine (c.p.m.)	Inhibition* (%)	Steroids	Conc. (μg ml^{-1})	^{3}H-thymidine (c.p.m.)	Inhibition (%)	Stimulation† (%)
Δ^4-Androstene-3, 17-dione	0.1	3,521	62	Δ^4-Pregnen-21-ol-3, 20-dione	0.1	13,803		149
	1.0	2,098	78		1.0	14,358		156
	10.0	30	100		10.0	2,324	75	–
Δ^5-Androstene-3β, 17β-diol	0.1	3,198	65	Δ^4-Pregnen-11β, 21-diol-3, 20-dione	0.1	15,202		163
	1.0	2,898	69		1.0	14,573		157
	10.0	1,534	84		10.0	9,098	–	–
Δ^4-Androsten 17β-ol-3-one	0.1	4,478	52	Δ^4-Pregnen-21-ol-3, 11, 20-trione	0.1	3,129	62	
	1.0	1,805	71		1.0	3,300	65	
	10.0	(-)656‡	100		10.0	(-)716	100	
Δ^5-Pregnen-3β-ol-20-one	0.1	924	90	Δ^4-Pregnen-11β, 17α, 21-triol-3, 20-dione	0.1	19,937		215
	1.0	(−)694	100		1.0	21,018		226
	10.0	(−)525	100		10.0	19,134		206
Δ^4-Pregnen-3, 20-dione	0.1	8,264	11	Dexamethasone	0.1	24,278		261
	1.0	7,008	25		1.0	24,481		263
	10.0	(−)286	100		10.0	18,985		204
17β-Oestradiol	0.1	4,088	56	FGF	50 ng ml^{-1}	9,283		
	1.0	2,097	78	Serum	8%	26,741		
	10.0	(−)680	100					

3T3 cells were plated and maintained as described in Fig. 2 (3.2 × 10^4 cells per 60 × 15 mm dish). On the day of the experiment, FGF (50 ng ml^{-1}) plus the different steroids were added to the dishes. The steroids were added in a final volume of 25 μl of ethanol. Serum was added to a final concentration of 8%. Thymidine incorporation was measured as described in Fig. 2 and the radioactivity incorporated into DNA in the presence of FGF was compared with that obtained in the presence of FGF plus steroids and in the presence of 8% serum. The incorporation of radioactivity in controls was 800 c.p.m.; this value has been deducted from the values obtained with FGF, FGF plus steroids and 8% serum.

* Inhibition values are expressed as percentage of the difference between FGF and control levels with control levels (800 c.p.m.) and below defined as 100% inhibition.

† Stimulation values are expressed as percentage of the FGF stimulation.

‡ In some cases the radioactivity incorporated into DNA when FGF plus steroids were present was less than that of controls; this is indicated by the sign (−).

4 shows, when increasing concentrations of FGF were added to resting cells they started to make DNA more actively. The minimal effective dose of FGF was about 0.1 ng ml^{-1} and plateau values were 5–10 ng ml^{-1} (Figs 4 and 5). Based on a molecular weight of 13,000 (ref. 8) this represents a concentration of 1×10^{-9}M. These concentrations are comparable with the physiological concentrations of polypeptide hormones in serum and are indicative of the potency of the FGF.

The maximal stimulation of DNA synthesis by FGF is about 30% of that observed with optimal serum concentration (Figs 4 and 5). This could indicate either that only 30% of the FGF-stimulated 3T3 cells enter the proliferative phase, or that the serum-containing medium stimulates more complete division, thus inducing additional DNA synthesis. The first hypothesis was rejected since autoradiography indicated that the entire 3T3 population was actively engaged in DNA synthesis when stimulated by FGF. The second hypothesis was explored by seeking factors which can potentiate the effect of FGF. It has been shown for many biological systems that glucocorticoids potentiate (permissive effect) the biological effect of polypeptide hormones[12]. Thrash and Cunningham have reported the stimulation of division of density inhibited fibroblasts by glucocorticoids[13] and Armelin has reported that glucocorticoids enhanced pituitary growth promoting activity[6]. These observations have led me to examine the effect of hydrocortisone and dexamethasone on FGF-stimulated DNA synthesis. Figure 5 shows that when 3T3 cells were maintained in the presence of FGF and hydrocortisone, the degree of incorporation of ^{3}H-thymidine into DNA became comparable with that found in the presence of serum. Furthermore, hydrocortisone did not potentiate the stimulatory activity of serum except when the serum was present in small concentrations. This can be interpreted as indicating that the concentration of hydrocortisone in serum is optimal to provoke maximal DNA synthesis. However, at low serum concentrations the hydrocortisone concentration is too low to be effective.

Progestogen, androgen and oestrogen were also tested for their permissive effect on the action of FGF. As Table 2 shows, androgen and oestrogen have an antagonistic effect on the incorporation of ^{3}H-thymidine into DNA. In the presence of high concentrations (10 μg ml^{-1}) incorporation could even decline to less than that observed with control 3T3 cells maintained without FGF. Progesterone in low concentrations had no marked inhibitory effect; however, when the concentration was increased to 10 μg ml^{-1}, ^{3}H-thymidine incorporation was inhibited completely. This effect of progesterone was interesting because the compound can compete for the receptor site of glucocorticoids[14].

In contrast to oestrogen, androgen and progestogen, glucocorticoids were agonistic with the exception of dehydrocortisone which was antagonistic. As expected from the previous results, dexamethasone and hydrocortisone were the most potent agonists and brought the incorporation of ^{3}H-thymidine to a level similar to that observed in the presence of high serum concentration.

If FGF and hydrocortisone are the only factors required for the control of 3T3 cell division, serum requirements for 3T3 cells can be replaced by those agents. As Fig. 6 shows, when 3T3 cells are maintained in the presence of 0.2% bovine serum albumin and hydrocortisone (0.5 μg ml^{-1}) they enter a resting stage. When FGF is added, DNA synthesis is initiated. The level of stimulation observed in the presence of an optimal concentration of FGF is comparable with that obtained with serum.

In summary, induction of 3T3 cell division requires only two agents, a polypeptide which has been purified from the pituitary gland and a glucocorticoid-hydrocortisone. Although the nature of the permissive effect of glucocorticoids on FGF activity can only be the object of speculation, glucocorticoids may be involved in the control of one or more components of the FGF effector system. An attractive possibility is that they modulate the receptor site for FGF; thus, in the absence of glucocorticoids, few or no receptor sites would be present, resulting in little activity of FGF. In their presence, the number of active sites would be induced maximally, leading to maximal activity of FGF. A similar hypothesis for the permissive effect of glucocorticoids on the lipolytic activity of luteinizing hormone[15] and thyroid stimulating hormone[16] has already been suggested.

It should be kept in mind that if glucocorticoids are needed for the induction of 3T3 cell division, that requirement does not necessarily apply to all types of cells. For L929 fibroblasts glucocorticoids are inhibitory[17].

Finally, it should be pointed out that in all the experiments only the initiation of 3T3 cell division and DNA synthesis for which I think the FGF associated with hydrocortisone is responsible have been investigated. The results do not exclude the possibility of the presence in serum of other factors which may affect cell plating, attachment and survival[18,19]. However, the acquisition of a highly purified polypeptide which triggers division of 3T3 cells *in vitro* should facilitate the study of the initial events which commit the cell to divide.

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1 VanWyk, J. S., Hall, K., Van den Brande, J. L., and Weaver, R. R., *J. clin. Endocr.*, **32**, 489 (1971).

2 Uthne, K., *Acta endocr. Copnh.*, Suppl. 173, (1973).

3 Clark, J., Jones, K., Gospodarowicz, D., and Sato, G., *Nature new Biol.*, **236**, 180 (1972).

4 Gospodarowicz, D., Jones, K., and Sato, G., *Proc. natn. Acad. Sci. U.S.A.* (in the press).

5 Corvol, M., Malemud, C. J., and Sokoloff, L., *Endocrinology*, **90**, 262 (1972).

6 Armelin, H., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2702 (1973).

7 Holley, R. W., and Kiernan, J. A., *Proc. natn. Acad. Sci. U.S.A.*, **60**, 300 (1968).

8 Gospodarowicz, D., *J. biol. Chem.* (in the press).

9 Gilman, A. G., and Nirenberg, M., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2165 (1971).

10 Pfeiffer, S. E., Herschman, H. R., Lightbody, R., and Sato, G., *J. Cell Physiol.*, **75**, 329 (1970).

11 Reisfeld, R. A., Lewis, U. J., and Williams, D. E., *Nature*, **195**, 281, (1962).

12 Jost, J. P., and Rickenberg, H. V., in *A. Rev. Biochem.*, **40**, 761 (1971).

13 Thrash, C. R., and Cunningham, D. D., *Nature*, **242**, 399, (1973).

14 Samuels, H. H., and Tomkins, G. H., *J. molec. Biol.*, **52**, 57 (1970).

15 Gospodarowicz, D., *Biochim. biophys. Acta*, **244**, 353 (1971).

16 Gospodarowicz, D., *J. biol. Chem.*, **241**, 1314 (1973).

17 Hackney, J. F., Gross, S. R., Aronow, L., and Pratt, W. B., *Molec. Pharmac.*, **6**, 500 (1970).

18 Temin, H. M., Pierson, R. W., and Dulak, N. C., in *Growth, nutrition and metabolism of cells in culture*, (edit. by Cristofalo, V. I., and Rothblat, G.,) **50** (Academic Press, New York, 1972).

19 Paul, D., Lipton, A., and Klinger, F., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 645 (1971).

Seafloor spreading theory and the odyssey of the green turtle

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Seafloor spreading theory seems to explain why the ancestors of a green turtle (Chelonia mydas) *subpopulation which now travels 2,000 km from Brazil to breed at Ascension Island, were induced to swim oceanwards for increasing distances during the gradual separation of South America and Africa in the earliest Tertiary.*

A SUBPOPULATION of the green turtle (*Chelonia mydas*) lives on the coast of Brazil but breeds and nests 2,000 km away on Ascension Island in the central equatorial Atlantic[1-6]. The feat of navigation involved in this odyssey has not been explained[5,7]. A puzzle of equal stature, and of more fundamental importance, arises in trying to account for the initial stages in the evolution of the adaptation[3,4]. Populations of the genus *Chelonia* have distinctive characteristics—enormous shoulder musculature together with its support, heavy fat deposits, and a peculiar jaw structure. The ecological regimen responsible for these characteristics is evidently the herbivorous feeding habit[1,8] and the need that this imposed for travel between a protected, shallow water pasture ground and the exposed shores on which suitable nesting beaches develop. Offshore and oceanic islands are likely to have good surf-built beaches and they almost always offer relative freedom from the nest-predators which plague mainland nesting beaches. In spite of these advantages, however, the Ascension colony has established the adaptations in the face of huge difficulties, inherent in the initial stages, which seem to make impossible demands on the process of natural selection. Seafloor spreading theory may bear directly upon this aspect, and it also offers clues to the navigation problem.

Turtles and central Atlantic opening

Marine turtles of *Chelonia* type inhabited the seas between 'North America' and northwestern Gondwanaland by the beginning of the late Cretaceous, about 100 million years (Myr) ago. Because of the cheloniid fossil record in this general area (refs 9–12 with the references provided there), we postulate that these ancient turtles included the ancestors of *Chelonia mydas*, even though its phylogeny cannot at present be reconstructed. (*Chelonia* has been found in Miocene sediments[9,10].) The northern coast of South America was a suitable habitat for these early turtles, providing a tropical to subtropical environment. There is fossil evidence that even then the herbivorous habit had evolved and that there was a separation of residence-pasture and breeding grounds[12]. Mammalian egg-predators no doubt acted as a limiting factor in turtle ecology at this time, augmenting the advantage in island nesting.

The opening of the equatorial Atlantic[13-16] (Fig. 1*a* and *b*), marking the final separation of South America and Africa at about 80 Myr ago, took place in steps: first, rift valley formation at about 110 Myr ago, or even earlier; second, sporadic but progressive ocean flooding with a strong west-to-east component by 90–80 Myr ago; and finally development of a ridge system which linked the spreading ridges of the North and South Atlantic and made of it a single ocean by 80 Myr ago.

Volcanic piles are a frequent feature of midoceanic ridges, and some may grow sufficiently to emerge as islands. As spreading

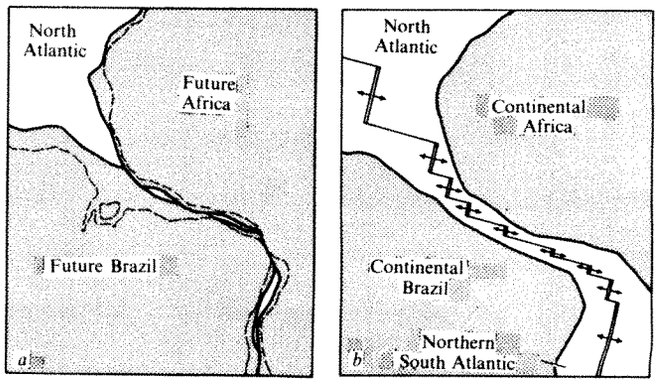


Fig. 1 *a*, Predrift reconstruction of equatorial 'South America' and 'West Africa', as parts of north-western Gondwanaland, based on the computer-tested bathymetric fit of Bullard *et al.* (ref 23). *b*, Reconstruction for the time of about 80 Myr ago. A 'Red Sea' connects the northern Atlantic with the younger southern Atlantic and its youthful spreading ridge. Spreading ridge detail is schematic.

continues, they become inactive and are carried outwards and downwards and become seamounts[17], the deepest being furthest from the ridge axis. They are sporadically replaced by new volcanoes on the ridge so that for the continental observer the volcanoes march seawards through time (Fig. 2). The ancestral turtles could thus migrate outwards with the volcanic islands, without the demands on the process of natural selection becoming excessive.

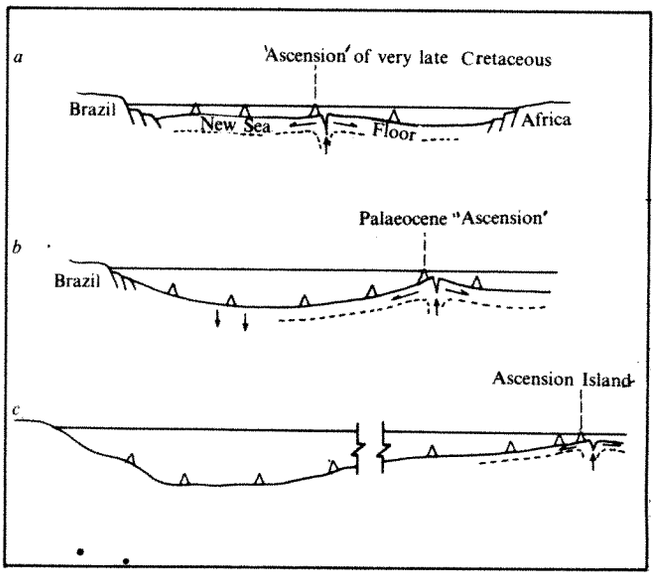


Fig. 2 The progressive appearance, then disappearance, of spreading ridge volcanoes as part of seafloor spreading mechanism. *a*, Approximately 70 Myr BP; *b*, approximately 60 Myr BP; *c*, approximately 1 Myr BP.

Turtle movement into the Brazil–West African channel

Before 100 Myr ago, turtle populations travelled between residence-pasture and breeding grounds along the shores of northern South America. By 90 Myr ago, a corridor had opened at the head of the gulf between Brazil and West Africa in line with the established longshore travel paths. By 80 Myr ago, the corridor was complete, much new coastline had been added, and there was a string of offshore islands. The exploitation of this oceanic channel by the turtles simply required repetitive extension of previous travel paths (Fig. 3). The bearings were WNW–ESE. This is close to a latitudinal course, and could be navigated relatively easily. Locating a particular beach on an island within this narrow sea or along its coasts would involve a longitudinal fix: local currents, rivers, even surfbeat, would offer individual signatures. It seems reasonable to assume that this pattern of travel, constant over a long period of time would become an established, heritable part of the turtle's behaviour.

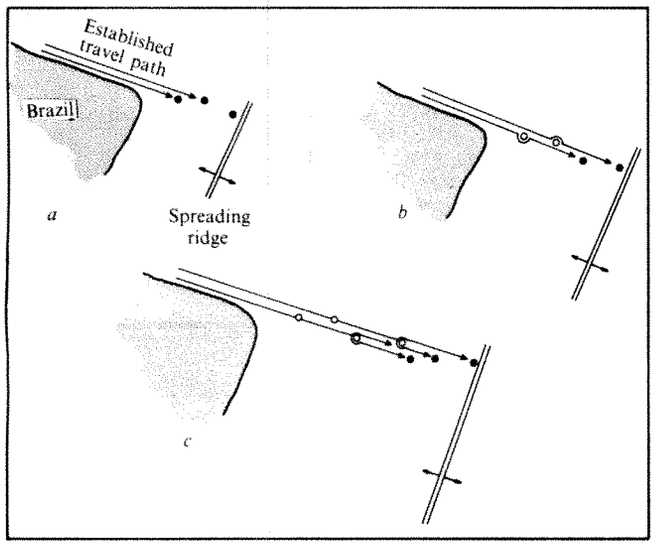


Fig. 3 As seafloor spreading proceeds, through stages *a*, *b* and *c*, new volcanic islands are generated near the ridge crest (double line). Older ones have moved out with the flanks of the ridge and gradually sink until they are submarine (open circle). As a former breeding ground becomes unusable, the turtle swims further on the same, or nearly the same, course.

By 80 Myr ago the Brazil–West Africa sea channel had connected with the northern South Atlantic and its active spreading ridge (Fig. 1*b*). The ridge volcanoes which had formed earliest, were close to those which had arisen or were still arising at the eastern end of the sea channel. For these new ridge islands to become included in the range of breeding grounds it was necessary only that some turtles extend their travel path, that is, simply stay on course, assuming either that the turtle was distracted from its prime target; or that the target had become submerged and unusable. On return trips the turtles would be guided to the final landfall by chemical cues, by surfbeat or even by direct vision. A degree of elasticity in the establishment and recognition of breeding grounds is necessary, but not much more than seems consistent with that found in the modern green turtle[18].

We envisage that by 70 Myr ago, the ancestors of the Ascension Island colony were making seaward breeding migrations of up to 300 km—a figure consistent with the spreading rate of 2 cm yr^{-1} (refs 14, 19). This process continued (Fig. 3) assuming there was sporadic creation of volcanic islands; Ascension Island is the last and youngest of them (less than 7 Myr old[19,20]). What was probably the penultimate island is now a seamount 15 km away, which is submerged to a depth of 1,500 m (ref 21).

We cannot establish the existence of a narrow swathe of seamounts connecting north-east Brazil with Ascension Island, because the detailed bathymetry is not well known. There is, however, a suggestion of a line of seamounts running south-eastwards from near Recife to about 30° W and then swinging east to Ascension (Fig. 4). This last limb is close to the trace of the Ascension Fracture Zone and its ridge[19]. The early Tertiary migration may have been controlled by volcanic islands that arose along the ridge.

Brazil to Ascension

We have argued that, since early in the Cainozoic era, the turtle has had an inherited tendency to swim a particular travel path, roughly WNW–ESE. This path can be followed by using the rising sun as a beacon to stay on latitude, an operation made simpler by the fact that the path is equatorial and the migration period is seasonal; turtles arrive at Ascension from February until May. Thus, for a December departure from the coast of northern Brazil the turtle heads directly into the rising sun (that is, ESE) and does the same every day. At night it rests below the equatorial current or even drifts eastwards with the counter-equatorial current[5]. The journey takes perhaps eight weeks. Because of the northerly drift of the sun at this time of the year, the general path followed from Brazil towards Ascension will describe a gentle arc, convex to the south, of the sort shown in Fig. 4. The required target is the intersection of the travel path with a plume of sensory clues arising from the island. Because of the equatorial current, which at this latitude has a southerly component, the stream line of solubles released from the island is favourably disposed to make the intersection and provide the turtle with a pathway to landfall; the stream line gives a longitudinal fix. The equatorial current also serves another important function, that of carrying the hatchlings, imprinted to Ascension, away from the ecologically unsuitable island[5]. The same requirements probably existed for the early Cainozoic populations, when the separation between Africa and South America was insufficient for development of an equatorial current. Reconstructions of late Cretaceous current systems[22] allow us to postulate an equally favourable smaller current system which would perform the same functions during the early stages of the migration to the islands.

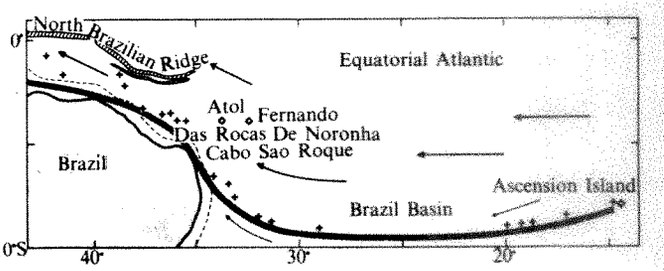


Fig. 4 The western equatorial Atlantic. The arrows indicate the generalised direction of the Equatorial Current (for the northern hemisphere summer). A possible line of seamounts (there are others) is indicated by the vertical crosses. Linking these is a hypothetical migration path (heavy pattern, still to be tested) taken by the Ascension turtles from Brazil to Ascension Island.

This navigatory scheme is simpler than that of Koch *et al.* (ref 7). In particular, it calls for a smaller plume from Ascension, in keeping with the size and nature of the island, and allows for recognition of Ascension emanations, as a new element, against the African background or 'noise' with which the equatorial current is loaded (the Congo contribution alone is massive). If the sun, in combination with chemoreceptive detection of the Ascension plume, is used as a navigational beacon, there would seem to be a corollary which can be examined: turtles from northern parts of the resident grounds would depart first and those from southern parts (south of Recif) would depart later. This is a necessary consequence of the northerly swing of the sun during the visitation period.

Inheritance of behaviour

We suggest that the process of racial learning is of the repetitive, stepping-stone type, which requires no radical change in behaviour at any point. The hypothesis puts the migration to Ascension Island in an evolutionary framework by allowing selective and adaptive processes to operate over a great period of time. Indeed, the period of time may be too long to be acceptable to some. If we are right it means that an extant species has inherited a vital behaviour pattern from ancestors that lived 40 Myr ago and more. In purely taxonomic terms these ancestral species were different, and perhaps very different, from *Chelonia mydas*. In the face of this somewhat unpalatable notion, we take comfort in the realisation that species are more than flesh and bones, especially bones. For the Ascension Island colony, we accept the seaward migration drive as an inherited feature that predates other morphological features conventionally used in systematics to define taxa at species and genus levels. We also accept the far reaching implications which lie behind this statement and its preamble, but we do not have space to discuss them here.

This article is the result, and therefore an acknowledgment, of the scholarly opportunities given to one of us (P.J.C.) during a year as Visiting Professor at the Hawaii Institute of Geophysics, University of Hawaii, where the Director, George Woollard, gave special help. A.C. received support from the National Science Foundation.

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[1] Carr, A., and Hirth, H., *Am. Mus. Novit.*, 2091 (1962).
[2] Carr, A., *Scient. Am.*, 212 (1965).
[3] Carr, A., in *Animal Orientation and Navigation* (edit. by Storm, R. M.), 35 (Proceedings of 27th Annual Biological Colloquium, Oregon State University Press, Corvallis, 1967).
[4] Carr, A., *So excellent a fishe* (Natural History Press, Doubleday, New York, 1967).
[5] Carr, A., in *Animal Orientation and Navigation* (edit. by Galler, S. R. *et al.*), 469 (National Aeronautics and Space Administration, SP-262, Washington DC, 1972).
[6] Da Costa, R. S., *Bol. Estud. Pesca (Braz.)*, **9**, 21 (1969).
[7] Koch, A. L., Carr, A., and Ehrenfeld, D. W., *J. theor. Biol.*, **22**, 163 (1969).
[8] Ferreira, M. M., *Arg. Estud. Biol. mar., Univ. Fed. Ceará*, **8**, 85 (1968).
[9] Bergounioux, F. M., in *Traité de Paléontologie* (edit. by Piveteau, J.), **5**, 487 (Masson, Paris, 1955).
[10] Hay, O. P., *Carnegie Inst., Washington, Spec. Publ.* 75 (1908).
[11] Zangerl, R., *Fieldiana: Geol. Mem.*, **3**, 279 (1960).
[12] Zangerl, R., and Sloan, R. E., *Fieldiana: Geol.*, **14** (2), 7 (1960).
[13] Dietz, R. S., and Holden, J. C., *J. geophys. Res.*, **75**, 4939 (1970).
[14] Larson, R. L., and Pitman, W. C., *Bull. geol. Soc. Am.*, **83**, 3645 (1972).
[15] Le Pichon, X., and Hayes, E. D., *J. geophys. Res.*, **76**, 6283 (1971).
[16] Maack, R., *Kontinentaldrift und Geologie des Sudatlantischen Ozeans* (Walter de Gruyter, Berlin, 1969).
[17] Menard, H. W., *J. geophys. Res.*, **74**, 4827 (1969).
[18] Carr, A., and Carr, M. H., *Ecology*, **53**, 425 (1972).
[19] van Andel, Tj. H., Rea, D. K., von Herzen, R. P., and Hoskins, H., *Bull. geol. Soc. Am.*, **84**, 1527 (1973).
[20] Bell, J. D., Atkins, F. B., Baker, P. E., and Smith, D. G. W., *Eos (Trans. Am. geophys. Un.)*, **53**, 168 (1972).
[21] Daly, R. A., *Proc. Am. Acad. Arts Sci.*, **60** (1925).
[22] Gordon, W. A., *J. Geol.*, **81**, 269 (1973).
[23] Bullard, E., Everett, J. E., and Gilbert-Smith, A., *Phil. Trans. R. Soc.*, A **1088**, 41 (1965).

LETTERS TO NATURE

PHYSICAL SCIENCES

Uranium in HR8911

In recent years there has been growing interest in analysing stellar spectra in order to observe *r*-process elements (which are synthesised by neutron capture on fast time scales). Cool Ap stars seem to be the most likely objects to display lines of such elements[1,2]. We report here preliminary results concerning some anomalies in the spectrum, which were obtained during analysis of the Cr-Eu-Sr peculiar star HR8911 (κ Piscium) and which show evidence for the presence of *r*-process elements in this object.

Four Mount Palomar spectrograms of HR8911 were measured, one of which was at 4.5 Å mm^{-1} dispersion, and the spectral lines identified from tracings obtained with a Hilger microphotometer. As only about 2/3 of the spectral lines belonged to common elements of A-type stars, we further examined the unidentified lines in the plate at higher dispersions to search for *r*-process elements. We compared the stellar wavelengths and intensities with those listed by Zeidel *et al.*[3]. It is important to stress that the following results concern a single spectrogram, whereas HR8911 is a spectrum-variable star and cursory inspection of the other plates shows considerable spectral variations. For example, in another plate lines of Fe I 43, usually strong in spectra of A-type stars, are much fainter than in the measured spectrogram.

Of the heavy elements synthesised in *r*-processes[4] the presence of osmium and uranium has been confirmed in our analysis. All unblended Os I lines with intensity greater than 50 in the intensity scale of Meggers *et al.*[5] have been found in the spectrum, together with many blended, and fainter lines, such as those observed in the spectrum of 73 Draconis[6]. Unblended Os II lines have not been observed. Almost all unblended lines of U II with intensity greater than 40 have been found in our spectrogram and the presence of many other blended lines is suspected. In addition, we remark that the two strongest U II lines (λ = 3,859.58 Å and λ = 3,854.66 Å) are unblended and clearly visible in the spectrogram.

Furthermore, there is some evidence for the presence of both neutral and singly ionised platinum in the atmosphere of this star, because the four strongest unblended lines of Pt I and some blended lines of Pt II were observed in the tracings. In addition, the strong Pt II ultraviolet line at λ = 3,551.37 Å is clearly visible in the other plates (10 Å/mm^{-1} dispersion).

Among the other *r*-process heavy elements, we found no lines of gold, mercury, lead and bismuth. There are, however, some lines coincidental with Ir I lines, the strongest at λ = 3,800.12 Å. This is unblended and clearly visible in the spectrogram.

In conclusion, it seems that HR8911 shows evidence for *r*-process elements, so it must be added to those Ap stars in which such elements have already been detected. Thus the complex problem of the origin of Ap stars requires a theory which accounts for the presence of these elements in many objects. The hypothesis of a supernova explosion of a nearby star[7] is an acceptable theory because the nucleosynthesis of the *r*-process elements requires a very high neutron density, such as that found in the interior of a highly evolved star. We notice further that in this case magnetic accretion[8], could have an important role.

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[1] Brandi, E., and Jaschek, M., *Publ. astr. Soc. Pacif.*, **82**, 847 (1970).
[2] Adelman, S., *Astrophys. J. Suppl. Ser.*, **26**, 226 (1973).
[3] Zeidel, A. N., Prokof'ev, V. K., Raiskii, S. M., Slavnyi, V. A., and Shreider, E. Ya., *Tables of spectral lines* (Plenum, New York, 1970).
[4] Seeger, P. A., Fowler, W. A., and Clayton, D.D., *Astrophys. J. Suppl. Ser.*, **11**, 121 (1965).
[5] Meggers, W. F., Corliss, C. H., and Scribner, B. F., *United States National Bureau of Standards Monograph* **32**, part 1 (US Government Printing Office, Washington, DC, 0000).
[6] Guthrie, B. N. G., *Observatory*, **89**, 224 (1969).
[7] Guthrie, B. N. G., *Astrophys. space Sci.*, **13**, 168 (1971).
[8] Havnes, O., and Conti, P. S., *Astr. Astrophys.*, **14**, 1 (1971).

Maxwellian relative energies and solar neutrinos

THE solar neutrino discrepancy is now regarded[1,2] as very serious. The general attitude is that the Sun is trying to tell us something but no one is quite sure what. I wish in this note to add to the list of possible *ad hoc* explanations; namely that the scarcity of ^{8}B neutrinos reflects a departure of the distribution of relative kinetic energies from the Maxwellian distribution. I have not been able to calculate a cause of the indicated depletion of the high energy tail of relative energies, so this explanation must be limited to the associated effect.

For an ideal gas (which the solar plasma is not) the Maxwellian distributions of single-particle energies can be shown[3] to be equivalent to a Maxwellian distribution for the motion of the centre of mass of the summed masses multiplied by a Maxwellian distribution for the relative motion of the reduced mass. Thermonuclear reactions are calculated with the last of these Maxwellian distributions. For a gas having long range interactions, such as the solar plasma, the distribution function cannot strictly be obtained in such a simple way. The particles undergo many-body collisions and the energy assignable to a particular pair of particles is not defined. A powerful theory could calculate a distribution of relative energies, but only by projecting out of the total distribution its two-particle projection. The usual argument is that the average Coulomb energy is smaller than the average thermal energy, so that an ideal gas is a good approximation.

Nonetheless we should perhaps be cautious, because the thermonuclear reactions involve improbable high energy fluctuations. For example, in a recent model[4] of the solar centre having $T_c = 14.9 \times 10^6$ K the most effective[3] energy E_o for the pp reaction is E_o (pp) = 4.58 kT, and the reaction ^{3}He (α, γ) ^{7}Be leading to production of ^{8}B involves pairs whose average relative energy is E_o (3, 4) = 17.4 kT — very far out on the tail of the distribution. The power for the Sun comes from energies of a few kT, whereas the neutrinos come from nearly 20 kT; that causes the ^{8}B neutrino flux to be strongly modified if the high energy tail of the two-particle distribution is depleted.

To illustrate these effects I have recalculated the Sun assuming all Maxwellian distributions are multiplied by the same factor $\exp f(E)$, where $f(E)$ is a slowly varying function of energy. I find that the ^{8}B flux from the Sun depends on d^2f/dE^2: if $d^2f/dE^2 < 0$, the ^{8}B flux is reduced and if $d^2f/dE^2 > 0$ it is increased. The findings are most easily discussed by expanding $f(E)$ in Taylor series as $f(E) = \beta_o - \beta_1 E - \beta_2 E^2 + \ldots$ and ignoring higher terms as having negligible importance near E_o. The constant β_o is set by requiring that the distribution function remain normalised, and is a function of β_1 and β_2.

The β_1 term has no physical effect although it might seem to have, since it does seem at first glance to reduce the high energy tail. This would be true at fixed T, but to maintain the Sun's luminosity at its known value, its central temperature would have to be increased sufficiently to counteract the effect of β_1 on the solar power. This increase in T raises the ^{8}B neutrino flux back to exactly the value it had for $\beta_1 = 0$. Thus β_1 may be ignored as having no effect. For the ^{8}B neutrinos to be reduced in a Sun of fixed luminosity, the deviation from Maxwellian must decrease faster than $\exp(-\beta_1 E)$ between E_o (pp) and E_o (3, 4); that is, d^2f/dE^2 must be negative.

Suppose then that $f(E) \approx \beta_o - \beta_2 E^2$ and imagine that β_2 is a small number so that no great violence is done to the distribution. It is convenient to think of $\beta_2 E^2$ as being $\delta(E/kT)^2$, where $\delta = \beta_2 (kT)^2$ is a smallness parameter for the deviation. My calculations show that values in the range $5 \times 10^{-3} < \delta < 10^{-2}$ are adequate to suppress the neutrino flux to Davis's limit[1]. Values of δ in this range would mean that the deficiency in the distribution function is only a few per cent at a few kT, and at $E = 10\,kT$ the rate of change of the distribution function with energy due to the β_2 term is still only 10% of the Maxwellian rate of change. These values of δ entail negligible corrections to the equation of state but large corrections to the ^{8}B neutrino flux.

Using the same[4] unperturbed model of the solar centre, I find that the ratio of PPI completions to PPII + III is

$$[\mathrm{PPI}/(\mathrm{PPII}+\mathrm{III})] \approx 10.36 \exp(117\delta) - 0.25 \qquad (1)$$

and is 10.11 for the unperturbed model. I also calculate that the ^{8}B neutrino flux stands in relation to the unperturbed flux as

$$\frac{\Phi_\nu(^8\mathrm{B}, \delta)}{\Phi_\nu(^8\mathrm{B}, \delta = 0)} \approx 10^{-100\,\delta} \qquad (2)$$

For the two values $\Phi = 5 \times 10^{-3}$ and 10^{-2}, the ^{8}B neutrino flux is reduced by factors of 3 and 10 respectively. The flux of neutrinos from ($e^- + {}^7\mathrm{Be} \rightarrow {}^7\mathrm{Li} + \nu$) is reduced by factors of 1.7 and 3.0 respectively in a straightforward application of equation (1). The ^{13}N neutrino flux, which scales as $\exp(-248\delta)$, is reduced by factors 3.5 and 12 respectively. Thus this *ad hoc* suggestion can substantially lower the neutrino detection rate even for sufficiently small value of δ that its value is otherwise almost inconsequential. The only other major consequence that comes readily to mind is that the equilibrium concentration of ^{3}He at the solar centre will have been increased by the factor $\exp(106\delta)$, which is about 2.7 at $\delta = 10^{-2}$.

These suggestions sound implausible because we are led to believe that the Maxwellian must be correct, even in a non-perfect plasma and even at the level of $\exp(-20)$; but the lack of a positive detection of solar neutrinos by Davis[1] is also implausible. I have been unable to treat the difficult aspects of many-body physics involved, so I write in the hope of more authoritative treatment from others.

I thank many colleagues who have urged me to publish this abbreviated account of a more detailed paper I circulated privately in 1971. This research was supported in part by the National Science Foundation.

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[1] Davis, R., jun. and Evans, J. C., *Proc. XIII int. Conf. Cosmic Rays* (Denver 1973).
[2] Bahcall, J. N., *Astr. J.*, **76**, 283 (1971).
[3] Clayton, D. D., *Principles of Stellar Evolution and Nucleosynthesis* chapter 4. (McGraw Hill, New York, 1968).
[4] Bahcall, J. N., Bahcall, N. A., and Ulrich, R. K., *Astrophys. J.*, **156**, 559 (1969).

Martian lee waves revisited

SEVERAL of the Mariner 9 television frames show standing-wave patterns in the clouds of the Martian atmosphere near surface features of high relief. These lee waves have been interpreted as high level condensation clouds in which the brightness variations are due to a combination of true lateral variations in the local concentration of condensed material and the shadows cast on lower levels by such scattering material. Here I demonstrate that the variation in slope of the mean scattering layer with respect to the local horizontal produces a photoclinometric brightness variation strong enough to explain the data with the invocation of quite modest relief in the cloud deck. Although some lee wave clouds in the later frames of the mission leave no doubt that they are mainly pressure trough condensations, lee wave clouds in the early dust-storm phase of the mission are probably largely the photoclinometric effect of vertical oscillations of approximately 40 m in the lines of flow past land protuberances. One interpretation of this result yields a wind velocity of 92 m s^{-1}.

I was particularly interested in a striking frame we chose for the preliminary report of the Mariner 9 mission[1]; a frame taken with the wide angle camera on orbital revolution No. 18 which showed an approximately sinusoidal brightness variation near the Martian terminator, superimposed on the broader brightness variation due to the angular progression of the Sun with respect to the local vertical. This apparent lee-wave pattern was highly periodic, with wavelength of about 40 km, and a general trend of isophotes running roughly parallel to the terminator. On Earth such patterns would be readily ascribed to the periodic genesis of clouds above the lifting-condensation-level as laminar flow lines rose and fell several times in gradually damped, near adiabatic, harmonic compressions and expansions excited by flow over a promontory of some type. Such an interpretation is readily verified for lee waves observed much later in the mission after the planet-wide dust storm abated[2].

I interpret the earlier lee wave in a different manner, in terms of the sinusoidal deformation in the laminar flow line corresponding to the mean scattering level in the top of the dust storm itself. The reason for this has nothing to do with the implausibility of the condensation cloud hypothesis, but rests on the demonstrability that the required deformation, giving rise to a corresponding brightness variation through a change of orientation of the normal vector to the cloud layer with respect to the solar direction, is extremely modest and therefore ought to be the dominant effect.

The development of inferred topography on the basis of the brightness distribution in the image of a surface exhibiting diffuse reflection, and a knowledge of the quantitative law of light scattering for the kind of surface under scrutiny, has been called photoclinometry by common agreement over the past few years. (The word 'photoclinometry' is due to J. F. McCauley from Greek roots *photos* and *clinos*.) An operational photoclinometric theory adapted to light scattering properties peculiar to the Moon was worked out by Watson[3] several years ago.

As part of the Mariner Mars programme I have been developing a generalised photoclinometric theory for application to Mars. The lee-wave deformation of the top of the dust storm is a test case for that theory, in part because the particular light scattering law is reliably known from the isophotal contours of Mars as seen by Mariner 9 before insertion into orbit[1], and also because the 'surface' in question, as an optically thick cloud top, was devoid of albedo variations. Photoclinometry can be unambiguously applied to the determination of a three-dimensional surface corresponding to a mean scattering level so long as the length over which significant optical thickness develops is small compared to the lateral distance over which significant relief develops (this is considerably less stringent a condition than that such an optically thick distance should be short compared to the actual relief itself). An analytical approximation yielding the most essential information about the relief was developed some time ago and shows what modest relief is actually implied.

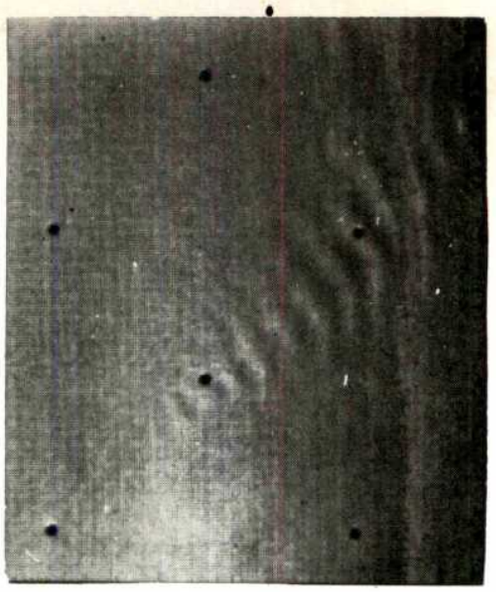


Fig. 1 Lee waves in the top of the great Martian dust storm of 1971, as photographed by Mariner 9. The gradual longitudinal dependence of scattered brightness has been approximately removed, using a computer programme developed by Pat Chavez of the USGS Flagstaff Computer Centre, leaving only the lee-wave effect which may then be greatly enhanced in contrast without partial saturation of the picture.

The planet-wide imagery showed that the planet was very nearly a Lambertian reflector under the dust conditions prevailing at the time. I shall use the Lambertian photometric function, cos i, where i is the incidence angle, in the following analysis. I shall also assume that the quantitative character of the hill and dale wrinkling in the upper dust layers of the Martian atmosphere is truly sinusoidal. Consider such ridge lines running parallel to the terminator and in a longitude interval narrow enough for the angle of the solar direction with respect to the local vertical (not surface normal) to be a constant i_o. If x is a linear distance toward the terminator, then the relative height of the mean scattering level under these assumptions is given by

$$y = A \sin (2\pi x/L)$$

where A is the vertical amplitude of the lee waves and L is the crest-to-crest separation. If i is the angle between the solar direction and the normal to the surfaces of constant dust density in the upper atmospheric layers, then

$$i = i_o + \tan^{-1} (\mathrm{d}y/\mathrm{d}x)$$

If the brightness of the surface is described by $B_o \cos i$, the following brightness variation is derived by simple trigonmetric analysis

$$B_o \cos i = \frac{B_o}{\sqrt{\left[1 + \left(\frac{\mathrm{d}y}{\mathrm{d}x}\right)^2\right]}} \left[\cos i_o - \left(\frac{\mathrm{d}y}{\mathrm{d}x}\right) \sin i_o\right]$$

Obtaining the derivative from the sine wave 'topography', then

$$B_o \cos i = \frac{B_o \cos i_o - \left(\frac{2\pi A B_o}{L}\right) \sin i_o \cos \left(\frac{2\pi x}{L}\right)}{\sqrt{\left[1 + \left(\frac{4\pi^2 A^2}{L^2}\right) \cos^2 \left(\frac{2\pi x}{L}\right)\right]}}$$

The denominator can be neglected for amplitudes up to 2.3 km. In this range the following simple result is obtained for the relation between the total relative contrast and the total relief

$$\Delta B / \langle B \rangle = 2\pi/L \, (\tan i_o) \Delta y_{max}$$

The maximum lee wave contrast is measured in Fig. 1 as 8% at a point where $i = 86°$. The refined ephemeris prediction, which would have placed this point slightly on the dark side of the terminator, was adjusted so that $i = 90°$ occurred just at

zero brightness level. All sources of error considered would probably not change the inferred relief by more than 35%, subject, of course, to the validity of the interpretation. The relief implied by the equation is 40 m.

The basic wind phenomenon at issue does not change under a photoclinometric interpretation. If meteorological parameters are to be inferred in part from image measurements, however, the effects discussed here must be dealt with. One of the simplest meteorological interpretations possible, if use is made of the insignificance of the figure of 40 m. in comparison to the length of the lee waves and the cloud height, is obtained by neglecting the crest-to-trough variations in flow velocity in the Bernouli equation, which is thereby justified. On this view the moving atmosphere can be viewed from a comoving reference frame as an adiabatic oscillator whose expansions and compressions undergo relatively little damping per cycle. The driving force is the buoyancy of the normal atmosphere for vertically displaced cells, from which an oscillator frequency is determined and thus, through the known length of the lee waves, a wind velocity of 92 m s^{-1}. This figure is in fair agreement with other lines of evidence, most notably the sizes of dust particles as inferred from scattering, cloud motions and fluid dynamical considerations, all of which suggest velocities of the order of tens of metres a second. Fuller details will appear elsewhere.

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[1] Masursky, H., *et al.*, *Science*, **175**, 294. (1972)
[2] Leovy, C. B., Briggs, G. A., Young, A. T., Smith, B. A., Pollack, J. B., Shipley, E. N., and Wildey, R. L., *Icarus*, **17**, 373 (1972).
[3] Watson, K., *U.S. Geol. Surv. Prof. Pap.* 599-*B*, B1 (1968).

Longitudinal effect in the response of total electron content to magnetic storms

IN a previous study[1] of the response of the ionospheric total electron content (TEC) data to magnetic storms, an average storm time behaviour of TEC for middle latitudes was deduced. On average TEC is enhanced during the first 24 h following the start of a storm, depressed for the next 24 h and near normal through the third 24 h. Another study[2] has shown a strong diurnal component in TEC behaviour with the maximum enhancement occurring in the dusk sector (1600 to 1800 LT). The present study of disturbed TEC behaviour generally agrees with that reported above but in addition indicates a longitudinal effect in the behaviour of both the storm time and diurnal components of disturbed TEC.

Data on TEC for 21 sudden commencement-type storms during 1969 for six viewing stations at middle latitudes are used to compute the average behaviour both in storm time and local time. No distinction is made regarding the severity of the storms used. The averages are computed as a percentage deviation of TEC from the average TEC on quiet days, calculated for each month using data from the ten magnetically quiet days of each month for each viewing station. In computing the averages the data from each storm are weighted to compensate for nonuniform distribution of storm sudden commencements in UT and are adjusted to compensate for deviation of prestorm values of TEC from the quiet day average TEC (ref. 3). I show the results of the storm time behaviour and the local time behaviour for each of these six stations.

The results of the storm time averages are presented in Fig. 1*a* for each of the viewing stations in the order of decreasing westward longitude. The usual initial enhancement in TEC is present in all the data during the first 10 to 15 h after the beginning of a storm. The feature of interest is the second enhancement in TEC which occur between 10 and 30 h after the start of a storm. This second enhancement is almost nonexistent at the easternmost station (Sagamore Hill) but it becomes more pronounced as one moves in the direction of increasing westward longitude (up in Fig. 1*a*). It actually becomes the larger of the two enhancements in TEC at the Stanford viewing station.

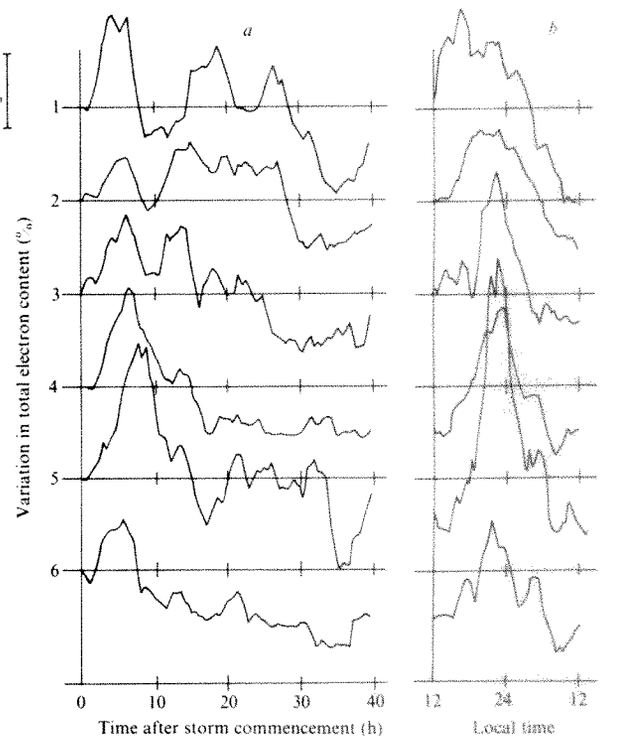


Fig. 1 *a*, Storm time averages of TEC for 21 storms during 1969 given in order of decreasing westward longitude for: 1, Cold Bay (49.8°N, 160.6°W); 2, Stanford (34.6°N, 125.1°W); 3, Fort Collins (37.0°N, 111.5°W); 4, Urbana (37.0°N, 86.6°W); 5, Rosman (32.6°N, 81.9°W); 6, Sagamore Hill (39.3°N, 71.0°W). *b*, Local time averages of TEC for 21 storms during 1969 given in order of decreasing westward longitude for the same stations.

The results of the local time averages are shown in Fig. 1*b*. All the averages show enhancements in TEC occurring during the afternoon and/or evening sectors of the first local time day of the storm. The characteristics of this enhancement also exhibit a longitudinal effect. The westernmost stations tend to have earlier starting and longer lasting afternoon and evening sector enhancements.

These two longitudinal effects are not readily explained by existing storm theories. Some longitudinal variations in the enhancements have been previously reported[4] but these were attributed to the uneven distribution of storms commencements in UT which would in turn result in a difference in the preferred local start times for each station. The weighted averaging used in this study should reduce this effect considerably.

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[1] Hibbard, F. H., and Ross, W. J., *J. geophys. Res.*, **72**, 5331 (1967).

[2] Papagiannis, M. D., Mendillo, M., and Klobuchar, J. A., *Planet. Space Sci.*, **19**, 503 (1971).
[3] Hearn, A. L., *Tech. Rept* No. 51 (University of Illinois, 1973).
[4] Jones, K. L., *J. atmos. terr. Phys.*, **33**, 379 (1971).

Strong electric fields above an ordinary rain cloud

ON September 6, 1968, in the course of a balloon flight designed to test our theory[1] that magnetospheric electric fields could be measured at balloon altitudes, we observed electric fields much larger than any we have ever observed in fair weather and which we usually attribute to thunderstorms, during the time that a rainstorm occurred under the instrumentation; however, various observations show that no lightning was being generated within any reasonable distance from our observations.

The apparatus is described in ref. 1; it is a set of three electrometers which measure the potential difference between pairs of conducting plates 2 to 3 m apart. Three orthogonal components of the electric field are measured. The apparatus also carries a magnetometer for measuring orientation and is rotated by a motor at one revolution in 6 min. Conductivity at altitude is periodically determined by momentarily shorting pairs of plates together, then allowing them to recharge through the atmosphere.

The balloon was launched at 2055 local standard time on September 5, 1968 (0255 September 6 UT) and reached its design ceiling of 91,000 feet about 1.5 h later. Usually, drifts in the electrometers are large for 0.5 to 1 h after reaching ceiling, possibly because of contamination of the atmosphere by emission of material from the balloon, but the system was quite well stabilised before the beginning of the observations reported here.

The pertinent observations are shown in Fig. 1. Beginning at 0815 UT all three components of the electric field began a marked change, increasing in magnitude to values which were too large for us to measure. During the onset of this event, at 0817 UT, the horizontal component of the field produced by the cloud (the difference from the pre-storm quiet field) was 150 mV m^{-1} at an azimuth of 30° and the vertical field was also 150 mV m^{-1} above the fair weather value. Data from times during which the conductivity was measured have been deleted from Fig. 1 except at 0945 UT where the recharging can be seen on the vertical field plot.

All components of the field remained off scale for about 1 h, except for occasional switching between the two extreme values of the horizontal measurement range caused either by rotation of the instrument or by field variations, and then

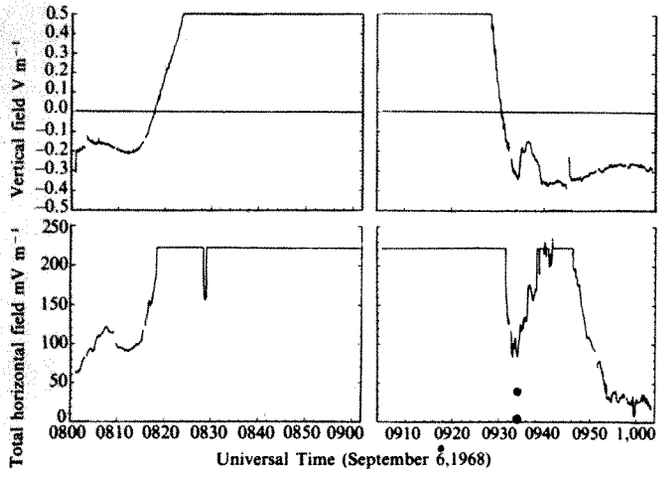


Fig. 1 Electric field variations detected by the balloon-borne instruments.

the field values returned to their 'normal' values over a period of 10 to 20 min. We believe that this phenomenon of switching establishes conclusively that the measurements are real and not the result of a failure in the instrument. Although the electrometers are mainly independent, they do have a common power supply, telemetry and logic system which conceivably might have malfunctioned. But the facts that the horizontal electrometers switch from positive off-scale to negative off-scale as the payload rotates, that they do this one at a time and not together and that the vertical instrument never switches sign, establish the effect as real. At 0932 UT, during the return to normal, the ratio of excess vertical to horizontal fields was 0.85.

The direction of the strong horizontal fields was determined at nine points in the storm, when one or the other of the horizontal axes switched between its extreme values as each of these axes became perpendicular to the fields (Fig. 2). Six of these points gave fields directed generally southwards and the remaining three points indicated westward fields. At the onset of the storm, the horizontal field pointed north, and at the end the field direction was variable, although from 0945 UT to 0951 UT there was an obvious eastward field. These measurements, as well as the brief resurgence of strong fields at the end of the storm, suggest that more than one charged region was present.

During these observations the balloon remained nearly fixed in position about 60 miles north-east of Minneapolis, at 45° 32′N, 92° 02′W. This position was within easy radar range of the US National Weather Service 10-cm wavelength

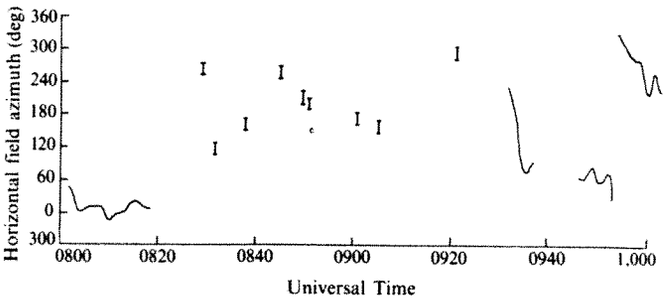


Fig. 2 Horizontal electric field direction.

radar at the Minneapolis—St Paul International Airport and the Weather Service supplied us with the results of their radar observations (Fig. 3). Neither lightning nor thunderstorms were reported within range of the radar in any direction.

Throughout the night, nonetheless, our local v.l.f. monitor recorded strong spheric activity. Observers under the balloon reported no thunder or lightning; thus we attribute the spherics to an intense thunderstorm system that was over the eastern United States at the time, received by means of the enhanced v.l.f. propagation which occurs at night.

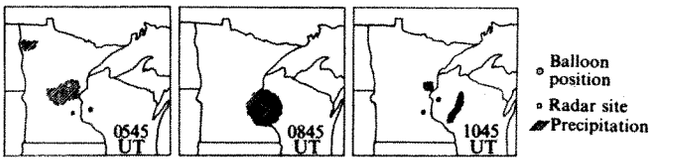


Fig. 3 Precipitation detected by 10-cm radar.

There was, however, a raining cloud some 200 km in diameter, which moved under the balloon when the large fields were observed. The cloud was visible on radar and the balloon recovery team reported rain, as did the tracking team in Minneapolis. The tops of the rainstorm clouds, as reported by radar, were at 18,000 feet. Field-mill measurements from the ground at Minneapolis indicated there was no lightning as the cloud passed overhead (G. Freier, private communication).

Our measurements are consistent with the formation of several bipolar charged regions of the cloud consisting of positive charges located above larger negative charges. The charged condition lasted for about an hour before dissipating.

Conductivity measurements near the end of the storm gave

$$4\pi\sigma = 0.90\ s^{-1}\ (e.s.u.)$$

at the balloon altitude of 27.6 km. This value is in the general range of fair weather values. Using a model of a charged cloud consisting of two unequal charges at different heights, imbedded in an atmosphere with conductivity varying exponentially with height[2-4], we find that typical horizontal and vertical field data may be fitted by an upper charge of 21 C and a lower charge of −35 C, located 50 km from the balloon. With the measured conductivity, the air-earth current due to the storm is 0.12 A.

We conclude that since we have observed strong electric fields above a cloud which did not generate lightning, there must be some process other than lightning which limits charge growth. This work has been supported by a grant from the National Aeronautics and Space Administration.

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[1] Kellogg, P. J., and Weed, M., *Planetary Electrodynamics* (edit. by Coroniti, Samuel C., and Hughes, James), **2**, 431, Gordon and Breach, Editors 1969.
[2] Holzer, R. E., and Saxon, D. W., *J. geophys. Res.*, **57**, 207 (1952).
[3] Anderson, F. J., and Freier, G., *J. geophys. Res.*, **74**, 5390 (1969).
[4] Gunn, R., *Terr. Magn. Atmos. Elec.*, **40**, 79-106 (1935).

Possible new effects in solid state physics

IT has been suggested[1,2] that the electric properties of small enough metallic particles will differ in important ways from those of the bulk material. To test such properties Kreibig and Fragstein[3] made nearly spherical silver particles of given radius R, with 30 Å $< 2R <$ 500 Å. This article is concerned with the prediction of new effects which would be observed if particles of similar size consisting of doped and possibly amorphous semiconductors could be produced. The theoretical and experimental background is discussed by Mott and Davis[4].

The simplest example of the effect to be discussed is perhaps activated hopping, for instance in an n-type compensated semiconductor. The disorder can localise the electron states and an activation energy W, provided by interaction with phonons, is necessary for transport. For strongly localised states $W \sim N(E)\ (R_D^3)^{-1}$, where $N(E)$ is the density of states and R_D is the average separation between donors. For typical specimens of interest 100 Å $< R_D <$ 400 Å (ref. 4, page 160) and it may therefore be possible to produce particles such that the particle radius $R < R_D$. In this case $W \sim R^{-3}$ independent of donor concentration. This relationship should be even more easily satisfied in the case of weak localisation, in which case $W \sim (\alpha')^3/N(E)$, where the value of α' is such that $(\alpha')^{-1} > R_D$ (ref. 4 page 41). In this again we should obtain $W \sim (N(E)R^3)^{-1}$. A possible experimental test for such effects is the alternating current conductivity (ref. 4, page 51). The hopping distance R_ω, which makes a maximum contribution at frequency ω, is given by $R = (1/2\alpha) \ln(\nu_{ph}/\omega)$ where α is of the order of the inverse Bohr radius and ν_{ph} is a phonon frequency (ref. 4, page 56). The equation $R = \frac{1}{2} \propto \ln(\nu_{ph}/\omega)$ can be used to define a critical frequency ω_c for ω, such that for $\omega < \omega_c R < R_\omega$ but for $\omega > \omega_c$ $R_\omega < R$. Thus, if $W \sim R^{-3}$ and $\omega > \omega_c$, then $\sigma(\omega) \sim R^6\omega^{0.8}$; whereas for $\omega < \omega_c$, $\sigma(\omega) \sim R^{10}\ \omega$ on the simple theory. This dependence on R and ω could be tested experimentally.

A more dramatic effect seems possible if the current theory of the metal-insulator transition in disordered systems, such as compensated semiconductors, is considered. If the mean square fluctuation of potential is denoted by U_o^2 and J denotes the bandwidth, then the equation $U_o > 5J$ is believed to be approximately correct for localisation (ref. 4, page 26). A distance a_E can be defined such that $U_o = 1/N(E)\ a_E^3$ (ref. 4, page 26). For a given energy, and $a_E > R$ this equation becomes $U_o = 1/N(E)\ R^3$. It follows that for sufficiently small particles the value of U_o will be large enough for the states to become localised so that a metal-insulator transition will occur. The critical value of R will depend on the energy but will be of the order of R_D at the centre of the band. By the same reasoning it is possible that the region of localised states at the edge of the conduction band in amorphous semiconductors will extend over a greater region of energy in small particles.

I thank N. C. Wickramasinghe for pointing out the importance of studying the properties of small particles.

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[1] Frohlich, H., *Physica's Grav.*, **6**, 406 (1937).
[2] Kubo, R., *J. phys. Soc. Japan*, **17**, 975 (1962).
[3] Kreibig, U., and Fragstein, C. V., *Z. Phys.*, **224**, 307 (1969).
[4] Mott, N. F., and Davis, E. A., *Electronic Processes in Non-Crystalline Materials* (Clarendon Press, Oxford, 1971).

Possible origin of Prandtl's mixing-length theory

PRANDTL'S hypothesis[1,2] about turbulent motion in a simple shear layer proposes that the typical values of the fluctuating velocity components in the x and y directions, u and v, are each proportional to $l\delta U/\delta y$ where l is the mixing length (*Mischungsweg*). Prandtl[2,3] says that l "may be considered as the diameter of the masses of fluid moving as a whole in each individual case; or again, as the distance traversed by a mass of this type before it becomes blended in with neighbouring masses. . . ."; and also that l is "somewhat similar, as regards effect, to the mean free path in the kinetic theory of gases". It follows that the Reynolds shear stress $-\rho_{uv}$ is proportional to

$$\rho l^2(\delta U/\delta y)^2$$

and l is defined so that the constant of proportionality is unity. Prandtl, however, described this expression as "only a rough approximation".

Later work (ref. 4, and see ref. 5 for a summary) has shown that the formula for the mixing-length can be derived, using dimensional analysis, by assuming 'local equilibrium' between the turbulence and the mean flow. The only known example of a region of local equilibrium is the wall layer with $l = Ky$: experimentally $K \approx 0.41$. In a self-preserving (self-similar) flow with a single length scale δ, dimensional analysis gives $l/\delta = f(y/\delta)$. These results of dimensional analysis apply to very restricted cases (which were, however, used historically to test the mixing-length theory) and they have no relation to Prandtl's original derivation. In more general cases the quantity l defined by Prandtl has no simple connection with any meaningful scale of the mean flow or the turbulence.

Almost any method of flow visualisation contradicts Prandtl's idea of momentum exchange via lumps of fluid ('*Flüssigkeitsballen*'). Turbulence is an assembly of tangled vortex lines and sheets, dominated by the essentially three-dimensional phenomenon of vortex stretching. If a lump of fluid happens to move away from its former surroundings as an entity, it interacts strongly with those surroundings by the interconnecting vortex lines.

It seems that Prandtl could reconcile his idea with the results of flow visualisation because the predominant method of flow visualisation used in the early years[2,6] of Prandtl's Institute at Göttingen, during the development of the mixing length theory, was the Ahlborn method—the observation of

particles sprinkled on the free surface of a water flow. Many papers and books in which appear photographs taken at Gottingen show that this method of visualisation is very effective in showing up vorticity normal to the free surface. Indeed, because the free surface acts as a plane of symmetry the vorticity vector is constrained to meet the free surface at right angles. The constraint clearly influences a given eddy to a depth below the surface of the order of the eddy size. It therefore affects all sizes of eddy and not merely the smallest, which contribute most to the vorticity. The three-dimensional character of the turbulence is destroyed near the free surface, leaving only two-dimensional circulations like those seen in, for example, Fig. 36 of ref. 7 (which are similar to the two-dimensional circulations of synoptic scale in the atmosphere, seen in satellite photographs of cloud patterns). The photographs of turbulent channel flow reproduced as Figs 39 and 40 of ref. 7 also show exaggerated two-dimensional circulations, quite unlike our modern concept of turbulent motion but amply justifying the name *Flüssigkeitsballen*: it must surely have been photographs like these that provided the inspiration for Prandtl's mixing-length theory of 1925. In 1934 Prandtl and Tietjens (ref. 7, art. 155) commented that "In the case of a three-dimensional fluid motion, it is necessary to study the flow not only on the surface but also in the interior of the fluid". It is, however clear from their inclusion of free-surface visualisations of turbulence that they did not fully appreciate the untypical nature of the motion in the plane of symmetry formed by the free surface.

It seems very likely that the mixing length concept is a good model for two-dimensional eddying motions like synoptic-scale atmospheric circulations: it should also be applicable to highly stable stratified flows in which vertical motion is suppressed.

These remarks are offered not as a criticism of Prandtl but rather to exonerate him from the criticism that the mixing length concept ignores the facts: it is an excellent description of the flow patterns seen on a free surface. Unfortunately, however, these patterns are not representative of turbulence in the interior of a fluid.

I thank Professors G. K. Batchelor and R. E. Luxton for their suggestions and discussions.

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[1] Prandtl, L., *Z. angew. Math. Mech.*, **5**, 136–139 (1925).
[2] Tollmien, W., Schlichting, H., and Görtler, H., *Ludwig Prandtl Gesammelte Abhandlungen* (edited by Springer, Berlin, 1961).
[3] Prandtl, L., *Proc. second int. Congr. appl. Mech., Zurich* (1926); translation quoted from Aeronautical Research Council paper, Ae. Techl. 341 (1927).
[4] Batchelor, G. K., *J. aeronaut. Sci.*, **17**, 441–445 (1950).
[5] Bradshaw, P., *Aeronaut. J.*, **76**, 403–416 (1972).
[6] Prandtl, L., *Jl. R. aeronaut. Soc.*, **31**, 720–741 (1927).
[7] Prandtl, L., and Tietjens, O., *Applied Hydro-and Aero-mechanics* (McGraw-Hill, New York, 1934).

Field evidence relating to the origin of ~ 3,000 Myr gneisses in southern West Greenland

SOME prominence has been given recently to the results and implications of isotope age studies[1–4] in the early Precambrian rocks of the Godthåb region, southern West Greenland. The studies were initiated after field investigations[5] suggested that the Godthåbsfjord region may contain outcrops of the oldest rocks in the earth's crust. McGregor[5] has grouped these rocks into an older and a younger set of gneisses (respectively the Amîtsoq and Nûk gneisses) separated in time by dykes (the Ameralik dykes), metavolcanic and metasedimentary rocks (the Malene supracrustals) and stratiform meta-anorthosites. Among conclusions drawn from the isotope work is that based on initial ratios of $^{87}Sr/^{86}Sr$ that the parents of the Nûk gneisses in the Godthåbsfjord area cannot have been derived by partial or complete melting of the older Amîtsoq gneisses[3]. We wish to comment on the applicability of this conclusion to the Nûk gneisses mapped in the area immediately to the south.

A team from the University of Exeter, supported by the Geological Survey of Greenland and the Natural Environment Research Council, began work in 1972 on the Buksefjorden map sheet (63 V 1, Danish Geodetic Institute), which overlaps the south of the Godthåbsfjord region, (ref. 5, Fig. 17) and the summer of 1973 has seen the completion of 1:20,000 mapping of a coastal area about 15 km wide extending more than 60 km south from Ameralik to Sermilik[6,7] (Fig. 1). Lithological units recognised by McGregor[5] in the Godthåbsfjord region extend south of Buksefjorden and we agree broadly, but not in detail, with the interpretation of the sequence of events in the evolution of the Archaean rocks of West Greenland listed in the most recent publication concerning isotopic ages[4]. It is pertinent to point out that we cannot accept McGregor's terminology[5] in its entirety.

Discordant, but intensely deformed, amphibolitic Ameralik dykes occur in leucocratic Amîtsoq gneisses at granulite and high amphibolite facies on the northeast of Amitsuarssugssuaq and at the entrance to Alangordlia (Fig. 1). The Amîtsoq gneisses and Malene amphibolites in Amitsuarssugssuaq are both injected and variably migmatised by granitic (s.l.) masses and gneisses which in the sense of McGregor are Nûk gneisses. The effect of this injection and migmatisation on the Ameralik dykes is to bring about a progressive loss of coherence until the dykes become strongly attenuated wisps which locally have recrystallised to coarse hornblendites. The wisps ultimately become assimilated by the younger gneisses. The Amîtsoq

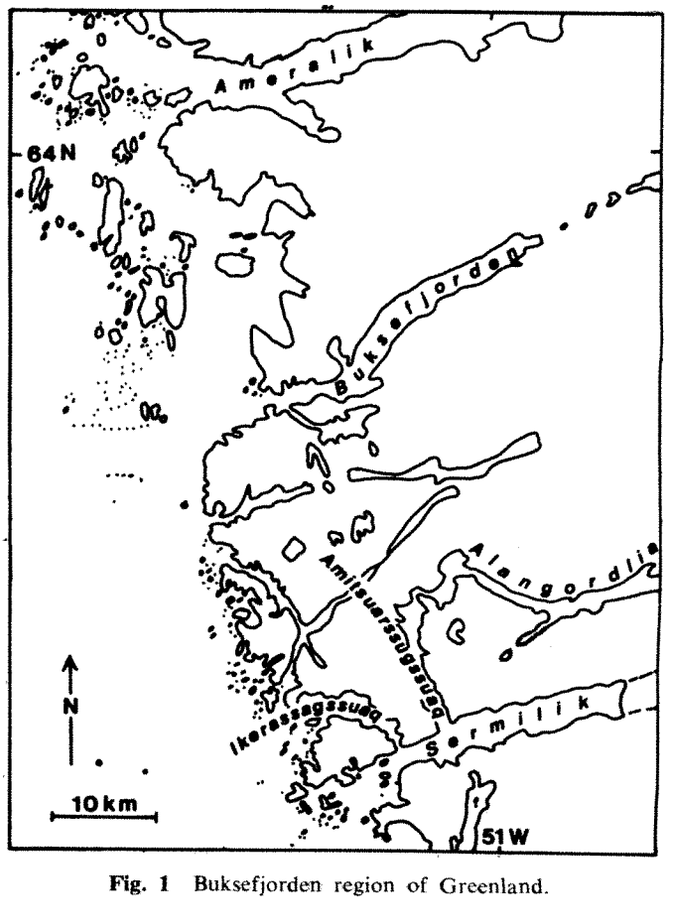


Fig. 1 Buksefjorden region of Greenland.

gneiss host to the dykes is also similarly reduced to xenolithic wisps and schlieren which merge completely into the younger gneiss. Similar effects are visible in coast outcrops around Ikerasagssuaq (Fig. 1). North of this inlet and around Amitsuarssugssuaq, the Amîtsoq gneisses, which for the most part are heavily migmatised with only rare Ameralik dykes, form linear strips within the Nûk gneisses, a relationship which suggests they have peeled off larger areas and are in an interrupted stage of incorporation into newer gneiss. Injected Nûk gneiss also occurs throughout the Buksefjorden region mapped so far. Original igneous textures survive only rarely: a hornblende granodiorite, for example, injected along a boundary between Amîtsoq gneiss and Malene amphibolite in east Amitsuarssugssuaq, retains igneous textures in the north but becomes gneissose with acid ptygma in the south. Much of the Nûk gneiss elsewhere in the Buksefjorden region contains enclaves of Amîtsoq gneiss (some clearly xenolithic) in various states of preservation. Modifications of the younger gneiss and its inclusions to new, banded gneisses have taken place in association with straight belt formation, for example in Amitsuarssugssuaq. This we regard as synchronous with the granulite facies metamorphism which has given a $^{207}Pb/^{206}Pb$ isochron of 2,850±100 Myr. (ref. 4).

The field evidence in the Buksefjorden region shows irrefutably that Nûk gneisses have been derived not only from injected material, but also by substantial remobilisation and incorporation of Amîtsoq gneiss. Our evidence is thus at variance with the conclusions drawn from isotope studies in the Godthåbsfjord region by Pankhurst *et al.*[3], that the Nûk gneisses "cannot have been derived by partial or complete melting of older rocks similar to the Amîtsoq gneisses." It has also been pointed out[4], that Sr and Pb isotope evidence suggests the ~ 2,800 Myr. granulite terrane in West Greenland does not represent a significant reworking of older crust (>100–200Myr.) such as the ~ 3,700 Myr. old gneisses of the Godthåb area, and they conclude that a significant addition of crustal material from an upper mantle source must have taken place in West Greenland between ~ 3,000–2,800 Myr. ago. The fact that Amîtsoq gneisses are common in the granulite facies terrane seen so far in Alangordlia and Sermilik (Fig. 1) does not in our view support this conclusion and we suggest, on the basis of field evidence for the late Archaean tectonometamorphic evolution of the Buksefjorden region to be published elsewhere, that events associated with the granulite facies metamorphism and later intrusion of the Qorqût granite suite[5] and related rocks may mark a stage as significant as the Nûk invasion postulated by McGregor in the addition of mantle-derived material to the Precambrian crust in West Greenland.

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1 Black, L. P., Gale, N. H., Moorbath, S., Pankhurst, R. J., and McGregor, V. R., *Earth planet. Sci. Lett.*, **12**, 245 (1971).
2 Moorbath, S., O'Nions, R. K., Pankhurst, R. J., Gale, N. H., and McGregor, V. R., *Nature phys. Sci.*, **240**, 78 (1972).
3 Pankhurst, R. J., Moorbath, S., and McGregor, V. R., *Nature*, **243**, 24 (1973).
4 Black, L. P., Moorbath, S., Pankhurst, R. J., and Windley, B. F., *Nature phys. Sci.*, **244**, 50 (1973).
5 McGregor, V. R., *Phil. Trans. R. Soc.*, **A/273**, 343 (1973).
6 Chadwick, B., and Coe, K., *Rapp. Greenland geol. Surv.*, **55**, 32 (1973).
7 Chadwick, B., Coe, K., Gibbs, A. D., Sharpe, M. R., and Wells, P. R. A., *Rapp. Greenland geol. Surv.* (in the press).

Hardening of tungsten carbide by irradiation

We report here a remarkable degree of hardening produced in standard tool-making grades of sintered tungsten carbide in a cobalt matrix by irradiation with charged particles.

Flat, rectangular blocks of three different grades (Wimet H, N and CXT) were bombarded with up to 5 $\mu A\ cm^{-2}$ of 32-MeV ^3He ions in the Birmingham University 150-cm cyclotron. Each block was partially screened by thick aluminium plate which could be moved to expose increasing areas at intervals of 15 min. Each sample was thus divided into 10 separate areas which had been bombarded for periods of 0 to 135 min. The maximum dose given was therefore 1.6×10^{17} ^3He ions cm^{-2} or 3×10^{11} rad. The ions penetrated to a depth of about 140 μm. The temperature of the block did not rise above 400° C during bombardment.

The hardness of each area after bombardment was measured using a Vickers Hardness testing machine, with a pyramidal diamond indentor, a load of 20 kg, and a 17-mm objective lens for measurement of the indentation.

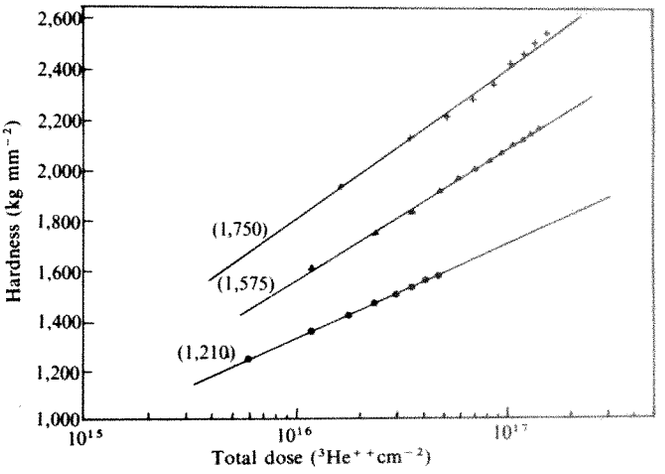


Fig. 1 Variation of Vickers hardness of three grades of Wickman Wimet tungsten carbide with bombardment of 32-MeV ^3He ions. The bracketed figures at the base of each line show the hardness of the unirradiated part of the sample. +, grade H; ▲, grade N; ●, grade CXT (Wickman Wimet grade).

The results are shown in Fig. 1. In the range within which measurements were made the hardness is a logarithmic function of dose, and about 30% increase in hardness was given to the hardest material by a dose of the order of 10^{17} ions cm^{-2}. At this density some 50 or so ions will pass through each atom of tungsten or of cobalt in the surface of the block and extensive damage to the crystal matrix of either material can be expected.

Further details and a more extensive series of measurements, together with measurements of other properties of the irradiated material will be published elsewhere.

We thank Dr E. M. Trent for suggesting the measurement of hardness as an indication of the changes produced by bombardment and for permission to use the necessary equipment; and Wickman Wimet Ltd for providing the samples.

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BIOLOGICAL SCIENCES

Activation of maternal mRNA in the absence of poly(A) formation in fertilised sea urchin eggs

THE protein synthesis which follows fertilisation of sea urchin eggs depends largely on genetic templates synthesised much earlier during oogenesis[1]. In the first 2 h after fertilisation the amount of protein being made increases steadily as the preformed mRNA molecules gradually become available for translation[2,3]. During this period of mRNA activation, the polyadenylic acid content of the embryo increases to more than twice the level present in unfertilised eggs[4–6]. This fertilisation-stimulated polyadenylation occurs in the cytoplasm on RNA stored in the egg[6] and is essentially complete by the time of the second cleavage. Soon after fertilisation the poly(A)-containing RNA moves from the post-ribosomal supernatant to the ribosomal-polysomal fraction of embryos[5,6], which suggests that polyadenylation is important in the activation of stored mRNA molecules. This is consistent with other reports that these tracts may play a role in the translation of viral RNAs[7,8] and eukaryotic mRNAs[9,10]. We have therefore investigated whether the formation of poly(A) after fertilisation is necessary for the activation of maternal mRNA. We measured the increase in protein synthesis and active mRNA while poly(A) synthesis was repressed and found a substantial activation even when poly(A) formation could not occur.

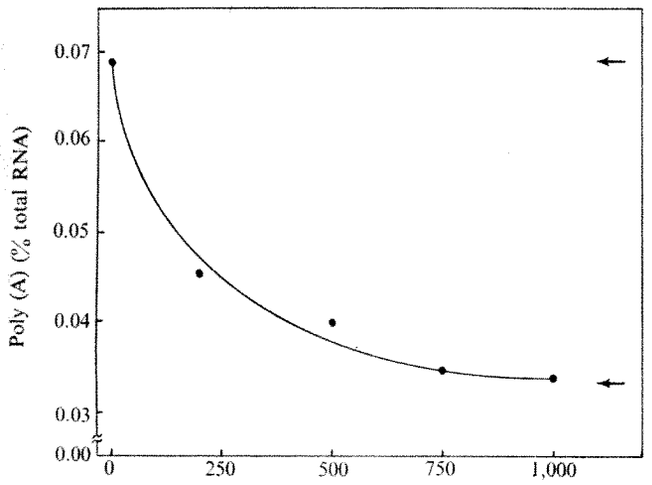


FIG. 1 Inhibition of polyadenylation by 3′-deoxyadenosine. Eggs were collected and washed by conventional procedures and maintained for 1 h on a rotary shaker at 21° C (ref. 2) in artificial seawater (ASW) containing 3′-deoxyadenosine (Cordycepin, Sigma) at the concentration indicated. The eggs were then inseminated with a 1:5,000 final dilution of sperm (95% fertilisation or greater was acceptable) and the embryos were allowed to develop for 2 h, at which time they were in the four-cell stage. Unfertilised eggs and embryos were washed once in cold acid seawater[3], once in a 0.1M Tris-HCl buffer, *p*H 9.0, containing 15% sucrose, and homogenised in 10 volumes of 0.1M Tris-HCl, *p*H 9.0, made 0.5% with sodium dodecyl sulphate[13]. RNA was extracted from the homogenates with phenol and chloroform in a method essentially the same as that used by Slater *et al.*[4] The ethanol-precipitated RNA, redissolved in hybridisation buffer, was washed twice with ether before being measured by $A_{260\ nm}$. The poly(A) content of the RNA was assayed according to the ^{3}H-poly(U) hybridisation technique developed by Gillespie *et al.*[11] and applied successfully to sea urchin RNA by Slater, *et al.*[4] Excellent results were obtained using 10 μg sea urchin RNA per aliquot hybridised with 0.8 μg of commercially prepared ^{3}H-poly(U) (Schwarz/Mann), specific activity 23.6 Ci mol^{-1}. The upper and lower arrows indicate the poly(A) content of normal 2 h embryos and unfertilised eggs respectively.

TABLE 1 Poly(A) assay of *Arbacia* eggs and embryos

RNA	ng poly(U) hybridised per μg RNA	poly(A) (% total RNA)
Unfertilised eggs	0.357 ± 0.041 (8)	0.033
Four-cell embryos	0.739 ± 0.283 (7)	0.069
Four-cell embryos in 3′-deoxyadenosine	0.364 ± 0.054 (3)	0.034
Four-cell embryos in adenosine	0.725 (1)	0.068

The concentration of nucleosides was 1 mg ml^{-1} artificial seawater. Cells were treated and poly(A) content assayed as in Fig. 1. The numbers in parentheses indicate the number of experiments performed.

We assayed the poly(A) in *Arbacia punctulata* using the ^{3}H-polyuridylic acid hybridisation technique of Gillespie *et al.*[11] and observed as reported for other species a doubling of poly(A) between fertilisation and 2 h, when the embryos are in the four-cell stage[4,6]. Further increase was not observed for several hours. Poly(A) formation was inhibited by 3′-deoxyadenosine (Cordycepin), which blocks polyadenylation[4,10,12] by terminating chain elongation. Sea urchin eggs were maintained in artificial seawater containing various concentrations of the adenosine analogue for 1 h, inseminated, allowed to develop in the continuous presence of the drug for 2 h, and assayed for poly(A) content. Increasing concentrations of the drug were found to inhibit the poly(A) formation progressively (Fig. 1), but extensive inhibition required relatively high concentrations. In seawater containing 200 μg 3′-deoxyadenosine ml^{-1}, 67% of the postfertilisation polyadenylation was suppressed; 750 μg ml^{-1} prevented 96% of the increase in poly(A). Similar large doses of adenosine did not affect poly(A) titres (Table 1). Embryos allowed to continue growth in any concentration of 3′-deoxyadenosine up to 1 mg ml^{-1}, although delayed 30–60 min by late cleavage stages, did not arrest until blastula stage. Developmental arrest at this stage is expected since mRNAs transcribed during cleavage seem to be required for gastrulation and morphogenesis[1] and 3′-deoxyadenosine disrupts mRNA formation[12]. It is surprising that such massive doses of the drug did not produce more general toxic effects.

Embryos in which the post-fertilisation addition of poly(A) to the maternal mRNA had been suppressed were tested for their ability to synthesise proteins. After 2 h of development in the inhibitor, they were incubated for

TABLE 2 Protein synthesis in poly(A)-inhibited embryos and controls

Cells	Total incorporation (%)	Incorporation into nascent protein (%)
Unfertilised eggs	6.8 ± 0.03	1.7
Four-cell embryos	31.1 ± 2.9	8.6
Embryos in 3′-deoxyadenosine, 200 μg ml^{-1}	31.9 ± 1.3	9.1
Embryos in 3′-deoxyadenosine, 750 μg ml^{-1}	25.9 ± 2.8	7.4

Cultures of 3 × 10^5 embryos were treated with 3′-deoxyadenosine at the concentrations indicated as described for Fig. 1. Two hours after fertilisation, embryos were incubated for 5 min with 5 μCi ^{3}H-leucine (30 Ci mmol^{-1}). Total soluble radioactivity and radioactivity incorporated into protein was determined by methods described previously[2]. The standard deviation indicated is from three experiments in each case. Incorporation into nascent proteins was determined by preparing polysomes[2,3] from embryos given ^{3}H-leucine as just mentioned and dividing the total radioactivity in the polysome portion of the gradient by the total soluble radioactivity in the sample layered onto the sucrose gradient.

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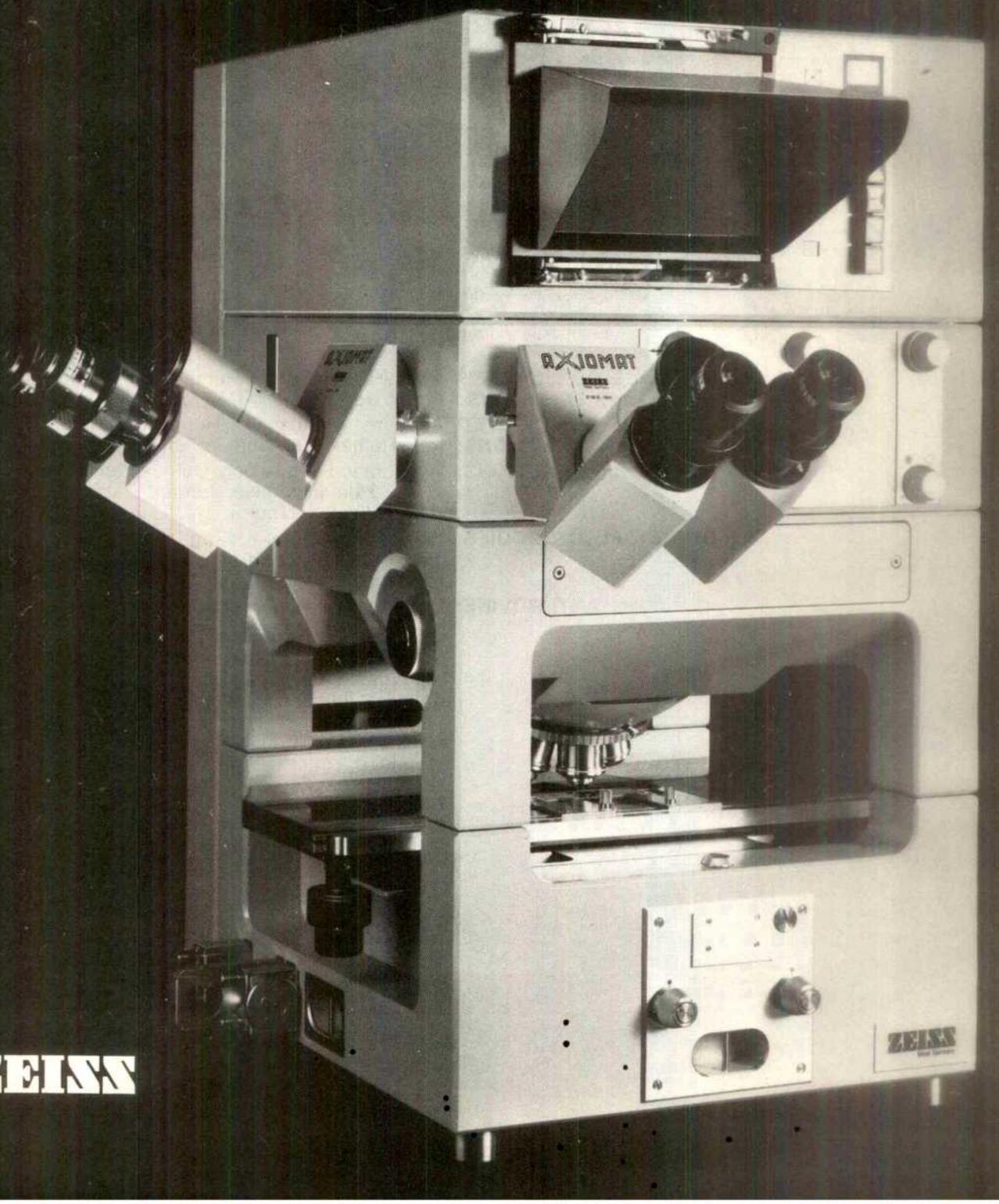
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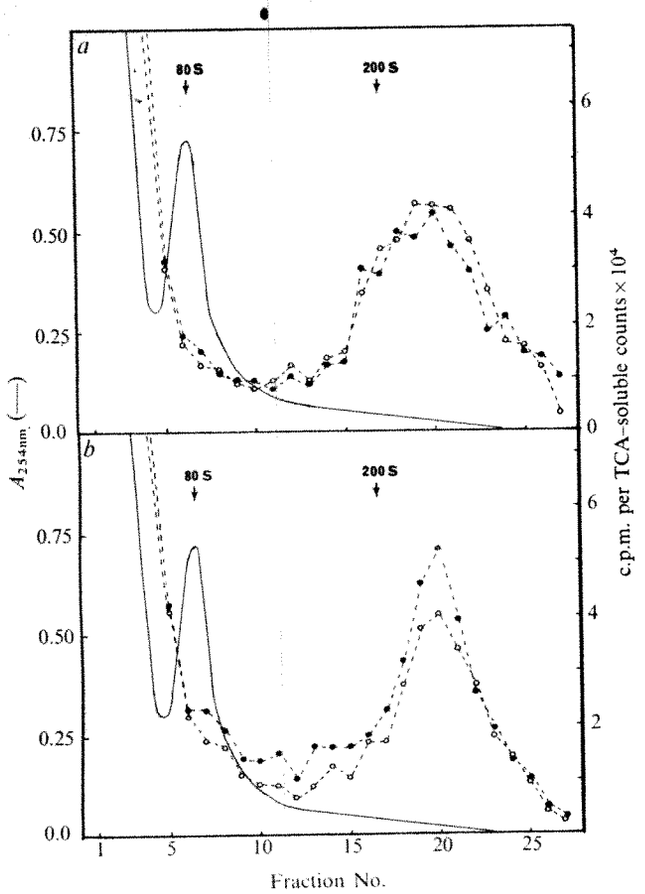


FIG. 2 Comparison of polysomes from normal embryos and embryos grown in 3′-deoxyadenosine at 200 μg ml^{-1} (*a*) and 750 μg ml^{-1} (*b*). A culture of 3 × 10^5 embryos was treated with the adenosine analogue as described in Fig. 1. At 2 h of development, the treated embryos and an equal number of control embryos were incubated for 5 min with 5 μCi ^{3}H-leucine (30 Ci mmol^{-1}). Polysomes were prepared from each culture with a high salt buffer as previously described[2,3] and centrifuged on 30 ml, 10–35% sucrose gradients for 3 h at 22,000 r.p.m. in a Beckman SW25 rotor (5° C). ●, Control embryos; ○, treated embryos.

5 min with ^{3}H-leucine and the rate of protein synthesis was estimated as the percentage of the soluble radioactive pool which had been incorporated into trichloroacetic acid-precipitable protein. This estimate depends on the assumption that the inhibitor does not alter the size of the pool. As Table 2 shows, incorporation at 2 h of development was completely insensitive to 200 μg ml^{-1} 3′-deoxyadenosine, which inhibits two-thirds of the normal poly(A) increase (Fig. 1). Increasing the level of drug to 750 μg ml^{-1}, to produce complete inhibition of the postfertilisation polyadenylation, had only a small effect on incorporation. It seems that protein synthesis can increase after fertilisation even without poly(A) formation.

The activation of protein synthesis when fertilisation takes place involves the translation of additional mRNA, which associates with ribosomes to increase the number of polysomes[3]. The ability of mRNA to enter the polysomes in sea urchins lacking the fertilisation-stimulated poly(A) sequences was investigated by preparing polysomes from the 3′-deoxyadenosine-treated embryos. Polysomes were measured as the percentage of the total soluble radioactive leucine incorporated into nascent proteins during a 5 min incubation with ^{3}H-leucine[2]. There is no diminution in size or number of polysomes from embryos in the adenosine analogue at a concentration of 200 μg ml^{-1}, as compared with polysomes from untreated embryos (Fig. 2*a*). When the dosage was increased to 750 μg ml^{-1} to eliminate all poly(A) formation, polysome size was not affected, although a slight decrease in number of polysomes was apparent in embryos grown in this high level of inhibitor (Fig. 2*b*). The quantitative results of these measurements of nascent proteins on the polysomes (Table 2) confirm the relative insensitivity of the activation of maternal mRNA to suppression of polyadenylation.

Polysomes from 2 h embryos apparently normally contain mRNA which has been polyadenylated as a result of fertilisation[5,6]. Our study suggests however that there is no direct correlation between the addition of these sequences and the ability of the mRNA to form polysomes. The finding that the postfertilisation synthesis of poly(A) is superfluous to the normal activation of maternal mRNA, coupled with the fact that it occurs in the cytoplasm[4,6], raises again the question of a role for poly(A) on mRNA. Evidence is accumulating[5–8,14] that the result of polyadenylation may primarily be the conferral of a higher degree of stability to mRNA during its intracellular transport and translation. The findings presented here are not incompatible with the idea that maternal RNA templates which have been polyadenylated are more stable than those without poly(A).

This study was performed in the Fertilization and Gamete Physiology Training Program at the Marine Biological Laboratory, Woods Hole, supported by a grant from the National Institutes of Health.

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1 Gross, P. R., in *Current Topics in Developmental Biology* (edit. by Moscona, A. A., and Monroy, A.), **2**, 1 (Academic Press, New York, 1967).
2 Humphreys, T., *Devl. Biol.*, **20**, 435 (1969).
3 Humphreys, T., *Devl. Biol.*, **26**, 201 (1971).
4 Slater, D. W., Slater, I., and Gillespie, D., *Nature*, **240**, 333 (1972).
5 Slater, I., Gillespie, D., and Slater, D. W., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 406 (1973)
6 Wilt, F., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2345 (1973).
7 Johnston, R. E., and Bose, H. R., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1514 (1972).
8 Armstrong, J. A., Edmonds, M., Nakazato, H., Phillips, B. A., and Vaughan, M. H., *Science*, **176**, 526 (1972).
9 Darnell, J. E., Wall, R., and Tushinski, R. J., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1321 (1971).
10 Mendecki, J., Lee, S. Y., and Brawerman, G., *Biochemistry*, **11**, 792 (1972).
11 Gillespie, D., Marshall, S., and Gallo, R. C., *Nature new Biol.*, **236**, 227 (1972).
12 Darnell, J. E., Philipson, L., Wall, R., and Adesnik, M., *Science*, **174**, 507 (1971).
13 Lee, S. Y., Mendecki, J., and Brawerman, G., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1331 (1971).
14 Perry, R. P., Greenberg, J. R., Kelley, D. E., LaTorre, J., and Schochetman, G., in *Gene expression and its regulation* (edit. by Kenney, F. T., *et al.*), 149 (Plenum, New York, 1973).

Triiodothyronine influences nuclear and extra-nuclear DNA in cultured human kidney cells

THE T-1 cell line, derived from normal human kidney epithelial cells and established over 15 yr ago by van der Veen *et al.*[1], has been found sensitive to and specific for the two thyroidal hormones, thyroxine (T_4) and triiodothyronine (T_3), as manifested by parameters reflecting morphology, proliferation, colony formation, and metabolism of lipids,

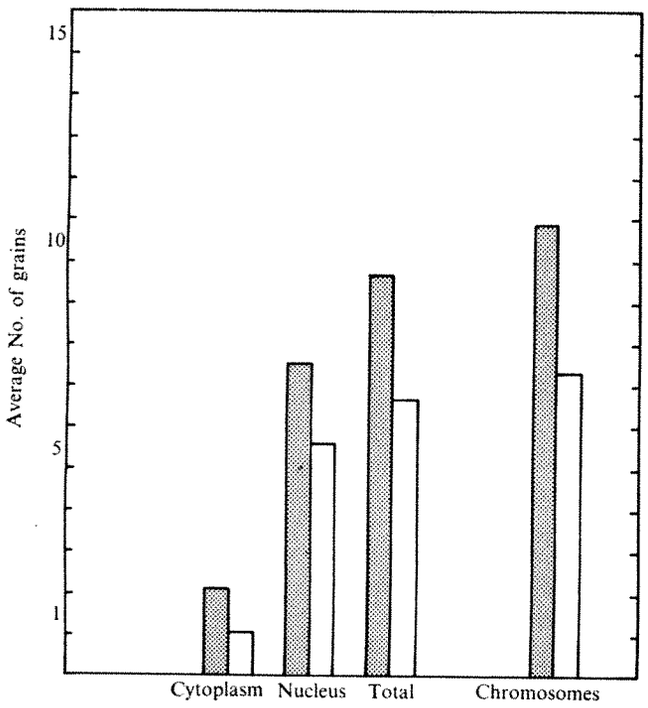


FIG. 1 Results of representative studies of ^{3}H-actinomycin D uptake by triiodothyronine (T_3) stimulated and control T-1 human kidney cells. Approximately 2×10^5 single cells were introduced into 100 mm Petri dishes (in duplicate) containing two microscope slides per dish. Cells stimulated for about 2 d with T_3 (8.90×10^{-7} M) as well as controls were labelled for 20 min with ^{3}H-actinomycin D (specific activity, 6.5 Ci mmol^{-1}) diluted to 0.5 μCi ml^{-1}, before rinsing with Hanks' balanced salt solution (BSS) and fixing with 50% ethanol–Hanks' BSS followed by absolute ethanol. Metaphase spreads of similarly treated and labelled cells were prepared by incubation with colchicine (25 μg ml^{-1}) for 16 h; hypotonic saline treatment and acetic alcohol (1:3) fixation completed the procedure. For autoradiography, cellular preparations and chromosome spreads were covered with Kodak NTB-2 emulsion, exposed for 14 and 11 d, respectively, followed by photographic processing and staining. Plotted are mean grain counts corrected for background, obtained by scoring 400 cells and 200 chromosome spreads; for each determination, the standard error of the mean was less than 5% of the indicated mean. □, control; □, T_3 stimulated.

proteins, and nucleic acids[2–6]. The cultured kidney cells therefore constitute a useful model for studying the peripheral actions elicited by these thyroidal hormones. Previous investigations strongly implicate the nucleus of the T-1 cell as being significantly involved in the response to T_4 and T_3; thus, thyroidal hormones affect nucleolar number, nucleolar RNA, and nuclear uptake of tagged T_4 (ref. 7). Here we report that T_3 acts on DNA throughout the T-1 cell in promoting the localisation of ^{3}H-actinomycin D, a labelled antibiotic that binds specifically to DNA[8,9].

For these determinations, autoradiographs were prepared of T-1 cells to evaluate ^{3}H-actinomycin D uptake by cytoplasm, nucleus, and chromosomes. Routinely, these cultured cells are grown as monolayers in Eagle's MEM supplemented with 10% foetal bovine serum, penicillin and streptomycin in 95% air–5% CO_2 incubators maintained at 37° C. For autoradiography, single cells in the exponential phase of growth, obtained by release and dispersal with trypsin, were overgrown onto microscope slides and, except for controls, stimulated for 2–3 d with T_3 (8.9×10^{-7} M) before tritium labelling; to similarly demonstrate the ^{3}H-actinomycin D associating with chromosomes, metaphase spreads of the labelled T-1 cells were made after incubation with colchicine for 5–16 h. After rinsing, fixing, and covering the slides with photographic emulsion, the exposed and processed autoradiographs were scored by counting developed grains. For making background corrections, autoradiographs of unlabelled preparations were scored. In this manner, cellular ^{3}H uptake was determined (five independent experiments); also assessed was the number of grains associated with chromosomes (three separate experiments). Data derived from representative studies are plotted in Fig. 1.

Evidently, T_3 stimulation of the T-1 cells results in a marked augmentation in binding of tagged actinomycin D, especially to chromatin, as judged by the enhanced nuclear radioactivity of interphase cells and by the label found incorporated in the chromosomes at metaphase arrest. Also noteworthy is the rise in the extra-nuclear localisation of the tagged antibiotic effected by T_3, possibly reflecting interaction between hormone and the mitochondrion[10], a cytoplasmic organelle containing DNA and concerned with respiration and energy transfer.

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1 Veen, J. van der, Mets, L., and Mes, A., *Arch. ges. Virusforsch.*, **8**, 230–237 (1958).
2 Siegel, E., and Tobias, C. A., *Nature*, **212**, 1318–1321 (1966).
3 Siegel, E., and Morse, N. M., *Proc. Soc. exp. Biol. Med.*, **137**, 595–597 (1971).
4 Siegel, E., Lammert, J., and Siegel, E. P., *Proc. Soc. exp. Biol. Med.*, **139**, 1288–1291 (1972).
5 Siegel, E., *Endocrinology*, **91**, 580–585 (1972).
6 Siegel, E., *Proc. Soc. exp. Biol. Med.*, **144**, 455–459 (1973).
7 Siegel, E., and Tobias, C. A., *Science*, **153**, 763–765 (1966).
8 Brachet, J., and Ficq, A., *C. r. hebd. Séanc. Acad. Sci., Paris*, **258**, 6258–6260 (1964).
9 Epstein, B. S., *J. Cell Biol.*, **35**, 709–713 (1967).
10 Buchanan, J., Primack, M. P., and Tapley, D. F., *Endocrinology*, **87**, 993–999 (1970).

Cytochalasin selectively inhibits synthesis of a secretory protein, cellulase, in *Achlya*

IN an effort to elucidate the possible relationship between cytoplasmic streaming and secretion we used the male strain E87 of the water mould *Achlya ambisexualis*[1]. Cellulase secreted by *Achlya* presumably modifies the plasticity of the cellulosic walls of this fungus during morphogenesis[2]. Synthesis of this enzyme can be induced by the steroid hormone antheridiol, by amino acids[2], or by shake induction (mechanical agitation) as reported here. In attempts to arrest streaming we used colchicine and vinblastine, which have been employed to disrupt microtubule function, and cytochalasins, whose reversible effects include inhibition of streaming[3], of macromolecular secretion[4], of uptake of small molecules such as glucose[5], and destruction of microfilaments[6].

Achlya mycelia were grown for 5 d in defined medium[7] aerated by bubbling. After filtration, 1 g of mycelium was incubated in replicates in 10 ml of fresh growth medium lacking glucose. Glucose-free growth medium was used during shake induction, which is not dependent on exogeneous glucose, to avoid the possibility that cytochalasin effects might be due to inhibition of glucose uptake. After 2 h incubation at room temperature on a reciprocating shaker at 120 cycles min^{-1}, 1 ml samples of medium and of aqueous mycelial extract were assayed viscometrically for cellulase using carboxymethyl cellulose as substrate[8]. The aliquot of mycelial

TABLE 1 Recovery of cellulase synthesis and secretion in *Achlya* after cytochalasin A (CA) treatment

Treatments	CA in medium*	Cellulase activity units Medium†	Mycelium‡
1 h static preincubation	−	2.4	24.8
		2.6	21.8
1 h static preincubation	+	0.0	16.0
		0.0	16.1
1 h static preincubation,	−, −	26.4	39.0
followed by		24.4	39.4
2 h shake induction in	+, +	0.9	14.2
fresh medium		2.9	14.9
	+, −	12.2	20.5
		11.6	26.6

* Glucose-free growth medium, with 2% dimethylsulphoxide, without (−) or with (+) CA at 20 μg ml⁻¹.
† Values represent duplicate treatments.
‡ Mycelial cellulase levels at the start of preincubation were 14.8 and 15.1 units.

extract used for the assay represented 0.5 g fresh weight of mycelium. One unit of cellulase was defined as that amount which gave 1% reduction in the flow time of the enzyme-substrate mixture during a 1 h incubation at 37° C. Cytochalasins (Aldrich Chemical Co., Milwaukee, Wisconsin) were dissolved in dimethyl sulphoxide to give 1% (w/v) stock solutions.

In experiments with *Achlya*, a wide range of colchicine (1×10^{-4} M to 2.5×10^{-3} M) or vinblastine (5×10^{-5} M to 3.3×10^{-4} M) concentrations failed to inhibit streaming or cellulase secretion. Cytochalasin A (CA) inhibited both cellulase secretion and synthesis (Fig. 1). At 20 μg ml⁻¹ cytoplasmic streaming was rapidly and completely arrested. Cytochalasin B (CB) at this concentration failed to inhibit streaming, and its effect on cellulase synthesis ranged from mild inhibition to stimulation. These differential responses to cytochalasins are consistent with those of Betina *et al*[9], who reported that CA, but not CB, is fungistatic and morphogenically active on fungi.

The effects of CA are reversible. Streaming recovers within 30 min of transfer to inhibitor-free medium. Mycelium preincubated for 1 h in CA, then transferred to fresh medium for 2 h of shake induction, is capable of cellulase synthesis and secretion (Table 1). The CA-free controls demonstrate shake induction of cellulase. Mycelium maintained in CA shows little secretion of cellulase, and no increase in tissue

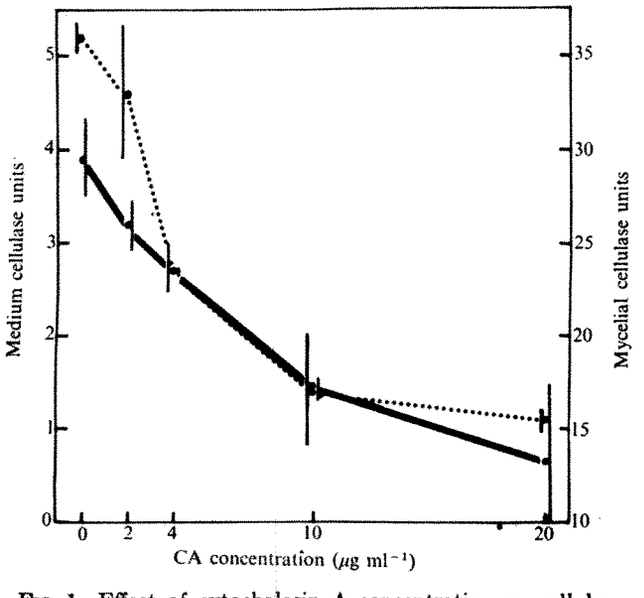


FIG. 1 Effect of cytochalasin A concentration on cellulase secretion and on mycelial cellulase. ●···●, Medium cellulase; ●———●, mycelial cellulase.

TABLE 2 Effect of cytochalasin A and cycloheximide on ^{14}C-leucine incorporation into mycelial protein, and on final radioactivity of medium

Experiment	Treatment*	Total medium activity c.p.m. ml⁻¹ after 4 h	Tissue protein c.p.m./100 μg after 4 h
I	Control	2209	3781
		2248	4552
	Cytochalasin	2785	4459
	A(20 μg ml⁻¹)	2644	5181
	Cycloheximide	2077	215
	(1 μg ml⁻¹)	2214	244
II	Control	312	174
		328	180
	Cytochalasin	376	196
	A(20 μg/ml⁻¹)	398	299
	Cycloheximide	298	64
	(1 μg ml⁻¹)	306	60

* All treatments were in duplicate, and contained 1 g fresh weight of mycelium in 10 ml glucose-free growth medium containing 2% dimethylsulphoxide and 0.4 mM leucine. The specific activities of the DL-leucine-1-^{14}C used in experiments I and II were 8.5×10^4 c.p.m. μmol⁻¹, and 7.9×10^5 c.p.m. μmol⁻¹ respectively. The 4 h incubation included 1 h static treatment and 3 h of shaking. Radioactivity was determined using 15 ml of a solubilising liquid scintillation fluid[13]. Medium was sampled after incubation to determine total radioactivity. Mycelia were rinsed twice with 30 ml of unlabelled leucine medium, and ground with sand in 0.5 N NaOH containing 10 mM leucine. The homogenates were acidified with 2 drops concentrated HCl, stored overnight at 0°C, and centrifuged for 15 min at 5,000*g*. Pellets were washed twice with 2 ml 10% trichloroacetic acid containing 10mM leucine, and resuspended in 0.5 N NaOH to a final volume of 2.1 ml. Aliquants were used for liquid scintillation counting, and for protein determination[14]. In *Achlya*, cycloheximide is a known inhibitor of protein, including cellulase, synthesis[12].

enzyme, when compared with the level at the start of preincubation.

The possibility that CA acts as a general protein synthesis inhibitor was excluded by the results in Table 2, which, in agreement with other reports[3,10] show no cytochalasin inhibition of labelled leucine uptake from the medium, or incorporation into protein. Failure of CA to inhibit general protein synthesis in *Achlya* indicates that the selective inhibition of cellulase synthesis may be a secondary effect, resulting from inhibition of secretion.

Previous work using cytochalasins has shown a correlation between streaming and plant cell growth[3,11]. Our results indicate the existence of coupling, not only between streaming and secretion, but between secretion and the synthesis of secretory protein, and raise the possibility that synthesis of certain proteins may be regulated at the level of secretion.

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1 Raper, J. R., *Proc. natn. Acad. Sci. U.S.A.*, **36**, 524 (1950).
2 D. des S. Thomas, and Mullins, J. T., *Science*, **156**, 84 (1967).
3 Bradley, M. O., *J. Cell Sci.*, **12**, 327 (1973).
4 Schofield, J. G., *Nature new Biol.*, **234**, 215 (1971).
5 Estensen, R. D., and Plagemann, P. G. W., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1430 (1972).
6 Wessels, N. K., Spooner, B. S., Ash, J. F., Bradley, M. O., Luduena, M. A., Taylor, E. L., Wrenn, J. T., and Yamada, K. M., *Science*, **171**, 135 (1971).
7 Mullins, J. T., and Barksdale, A. W., *Mycologia*, **52**, 352 (1965).
8 Bell, T. A., Etchells, J. L., and I. D. Jones, *US Dept. Agric. ARS*, **72**, 5 (1955).
9 Betina, V., Mičeková, D., and Nemec, P., *J. gen. Microbiol.*, **71**, 343 (1972).

[10] Zigmond, S., and Hirsch, J. G., *Science*, **176**, 1432 (1972).
[11] Mascarenhas, J. P. and Lafountain, J., *Tissue Cell*, **4**, 11 (1972).
[12] Kane, B. E., Reiskind, J. B., and Mullins, J. T., *Science*, **180**, 1192 (1973).
[13] Warner, A. H., and D. K. McClean, *Devl Biol.*, **18**, 278 (1968).
[14] Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265 (1951).

Neurotransmitter synthesis in inner ear and lateral line sense organs

HAIR cells of inner ear sense organs and of lateral line organs in fish are innervated by afferent nerve fibres, to which they transmit information about mechanical stimuli. Efferent inhibitory nerve fibres, which originate in the central nervous system, also innervate most hair cells at their base (Fig. 1*b*). Recent evidence indicates that both these synapses are chemical; excitatory postsynaptic potentials (e.p.s.p.s) have been recorded in afferent nerve terminals in response to mechanical stimulation[1,2] and inhibitory postsynaptic potentials (i.p.s.p.s) have been recorded in hair cells on stimulation of the efferent fibres[2]. Furthermore, several findings suggest that acetylcholine is the efferent transmitter: i.p.s.p.s in hair cells, as well as the ensuing inhibition of afferent discharge, can be blocked by curare[2–5]; both acetylcholine esterase and choline acetyltransferase have been found in the efferent fibres[6–9]. The nature of the afferent transmitter however has not been elucidated, although catecholamines[10] and glutamate[11] have been implicated.

We have investigated possible neurotransmitters in the inner ear and lateral line, especially at the afferent synapse. Three preparations were used: (1) the basilar papilla, an auditory organ in the bullfrog (*Rana catesbeiana*) which has only afferent innervation[12,13] (Fig. 1*a*); (2) the lateral line canal organ of a teleost fish (toadfish, *Opsanus tau*) from which both the afferent and efferent innervations[14,15] have been studied with intracellular recordings[2]; (3) the crista ampullaris of the semicircular canal in the skate (*Raja ocellata*), which has both afferent and efferent innervations and can be used for pharmacological studies.

We used a method based on the finding that cells which use a particular transmitter can synthesise and accumulate this transmitter in high concentrations[16]. Tissues were incubated in a medium containing radioactive precursors of noradrenaline, dopamine, γ-aminobutyric acid (GABA), acetylcholine (ACh) and serotonin. Tissue extracts were then fractionated by high-voltage electrophoresis and the radioactive products were identified[17,18]. The activities of the enzymes involved in the synthesis of particular transmitters were also measured. Preliminary pharmacological experiments were also performed.

Three to eight organs from one of the three types described above were dissected out and incubated at 20° C for 6–18 h in 30 μl of medium (Leibovitz medium for frog (260 mOsmol) and toadfish (320 mOsmol), skate Ringer for skate) containing 100 μCi ml^{-1} of one or a combination of electrophoretically purified L-^{3}H-glutamic acid (generally labelled, 2.5 Ci mmol^{-1}), methyl-^{3}H-choline chloride (6.7 Ci mmol^{-1}) and L-3,5-^{3}H-tyrosine (2.5 Ci mmol^{-1})[17,18]. In two experiments, toadfish lateral line canal organs were incubated with L-methylene-^{14}C-tryptophan (54.5 Ci mol^{-1}). After incubation, the tissue was first washed for 1 min in 2 ml Ringer and then homogenised with a ground glass homogeniser in 20 μl 0.47 M formic acid–1.4 M acetic acid containing unlabelled precursors and transmitters. A 10 μl sample of the homogenate and 10 μl of the labelled medium

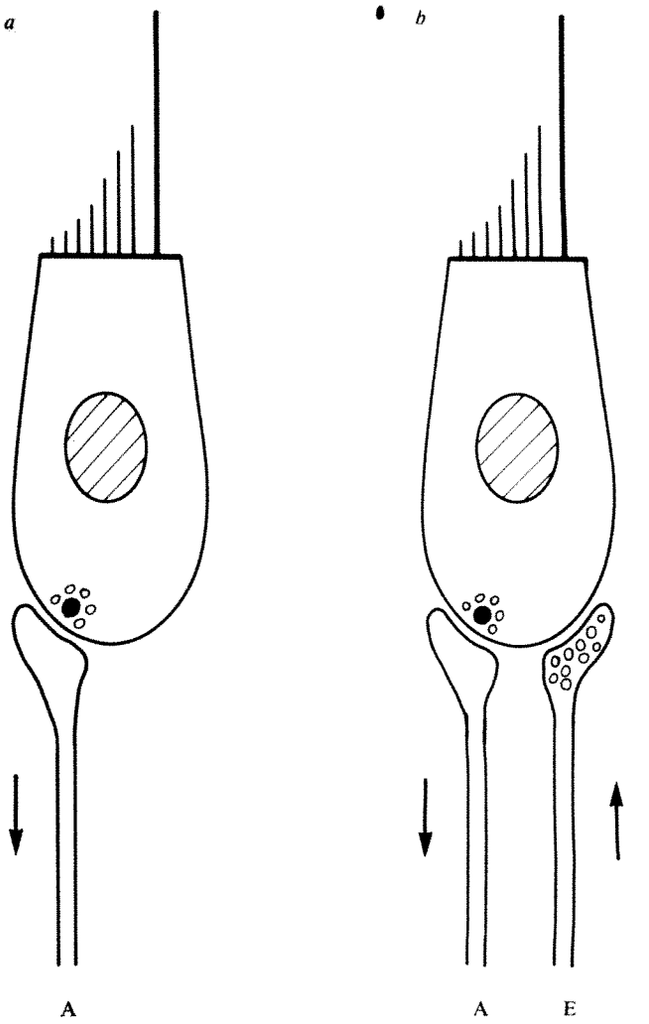


FIG. 1 Schematic diagram of hair cells and their innervation in (*a*) the frog basilar papilla, and (*b*) the lateral line canal organ and the semicircular canal ampulla.

TABLE 1 Biosynthesis of possible neurotransmitters in inner ear and lateral line sense organs

Incubations	^{3}H-Noradrenaline	^{3}H-Dopamine	^{3}H-GABA	^{3}H-Acetylcholine
Frog basilar papillae	30 ± 26	32 ± 18	562 ± 85*	49 ± 15
Frog medium	32 ± 20	35 ± 20	74 ± 39	55 ± 20
Toadfish lateral line organs	30 ± 18	15 ± 9	2,350 ± 340*	556 ± 84*
Toadfish medium	28 ± 15	16 ± 10	122 ± 56	53 ± 27
Skate semicircular canal ampullae	33 ± 22	26 ± 16	1,252 ± 315*	145 ± 45*
Skate medium	30 ± 18	26 ± 16	65 ± 35	26 ± 16
Medium without incubation	29 ± 6	18 ± 8	56 ± 25	28 ± 15

The tissues were incubated in media containing ^{3}H-tyrosine, ^{3}H-glutamate and ^{3}H-choline for 16 h. The numbers represent means ± s.d. of radioactive c.p.m. for 10 μl homogenate or medium for three experiments.
* Value indicates that the synthesis in tissue is significantly different from background.

were used for high voltage paper electrophoresis (6,000 V, 1.5 h, *p*H 1.9)[17,18]. After electrophoresis, the paper was dried, and ascending chromatography was applied perpendicular to the direction of electrophoresis to confirm the identities of the products (solvent for catecholamines and serotonin was: methyl ethyl ketone: propionic acid: water (200:65:55); solvent for GABA and acetylcholine was: *n*-butanol: acetic acid: water (60:15:25))[19]. After chromatography, the paper was dried and the spots corresponding to the precursors and transmitters were revealed by staining[17]. The regions corresponding to the appropriate transmitters and labelled precursors were cut into 1-cm squares and placed in scintillation vials each containing 1 ml 0.01 M HCl. After 2 h, 9 ml of Aquasol was added to each vial and the radioactivity was measured by scintillation counting.

In the basilar papilla and the lateral line canal organ the presence of glutamate decarboxylase (EC 4.1.1.15)[20] and choline acetyltransferase (EC 2.3.1.6)[18] was also investigated. For measurement of glutamate decarboxylase activity, organs were homogenised in 40 μl of L-^{14}C-glutamate (4 μCi, uniformly labelled, 255 Ci mol^{-1}), 2-mercaptoethanol (10 mM), pyridoxal phosphate (1 mM), and KH_2PO_4 (200 mM, *p*H 7.3)[20]. For determination of choline acetyltransferase activity, the organs were homogenised in 40 μl of ^{3}H-acetyl-coenzyme A (4 μCi, 980 Ci mol^{-1}), physostigmine (0.5 mM), KCl (100 mM) EDTA (0.2 mM, neutralised), choline (5 mM) and KH_2PO_4 (10 mM, *p*H 7.2)[18]. Each mixture was divided into four aliquots and incubated at 20° C for 0–8 h. The incubations were stopped by adding 1 μl of 2M HCl to each aliquot. The reaction products were analysed by high voltage paper electrophoresis and the activities of labelled GABA and acetylcholine were measured by scintillation counting.

The experiments on transmitter biosynthesis indicated that the bullfrog basilar papilla with only afferent innervation synthesised GABA but no detectable acetylcholine, dopamine or noradrenaline (Table 1). On the other hand, the lateral line canal organ in the toadfish and the skate ampulla, which have both afferent and efferent innervation, synthesised GABA and acetylcholine but not dopamine, noradrenaline or serotonin. Although quantitative kinetics of enzymatic activities were not studied, our results indicated that the basilar papilla (Fig. 2*a*) contained glutamate decarboxylase but not choline acetyltransferase, whereas the lateral line canal organ contained both enzymes (Fig. 2*b*). These findings are consistent with the results of Table 1.

Thus, acetylcholine was synthesised in the lateral line canal organ and in the semicircular canal ampulla, both of which receive efferent nerve fibres, but not in the basilar papilla which lacks efferents. In addition, choline acetyltransferase was found only in organs with efferent innervation. This correlation strongly supports the notion that the efferent nerve fibres are cholinergic, as suggested earlier by physiological and biochemical[2–9] results. The fact that curare, which specifically blocks cholinergic synapses, abolishes inibition caused by the efferents but leaves afferent synaptic transmission unaffected, implies that a different transmitter is involved at the afferent synapse. Since GABA is synthesised by all three organs, all of which have afferent synapses that show the ultrastructural and physiological criteria for chemical transmission these results point to GABA as a putatitive afferent transmitter.

Our biochemical findings, however, do not exclude the possibility that the synthesis of GABA occurred in the afferent nerve fibres, non-neural elements or other unidentified neurones rather than in the hair cells. In an attempt to clarify this point, preliminary pharmacological experiments were performed on the isolated labyrinth of the skate[21]. In this preparation the horizontal crista ampullaris could be stimulated by hydraulic displacement of the cupula and impulses from single afferent fibres could be recorded for several hours[22]. The base of the ampulla, where the nerve fibres enter, could be perfused with test solutions. Picrotoxin, a specific blocking agent for synapses transmitting with GABA[16,23], was found to block spontaneous as well as evoked discharge in sensory nerve fibres at moderate concentrations of the drug (5×10^{-5} to 10^{-6}M). This blockage was partly reversible in some cases. These experiments will be reported in more detail elsewhere.

Taken together, these findings give support for acetylcholine as the inhibitory transmitter used by efferent fibres of the acustico-lateralis system. In addition, our results raise the possibility that GABA is the neurotransmitter at the excitatory synapse between the hair cell and the afferent fibre. This possibility is interesting because in synapses where GABA has been shown to be the transmitter, its action on the postsynaptic cell is inhibitory[16,24]. Thus, if future experiments substantiate our results, GABA would be an excitatory transmitter used by hair cells in the inner ear and lateral line sense organs.

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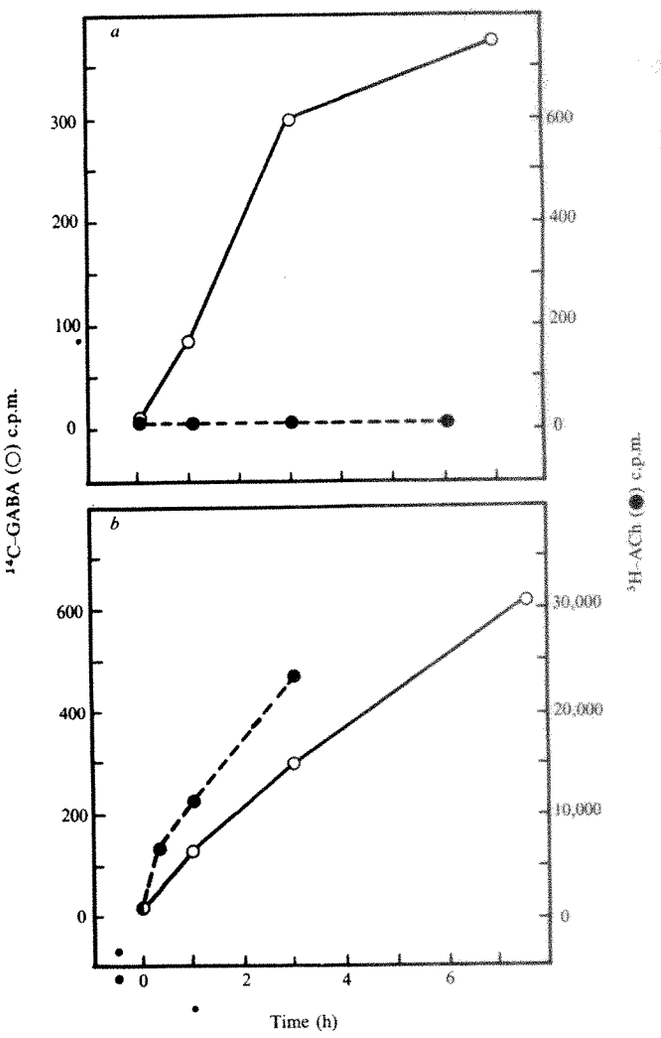


FIG. 2 Enzyme activities of glutamate decarboxylase (——) and choline acetyltransferase (– – – –) in the bullfrog basilar papilla (*a*) and the toadfish lateral line canal organ (*b*).

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1 Furukawa, T., and Ishii, Y., *J. Neurophysiol.*, **30**, 1377 (1967).
2 Flock, Å., Russell, I., *Nature New Biol.*, **243**, 89 (1973).
3 Fex, J., *Nature*, **213**, 1233 (1967).
4 Russell, I., *J. exp. Biol.*, **54**, 621 (1971).
5 Flock, Å., and Russell, I., *J. Physiol., Lond.*, **235**, 591 (1973).
6 Hilding, D., and Wersäll, J., *Acta Otolaryngol.*, **55**, 205 (1962).
7 Iurato, S., Luciano, L., Pannese, E., and Riale, E., *Acta Otolaryngol.*, Suppl. **279** (1971).
8 Russell, I., *J. exp. Biol.*, **54**, 643 (1971).
9 Jasser, A., and Guth, P., *J. Neurochem.*, **20**, 45 (1973).
10 Osborne, M. P., and Thornhill, R. A., *Z. Zellforschung*, **127**, 347 (1972).
11 Steinbach, A. B., and Bennett, M. V. L., *J. gen. Physiol.*, **58**, 580 (1971).
12 Robbins, R. G., Bauknight, R. S., and Honrubia, V., *Acta Otolaryngol.*, **64**, 436 (1967).
13 Frishkopf, L., and Flock, Å., *Acta Otolaryngol.*, **77**, 176 (1974).
14 Hama, K., *J. Cell Biol.*, **24**, 193 (1965).
15 Flock, Å., *Acta Otolaryngol.*, Suppl. **199**, 1 (1965).
16 Kravitz, E. A., in *The Neurosciences, A study program* (edit. by Quarton, G., Melnechuk, T., and Schmitt, F.), 433 (Rockefeller University Press, New York, 1967).
17 Hildebrand, J. G., Barker, D., Herbert, E., and Kravitz, E., *J. Neurobiol.*, **2**, 231 (1971).
18 Lam, D. M. K., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1987 (1972).
19 Mains, R., and Patterson, P., *J. Cell. Biol.*, **59**, 329 (1973).
20 Lam, D. M. K., *J. Cell Biol.*, **54**, 225 (1972).
21 Lowenstein, O., and Sand, A., *J. Physiol., Lond.*, **99**, 89 (1940).
22 Flock, Å., and Goldstein, M., *Biol. Bull.*, **145**, 433 (1973).
23 Robbins, J., and Van der Kloot, W. G., *J. Physiol., Lond.*, **143**, 541 (1958).
24 Krnjevic, K., *Nature*, **228**, 119 (1970).

Release of specific protease during mitotic cycle of L5178Y murine leukaemic cells by sublethal autolysis

SUBLETHAL autolysis is postulated to be the process by which degradative enzymes may modify cell plasma membrane macromolecules without causing cell death[1,2]. Malignant or virally transformed cells can be agglutinated by various agglutinins; their untransformed counterparts are not agglutinated, but normal cells become agglutinable upon mild treatment of the cells with a proteolytic enzyme[3]. Therefore, elevated levels of degradative enzymes may be important for maintenance of the malignant or transformed state through sublethal autolysis, which constantly modifies cell plasma membrane surfaces. This report shows that L5178Y cells release small amounts of a protease active at *p*H 7.8 and that release of this enzyme is almost exclusively in the M period of the L5178Y mitotic cycle. Rubin[4] reported that small amounts of trypsin or Pronase produced an overgrowth phenomenon in cells; that is, contact-inhibited cells were released from contact inhibition and proceeded through a subsequent round of mitosis. Rubin[4] has also isolated from cells infected with Rous sarcoma virus a similar non-dialysable, thermolabile overgrowth-stimulating factor.

The ability of lysosomal enzymes to modify the cell periphery has been documented and the control of cell growth through such modification has been reported[1-6]. Release of lysosomal enzymes during M phase in synchronous normal cells has been reported[3], a proteolytic activity associated with malignant cells mediating fibrinolysis has been said to be a general enzymatic change associated with transformation[7], and increased concentrations of proteases have been found in a number of virally transformed cells compared with their normal counterparts[1,8,9].

L5178Y cells (mouse lymphoma cell line) were grown in suspension culture in sealed containers in Fischer's medium[10] with 10% horse serum and were used in the exponential growth phase. Cell numbers were determined in a Coulter counter. Cells were synchronised by the method of Doida and Okada[11-14] by applying one treatment with excess thymidine followed by one treatment with colcemid and deoxycytidine. At each hour after release from the colcemid block 30 ml cell suspension were centrifuged out of solution at 1500*g* for 5 min, and the medium was assayed for proteolytic enzymes as given below. Simultaneously with the sampling for analysis, 3 ml cells were centrifuged out of solution, resuspended in 0.2 ml Fischer's medium containing 10 μCi of ^{3}H-thymidine (15 Ci mmol^{-1}) and incubated at 37°C for 5 min. The incubation was terminated with 10% trichloroacetic acid; the cell suspension was centrifuged and the insoluble pellet washed twice with 10% trichloroacetic acid and once with ethanol–diethyl ether (2:1 (v/v)) dissolved in 1 M NaOH, plated on a glass filter disk, and counted in a liquid scintillation counter. Counts per minute from zero-time incubations, which were precipitated immediately, were subtracted from these results. This radioactive incorporation of ^{3}H-thymidine is an indication of DNA synthesis in the cell and is a useful measure of synchrony and the S period. Protein was determined by the method of Lowry *et al.*[15]. Crystalline bovine serum albumin was used as a standard. Protein per cell increased linearly from about 0.9 mg per 10^7 cells to about 1.7 mg per 10^7 cells after about 9.5 h and then dropped back to 0.9 mg per 10^7 cells. In general, there was a doubling of the cells at 9.5–10 h after release from the M block. Further details of these methods have been given previously[12-14].

^{3}H-Acetyl haemoglobin was prepared as described previously[15-17]. ^{3}H-Acetic anhydride (400 Ci mol^{-1}) was reacted

TABLE 1 Influence of divalent cations and group-specific reagents on *p*H 7.8 proteinase of medium from exponential L5178Y cells

Addition	Final concentration (mM)	Relative activity (%) *p*H 7.8 proteinase
None	—	100
$CoCl_2$	1.0	58
$HgCl_2$	1.0	10
$PbCl_2$	1.0	80
$MnCl_2$	1.0	87
$MgCl_2$	1.0	100
$CaCl_2$	1.0	90
$CdCl_2$	1.0	10
$BaCl_2$	1.0	76
$CuCl_2$	1.0	22
$FeCl_2$	1.0	96
$ZnCl_2$	1.0	6
EDTA	1.0	96
Diisopropylphosphofluoridate	0.1	2
Cysteine	1.0	94
Mercaptoethanol	1.0	90
Sodium *p*-chloromercuribenzoate	1.0	24
2, 4′-dibromoacetophenone*	0.1	96
2-bromo-2-phenylacetophenone*	0.1	98
Iodoacetate	0.1	96
Ortho-iodosobenzoate	1.0	94

* Dissolved in dimethylsulphoxide diluted to 0.8% (v/v) in the assays.

The medium was incubated with the indicated compounds at the final concentration designated for 10 min at 25°C before the substrate was added. Data are given as relative activity; means from five independent determinations.

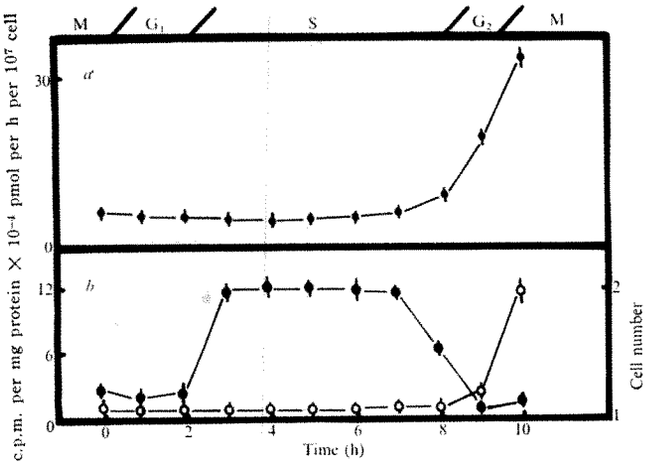


FIG. 1 Activity of released protease in synchronous L5178Y cells. Culture and synchrony of cells was as described previously[11–14]. Experiments were performed by collective cells in the indicated growth phase, removing the cells by centrifugation at 1500*g* for 5 min and analysing the proteolytic activity at *p*H 7.8 of the medium as described in the text. Appropriate medium controls were assayed simultaneously, and the activity was subtracted from the total activity. Data are pmol of ^{3}H-acetyl haemoglobin per 10^7 cells and are means ± 1 s.d. from five independent observations. *a*, Released proteolytic activity. *b*, DNA synthesis during cell mitotic cycle as measured by incorporation of ^{3}H-thymidine into acid-insoluble material (●) and cell number (Coulter counter; ○) in arbitrary units. A representation of the cell cycle is given above the panels[11–14]. Cells were released from mitotic block just before zero time.

with the Type I beef blood haemoglobin. The material was purified to a specific activity of 162 c.p.m. pmol^{-1} based on a molecular weight of 68,000 using the counting procedures described here. The specific activity of the material was 300 Ci mol^{-1}. Routinely in the assay systems described below, 50 μl (136 μg ^{3}H-acetylated haemoglobin; 2 nmol; 324,000 c.p.m.; 0.6 μCi) of the ^{3}H-acetylated haemoglobin was added per assay.

Routinely, 50 to 100 μl of medium or lyophilised medium made up to a smaller volume were added to 100 μl 0.1 M phosphate buffer, *p*H 7.8. As the substrate, 50 μl of ^{3}H-acetyl haemoglobin (2 nmol) were added. This mixture was incubated for 1 h at 37°C in a Dubnoff metabolic shaker. The reaction was terminated by placing the assay tubes in an ice-water bath (0–4° C) and adding 100 μl of 2.5% haemoglobin and 50 μl of 60% trichloroacetic acid (TCA). The precipitated material was removed by centrifugation at 5,000*g* for 5 min, and an aliquot of the supernatant fluid was plated on a glass fibre filter; the radioactivity was determined by counting in a liquid scintillation counter[17,18]. Activity is expressed as pmol of haemoglobin degraded per h per 10^7 cells. Suitable blanks consisting of medium without cells were added and incubated simultaneously.

Medium from exponentially growing L5178Y cells had 8 ± 1 pmol of haemoglobin per h per 10^7 cells. Activity of 5′-nucleotidase, esterase, and UDPase (all enzymes present in large amounts in L5178Y cells[12]) were undetectable in the medium from the same cells, indicating that the proteolytic activity was probably actually released from the cells and that cell lysis was not occurring. The proteolytic enzyme in the medium was very dependent upon *p*H: although a slight peak of activity occurred between *p*H 3 and 4 (2 ± 0.6 pmol per h per 10^7 cells), essentially no activity was present below *p*H 7.0 and above *p*H 8.6. Proteolytic enzymes can be classified in one of four groups: (1) serine proteinases, (2) acid proteinases, (3) thiol proteinases, and (4) metal activiated proteinases[13]. As demonstrated in Table 1, the thiol-specific reagents, iodoacetate and *p*-chloromercuribenzoate, failed to substantially inhibit the proteinase (although the *p*H 7.8 proteinase was inhibited by *p*-chloromercuribenzoate); however, $HgCl_2$ was inhibitory to the enzyme. The data of Table 1 clearly show the released protease to be a serine proteinase.

The data of Fig. 1 show the enzyme to be most active in the M phase of the mitotic cycle; indeed during division at 10 h the enzyme reached peak activity of 30 ± 2 pmol haemoglobin per h per 10^7 cells. The data clearly show that in these chemically synchronised cells a protease active at near physiological *p*H is exclusively released from the cell into the medium just subsequent to and during division. The role this enzyme plays in cellular regulation and control (if any) such as cell division, maintenance of the neoplastic state, overgrowth phenomena, sublethal autolysis, rounding up and lack of adhesion of cells during division, remains to be elucidated.

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1 Bosmann, H. B., *Biochim. biophys. Acta*, **264**, 339 (1972).
2 Bosmann, H. B., *Biochim. biophys. Acta*, **279**, 456 (1972).
3 Burger, M. M., In *Growth Control in Cell Cultures* (edit. by G. E. W. Wolstenholme and J. Knight) (Churchill and Livingston, London, 1971).
4 Rubin, H., *Science*, **167**, 1271 (1970).
5 Dingle, J. T., in *Lysosomes in Biology and Pathology* (edit. by J. T. Dingle and H. B. Fell), **2**, 421 (North-Holland, Amsterdam, 1969).
6 Weiss, L., *The Cell Periphery; Metastasis and Other Contact Phenomena*, (North-Holland, Amsterdam, 1967).
7 Reich, E., *Fedn. Proc.*, **32**, 2174 (1973).
8 Bosmann, H. B., *Expl Cell Res.*, **54**, 217 (1969).
9 Bosmann, H. B., Lockwood, T., and Morgan, H., *Expl Cell Res.*, **83**, 25 (1974).
10 Fischer, G. A., and Sartorelli, A. C., *Methods med. Res.*, **10**, 247 (1964).
11 Doida, Y., and Okada, S., *Expl Cell Res.*, **48**, 540 (1967).
12 Bosmann, H. B., *Biochim. biophys. Acta*, **203**, 256 (1970).
13 Bosmann, H. B., and Bernacki, R. J., *Expl Cell Res.*, **61**, 489 (1970).
14 Bosmann, H. B., *J. biol. Chem.*, **246**, 3817 (1971).
15 Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265 (1951).
16 Bernacki, R. J., and Bosmann, H. B., *J. Membrane Biol.*, **7**, 1 (1972).
17 Bosmann, H. B., *Int. J. Peptide Protein Res.*, **5**, 135 (1973).
18 Hartley, B. S., *A. Rev. Biochem.*, **29**, 45 (1960).

Exencephaly in the syndrome of trisomy No. 12 of the foetal mouse

EXPERIMENTAL[1] and morphological[2] evidence suggests that exencephaly and anencephaly represent different degrees of severity of cranioschisis. Their morphogenesis seems to involve a primary disturbance of the cephalic somites and notochord. Both conditions are experimentally inducible in several species by the action of different causal factors and teratogens[3–7]. Moreover, exencephaly has been observed occasionally in gene mutants, for example in the mouse[8], though rarely with complete penetrance in the affected strains[9], and apparent autosomal recessive inheritance of neural tube defects has been suggested to occur at least in some instances in man[10,11]. Therefore, it can be assumed that the aetiology of cranioschisis is at least complex and multifactorial involving environmental agents as well as

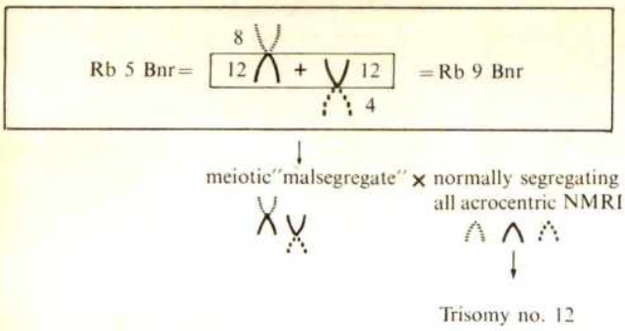


FIG. 1 The breeding of aneuploid zygotes by mating Rb5/Rb9 doubly heterozygous males with NMRI females.

genetic factors. But so far, defects belonging to this entity have not yet been observed in human or other mammalian chromosome mutation syndromes. In this respect it seems of interest to report the finding of exencephaly as constant and characteristic feature of trisomy of chromosome 12 in the mouse foetus.

Double structural heterozygotes possessing two metacentrics derived from Robertsonian changes and displaying monobrachial homology were used for inducing trisomy in the foetal offspring (A. G., D. Giers, and U. K., unpublished). Thus, for the study of trisomy No. 12, doubly heterozygous males with the Rb(8.12)5 and Rb(4.12)9 Bnr metacentrics (refs 12–14 and recommendations of subcommittee to consider rules for chromosome aberrations of the Committee on Standardized Genetic Nomenclature for Mice.) were selected and bred with NMRI (laboratory strain) females (Fig. 1). The two metacentrics share the chromosome arm which corresponds to mouse chromosome No. 12. In meiosis I they form a tetravalent (Fig. 2*a*) which is responsible for anaphase I malsegregation producing 43% chromosomally unbalanced, hypo- and hypermodal (Fig. 2*b*) spermatocytes II (A. G., D. Giers, and U. K., unpublished). On theoretical grounds it can be expected that among the aneuploid zygotes resulting from mating the Rb5/Rb9 double heterozygote with NMRI, the offspring with 41 chromosome arms in which both metacentrics had segregated together must correspond to the trisomy of the arm shared by them, that is of chromosome No. 12 (Fig. 1 and 2*b*).

Nineteen NMRI females mated with Rb5/Rb9 males were killed at day 10, 12, 13 to 14, and 19 (vaginal plugs controlled in the mornings; 'plug day' = day 1). Karyotypes were prepared from the total implant at day 10, and from the foetal membranes in the later stages of which the embryos were saved for morphological studies. At least 10 metaphases per implant were evaluated. At days 10 and 12 to 14, a total of 33 (21%) out of 156 living embryos were aneuploid (Table 1), of which 28 (28/33) showed 41 chromosome arms in the presence of the Rb5 and Rb9 metacentrics. Thus, they corresponded to trisomy No. 12. All of the 13 embryos of days 12 to 14 with the trisomy No. 12 karyotype which were morphologically studied exhibited fully developed exencephaly (Figs 3*b* and 4). The defect of the cranial vault is always complete and the brain protrudes beyond the cranium generally displaying a fairly good differentiation of the telencephalic hemispheres and of the diencephalic and rhombencephalic areas (Fig. 4). The eyes are remarkably small and hypoplastic. The size of most affected embryos is normal or only slightly subnormal. This is also true for the developmental stage characteristics, for instance development of the limbs and of the internal organs. In less than a fourth of the observed cases of cranioschisis, however, the development of the brain was more heavily affected, corresponding to more or less pronounced anencephaly (Fig. 3*c*). In addition these embryos were severely retarded and hypoplastic. At day 19, there were only three cases exhibiting exencephaly and trisomy No. 12, but the affected embryos were already dead and autolysed. A more detailed histological study of trisomy No. 12 describing the changes of the brain, the skull basis, the eyes and other organs, for instance the placenta, will be the subject of a further report.

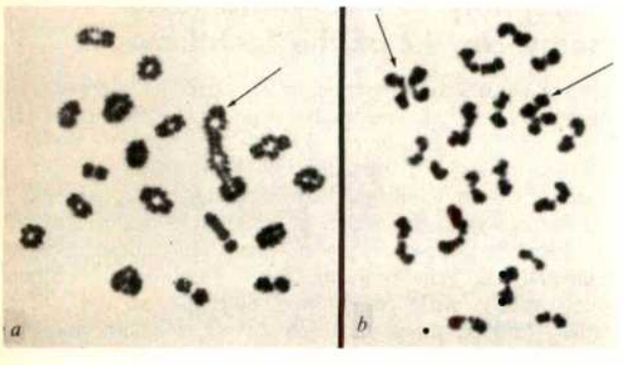


FIG. 2 *a*, Diakinesis (metaphase I) from Rb5/Rb9 male with tetravalent, *b*, Meiotic metaphase II with a hypermodal number of chromosome arms ($n = 21$) in the presence of the Rb5 and Rb9 metacentrics.

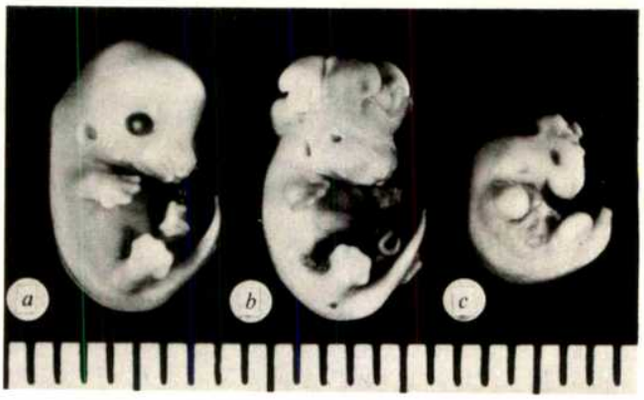


FIG. 3 Three embryos (on mm scale) of backcross progeny Rb5/Rb9Bnr male × NMRI female, day 13½; *a*, normal euploid littermate; *b*, trisomy No. 12 and exencephaly with microphtalmia; *c*, more severely affected and retarded trisomic embryo with anencephaly.

Two trisomic embryos (2/33 of days 10 to 14, one each of day 12 and 14) possessed only one metacentric (Table 1). It has to be assumed that in these cases the extra chromosome cannot be No. 12, but must be the result of meiotic malsegregation of a bivalent, though of undetermined origin. In fact, they were the only morphologically recognisable trisomics without cranioschisis. Their main external feature was marked hypoplasia. Moreover, at day 10, three triploid implants (3/33) were observed, all of them of maternal origin of the extra haploid set.

The present observations indicate that cranioschisis, and particularly exencephaly, is a constant feature of trisomy No. 12 of the mouse foetus. Exencephaly has never been encountered among the other autosomal trisomic conditions of the mouse so far studied in our laboratory (trisomy No. 1, 8, 10, 11, 13 and 15–17, A. G., D. Giers, and U. K. unpublished), or reported from other laboratories (trisomy No. 19, ref. 15). All these trisomic conditions cause death in the course of early or late foetal development, thus eventually leading to their elimination at least before term. In this respect, trisomy of chromosome No. 12 of the mouse can be well delineated and classified as displaying a characteristic

TABLE 1 Occurrence of aneuploidy and of macroscopically recognisable defects

		day 10		day 12		day 13/14		day 19	
No. of females		5		5		5		4	
No. of corpora lutea		65		73		65		57	
No. of resorptions		8		18		13		17	
Chromosome No.	$2n =$	40	41	40	41	40	41	40	41
No. of live normal embryos		35	—	46	—	42	—	33	—
No. of exencephalic embryos		—	18*	—	5†	—	8†	—	3§
No. of otherwise abnormal embryos					1‡	—	1‡	—	—

* Morphology not determined. 15/18 $2n = 41$ with two metacentrics. 3/18 triploid.
† $2n = 41$ in the presence of two metacentrics.
‡ $2n = 41$ with only one metacentric. No exencephaly; embryos hypoplastic and retarded.
§ Freshly dead or autolysed.

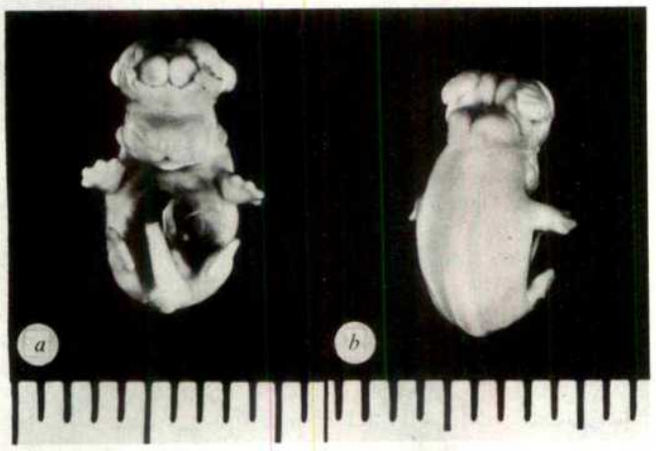


FIG. 4 Front *a* and back *b* view (on mm scale) of exencephalic embryo with trisomy No. 12. Developmental stage day 13½ to day 14.

exencephaly or cranioschisis syndrome compatible with survival until a late period of gestatation, possibly until day 17 or 18. The observation that its developmental profile surpasses at least the survival capacity of trisomy No. 1, 8, 11, 15 and 17 (A. G., D. Giers, and U. K., unpublished) lends support to the assumption that cranioschisis *per se* is not the primary cause of prenatal death in the trisomy No. 12 syndrome of the mouse.

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1 Marin-Padilla, M., and Ferm, V. H., *J. Embryol. exp. Morph.*, **13**, 1 (1965).
2 Marin-Padilla, M., *Virchows Arch. path. Anat. Physiol.*, **339**, 245 (1965).
3 Hamburgh, M., *Nature*, **169**, 27 (1952).
4 Giroud, A., and Martinet, M., *Acta anat.*, **44**, 358 (1960).
5 Kalter, H., and Warkany, J., *Am. J. Path.*, **38**, 1 (1961).
6 Renwick, J. H., *Br. J. prev. soc. Med.*, **26**, 67 (1972).
7 Poswillo, D. E., Sopher, D., and Mitchell, S., *Nature*, **239**, 492 (1972).
8 Grüneberg, H., *J. Genet.*, **52**, 52 (1954).
9 Lyon, M. F., Phillips, R. J. S., and Searle, A. G., *Genet. Res.*, **5**, 448 (1964).
10 Penrose, L. S., *J. ment. Defic. Res.*, **1**, 4 (1957).
11 Fuhrmann, W., Seeger, W., and Böhm, R., *Humangenetik.*, **13**, 241 (1971).
12 Gropp, A., Winking, H., Zech, L., and Müller, H.: *Chromosoma*, **39**, 265 (1972).
13 Gropp, A., and Zech, L. in *23rd Nobel Symp. Chrom. Identif.*, 118 (edit. by Caspersson, T., and Zech, L.) (Academic Press, 1973).
14 Cattanach, B. M., Williams, C. E., and Bailey, H., *Cytogenetics*, **11**, 412 (1972).
15 White, B. J., Tjio, J.-H., van de Water, L. C., and Crandall, C., *Cytogenetics*, **11**, 363 (1972).

Phosphorylation of selected serine and threonine residues in myelin basic protein by endogenous and exogenous protein kinases

THERE is considerable interest in phosphoprotein metabolism in the brain and the effects of neurotransmitters on the turnover of phosphate in membrane proteins[1]. Emphasis has been placed on the phosphorylation of proteins of synaptosomal membranes, but in addition myelin comprises a considerable proportion of the total substrate activity for a soluble protein kinase of brain[2]. In the following paper Miyamoto *et al.*[3] show that this kinase, which is stimulated by adenosine 3′, 5′-cycle monophosphate (cyclic AMP), phosphorylates the basic protein of myelin. Besides being a substrate for this soluble kinase the basic protein was found to be phosphorylated by an endogenous kinase of myelin[3]. Carnegie *et al.*[4] demonstrated that a protein kinase from rabbit muscle would selectively phosphorylate certain serine and threonine residues in the basic proteins of rat and human myelin. Soluble protein kinases from brain and muscle seem to phosphorylate similar sites in histones[5]. We present evidence here that the endogenous protein kinase of myelin phosphorylates the basic protein, but not the proteolipid protein, and that the site of phosphorylation by this endogenous kinase is quite different from the site phosphorylated by soluble protein kinase.

Myelin was prepared from fresh bovine spinal cord by a rapid method[6] and stored at −20° C. γ-^{32}P-ATP was prepared[7] with a specific activity of 2500 d.p.m. pmol^{-1}. Protein kinase was prepared from rabbit muscle[8] and detailed conditions for phosphorylation of proteins by this kinase are described by Kemp *et al.*[9]

Myelin (300 μl, 7.5 mg protein) was incubated with 50 μmol HEPES buffer[9] pH 7.5; 29.3 nmol γ-^{32}P-ATP; 10 μmol $MgCl_2$; 10 μmol KF, in a final volume of 1 ml, for 10 min at 37° C. Samples (50 μl) were removed and added to 1 ml of a solution of ATP (100 μM) in 50% trichloroacetic acid. The precipitate was collected on glass fibre disks, washed repeatedly with trichloroacetic acid and ATP solution, and finally with ether.

Other incubations were performed as above but with the

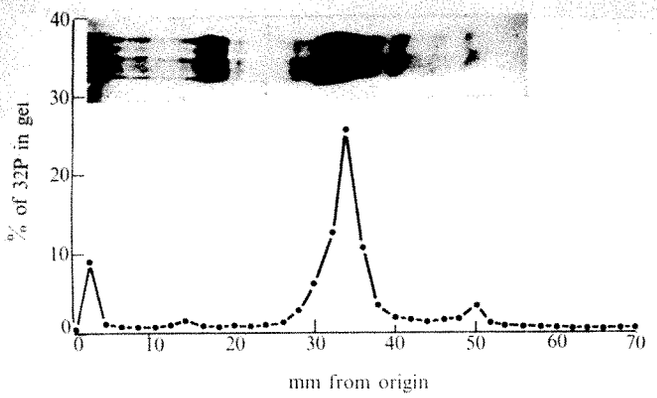


FIG. 1 Electrophoresis of myelin after phosphorylation by γ-^{32}P-ATP and endogenous kinase. Lyophilised myelin (about 10^5 d.p.m.) was dissolved in phenol-formic acid-water (2:1:1, by volume) and separated by electrophoresis in the same solvent in a 15% polyacrylamide gel for 4 h at 550 V. 28 MA. Gels were stained with amido black and 2 mm slices were taken starting from the cathodic side of the slot which is not shown in the photograph of the stained gel. The slices were counted for ^{32}P in a flow counter with an efficiency of 30.8%. Proteolipid protein is the band at 17.5 mm and basic protein that at 34 mm.

addition of either 2 μM cyclic GMP; 2 μM cyclic AMP; or 100 μl of rabbit muscle protein kinase (5.28 mg ml^{-1}) plus 2 μM cyclic AMP. The total ^{32}P incorporated (d.p.m. per mg protein) into the myelin under these conditions were as follows: myelin alone, 3.0×10^5; myelin plus cyclic AMP, 2.8×10^5; myelin plus cyclic GMP, 3.0×10^5; and myelin plus cyclic AMP and rabbit muscle kinase, 4.4×10^5. Thus cyclic AMP did not stimulate the endogenous kinase of bovine myelin. The addition of rabbit muscle kinase increased the total incorporation of ^{32}P into myelin. In other experiments, endogenous kinase did not appear to utilize γ-^{32}P-GTP as a phosphoryl donor.

Phosphorylated myelin was collected from the incubation mixture by centrifugation and washed with 2×10 ml water before lyophilisation. Samples of lyophilised myelin from the above incubation mixtures were dissolved in phenol-formic acid-water (2:1:1, by vol) and subjected to electrophoresis in this solvent[10] in a 15% polyacrylamide gel. Of the total radioactivity applied to gel 25% was not associated with any band of protein but was spread throughout the gel. After correction for this background 85% of the radioactivity was associated with the basic protein and none with the proteolipid protein (Fig. 1). Almost identical patterns, with no additional minor peaks or changes in the proportion (about 9%) of the protein of high molecular weight remaining at the origin, were obtained with myelin from the other incubation mixtures. In the sample with added kinase where the total incorporation of ^{32}P was increased, the pattern remained the same. Approximately 85% of the ^{32}P was incorporated into the basic protein.

Basic protein was prepared from each of the samples of lyophilised myelin by precipitation from chloroform-methanol[11]. The basic protein was collected by filtration and the recovery of radioactivity was similar to that found by gel electrophoresis. Some difficulty was experienced in quantitatively eluting the phosphorylated basic protein from filter paper. That the phosphorylated protein was indeed the myelin basic protein was determined by several criteria. Amino acid analysis agreed with that of a sample of myelin basic protein obtained from bovine spinal cord. Gel electrophoresis showed only traces of proteins other than the myelin basic protein. The main peak of radioactivity coincided with the myelin basic protein and less than 15% was associated with the contaminants, with no individual contaminant containing more than 6%. The phosphorylated proteins were digested with trypsin[12] (treated with N-tosyl-L-phenylalanyl-chloromethyl ketone) for 3 h at 37° C and peptide maps, stained with ninhydrin, were prepared as described in Fig. 2. These maps were identical with those from authentic basic protein.

From the specific activity of the phosphorylated protein approximately 25 mmol P were found to be incorporated per mol of basic protein. Autoradiography for 24 h was used to locate the ^{32}P-peptides. Analysis of the peptides was as described previously[12,4] and phosphoserine was identified by electrophoresis[13]. Myelin basic protein from bovine spinal cord was phosphorylated and digested as previously described for the human and rat protein[4].

The position of the main radioactive spots in a tryptic digest of ^{32}P-basic protein are shown in Fig. 2. The spots are labelled as described by Carnegie *et al.*[4] for the phosphorylated human and rat proteins. Spot A, from these proteins, was not found in the digest of the bovine protein. Spots B, C and F were obtained in sufficient yield for amino acid analysis, which enabled their identification (Table 1). Edman degradation of the material in spot C showed that serine-110, rather than serine-112, was phosphorylated. Spot B was derived from peptide 6 and 7 in the bovine protein. It is well known that acidic residues adjacent to a basic amino acid inhibit tryptic digestion. Spot B was not conclusively identified in the human and rat proteins although it was shown to contain phosphothreonine[4].

Spot G was not obtained in sufficient yield for accurate amino acid analysis. From a comparison of the effects of phosphorylation on the chromatographic and electrophoretic properties of peptides which were identified, however, it was possible to tentatively identify the parent peptides of spots G, K and O. The presence of these spots in digests of phosphorylated bovine, rat and human proteins suggested that they were derived from invariant regions of amino acid sequence[4]. Their low values of R_f and the presence of phosphoserine rather than phosphothreonine in hydrolysates of

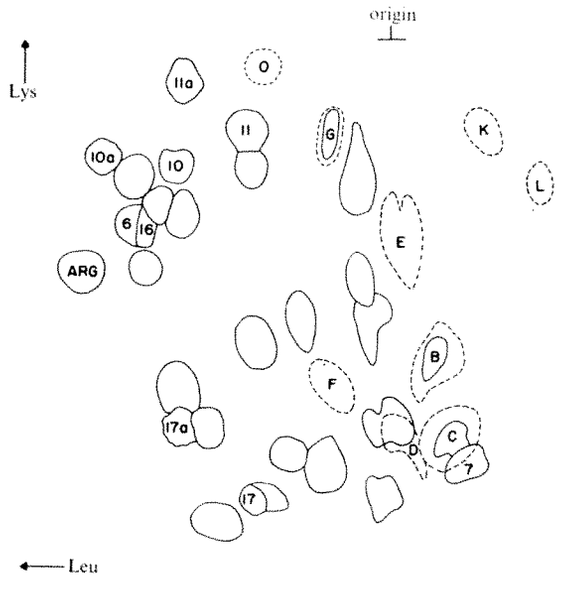


FIG. 2 Peptide map of phosphorylated bovine basic protein. The ^{32}P-protein was digested with trypsin and subjected to electrophoresis at *p*H 6.5 and chromatography in *n*-butanol-pyridine-acetic acid-water (15:10:3:12, by volume)[12]. Peptides reacting with ninhydrin (—) are numbered as in ref. 15 and those labelled 'a' (for example, 10a Arg-Gly-Ser-Gly-Lys) are derived from the failure of trypsin to digest a bond at the N-terminal end of the peptide (for example, 10 Gly-Ser-Gly-Lys). Auto radiography for 24 h was used to detect the ^{32}P-peptides. The main spots (- - - -) are shown and labelled as in ref. 4 as they had identical electrophoretic and chromatographic properties to the corresponding ^{32}P-peptides from phosphorylated human and rat proteins.

TABLE 1 Percentage distribution of radioactivity in peptides from a tryptic digest of ^{32}P-basic protein

^{32}P-Spot (see Fig. 2)	Derived from peptide No. (Fig. 2)	Amino acid sequence	^{32}P-Amino acid residue No. in protein	Incubation mixture with γ^{32}P-ATP: Myelin	Myelin + RMPK + cyclic AMP	Basic protein + RMPK + cyclic AMP
B	6 + 7*	His-Arg-Asp-Thr-Gly-Ile-Leu-Asp-Ser-Leu-Gly-Arg	Thr-34	1.1	1.7	20.9
C	17*	Gly-Leu-Ser-Leu-Ser-Arg	Ser-110	2.7	17.9	42.8
D	?	Unknown	Ser	0.9	4.0	8.9
E	?	Unknown	Ser	4.7	9.6	8.4
F	17a*	Gly-MMA-Gly-Leu-Ser-Leu-Ser-Arg	Ser-110	0.6	1.3	5.1
G	10a†	Arg-Gly-Ser-Gly-Lys	Ser-55	67.0	48.6	4.7
K	10†	Gly-Ser-Gly-Lys	Ser-55	5.4	3.4	1.1
O	11a†	Arg-Gly-Ser-Gly-Lys-Asp-Gly-His-His-Ala-Ala-Arg	Ser-55	8.2	6.7	0.8
Others	?	Unknown	Ser	9.4	6.8	7.3

Incubations with myelin were carried out for 10 min whereas the incubation of isolated myelin basic protein plus rabbit muscle kinase was carried out for 180 min to enable a direct comparison to be made with the phosphorylation of the human and rat proteins[4]; incubation for 10 min gave a similar distribution.

* Identified by amino acid analysis, and ref. 4.

† Identified by chromatographic and electrophoretic properties (see text). MMA, monomethylarginine; RMPK, rabbit muscle protein kinase. Others, minor spots which had been 0.1 and 3% of the total radioactivity on the peptide map.

these spots eliminated a large number of peptides from consideration. From a comparison of the change in electrophoretic mobility caused by phosphorylation of peptides which were identified, it was possible to estimate the molecular weights of the parent peptides of spots G, K and O (compare ref. 14). After this comparison only two serine residues, 8 and 55, remained to be considered. It is known that trypsin gives rise to three peptides[12,15] all containing serine-55 (Table 1) and these fit the calculated molecular weights of the parent peptides of G, K and O. Therefore, serine-55 was tentatively identified as a site of phosphorylation for the endogenous kinase especially as the sequence adjacent to it is invariant.

Phosphorylation of myelin basic protein by endogenous kinase occurs mainly at one serine residue, tentatively identified as 55 (Table 1). Radioactivity at this site represented 80% of the ^{32}P incorporated. When rabbit muscle protein kinase was incubated with myelin, phosphate was incorporated into serine residues 55 and 110. After allowance was made for the contribution of the endogenous kinase, it seemed that the rabbit muscle kinase was mainly phosphorylating serine-110. These results indicate that the two kinases have different specificities. The labelling pattern obtained with the endogenous kinase was unaffected by either cyclic AMP or cyclic GMP.

As indicated in Table 1, the longer term incubation of the isolated myelin basic protein with rabbit muscle kinase results in phosphorylation of both serine and threonine residues. Miyamoto *et al.*[3] have reported that incubation of isolated myelin basic protein with the brain cyclic AMP-dependent kinase also leads to phosphorylation of serine and threonine residues. It will be important to determine whether the specificity of *in vivo* phosphorylation of myelin basic protein resembles that obtained with endogenous myelin kinase or with exogenously added kinases.

With the isolated bovine basic protein and rabbit muscle protein kinase the distribution of ^{32}P was similar to that obtained with the human and rat proteins except that a major spot A, His-Gly-Ser-Lys, was not obtained with the bovine protein as substrate. The sequence in the bovine protein in this region is -Gly-Arg-Ser-Lys- due to the deletion of His-Gly[15]. No major spot containing this serine was found which may indicate that close proximity of the arginine residue to the serine prevented its phosphorylation. Myelin basic proteins are heterogeneous as a result of methylation of arginine-107 (refs 12, 15). As was found with the human and rat proteins, the rabbit muscle protein kinase preferentially phosphorylated serine-110 in the unmethylated form of the bovine protein[4]. It should be emphasised that this site remains accessible when the protein is complexed with other proteins and lipids of myelin. The accessibility of this region in myelin is supported by preliminary experiments which showed that arginine-107 could be readily methylated by methylase I (G. M. Jones and P. R. Carnegie, unpublished).

In summary, although there are 26 possible serine or threonines available for phosphorylation in the protein, only one of these (serine-55) is preferred by the endogenous kinase and one (serine-110) by the added kinase when the protein is complexed in myelin. Miyamoto *et al.*[3] found the basic protein could be phosphorylated *in vivo* but the sites of phosphorylation have not been identified; it cannot be assumed that they will be the same as those demonstrated *in vitro*.

The observation that the myelin basic protein can be specifically phosphorylated opens up new approaches to the study of the possible physiological role of this protein in normal[16], neurodegenerative[17] and neoplastic[18] conditions.

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1 Reddington, M., Rodnight, R., and Williams, M., *Biochem. J.*, **132**, 475–482 (1973).
2 Johnson, E. M., Maeno, H., and Greengard, P., *J. biol. Chem.*, **246**, 7731–7739 (1971).
3 Miyamoto, E., Kakiuchi, S., and Kakimoto, Y., *Nature*, **249**, 147 (1974).
4 Carnegie, P. R., Kemp, B. E., Dunkley, P. R., and Murray, A. W., *Biochem. J.*, **135**, 569–572 (1973).
5 Nishizuka, Y., Inoue, Y., Matsumura, S., Yamamura, H., Kumon, A., Nishiyama, H., and Shimomura, R., in *Abstracts of the Fourth Meeting, International Society Neurochemistry*, 61 (Tokyo, August, 1973).
6 Mockrasch, L. C., in *Methods of Neurochemistry* (edit. by Fried, R.), **1**, 1–29 (Marcel Dekker, New York, 1971).
7 Glynn, I. M., and Chappell, J. B., *Biochem. J.*, **90**, 147–149, (1964).
8 Walsh, D. A., Perkins, J. P., and Krebs, E. G., *J. biol. Chem.*, **243**, 3763–3765 (1968).
9 Kemp, B. E., Frosciò, M., and Murray, A. W., *Biochem. J.*, **131**, 271–274 (1973).

[10] Eng, L. F., Bond, P., and Gerstl, B., *Neurobiology*, **1**, 58–63, (1971).
[11] Gonzalez-Sastre, F., *J. Neurochem.*, **17**, 1049–1056 (1970).
[12] Carnegie, P. R., *Biochem. J.*, **123**, 57–67 (1971).
[13] Murray, K., and Milstein, C., *Biochem. J.*, **105**, 491–495 (1967).
[14] Offord, R. E., *Nature*, **211**, 591–593 (1966).
[15] Eylar, E. H., Brostoff, S., Hashim, G., Caccam, J., and Burnett, P., *J. biol. Chem.*, **246**, 5770–5784 (1971).
[16] Davison, A. N., and Peters, A. *Myelination* (Charles C Thomas, Springfield, Illinois, 1970).
[17] Carnegie, P. R., *Nature*, **229**, 25–28 (1971).
[18] *Nature*, **244**, 130–131 (1973).

In vitro and *in vivo* phosphorylation of myelin basic protein by cerebral protein kinase

A class of cyclic AMP-dependent protein kinases may mediate a wide variety of physiological functions through phosphorylation of enzyme molecules or other cellular proteins[1]. Certain hormones activate these kinases indirectly by causing the synthesis of cyclic AMP in particular organs. The myelin basic protein is a major protein of myelin in vertebrate brains and spinal cords. It has been purified to homogeneity and its amino acid sequence has been established[2,3]. Myelin is a cell membrane substance as evidenced from its ontogenic origin. We wished to know if the basic protein serves as a substrate for a cyclic AMP-dependent protein kinase.

Protein kinase and myelin basic protein were purified from bovine brain according to the method of Miyamoto *et al.*[4] and Oshiro and Eylar[5], respectively.

Myelin basic protein was purified to homogeneity, shown by its migrating as a single band on acrylamide disc gel electrophoresis at *p*H 4.3 (ref. 6). The substrate activity of the myelin basic protein was compared with that of various proteins which are known to be substrates for the protein kinase. As shown in Table 1, ^{32}P was incorporated into myelin basic protein as rapid as into histone, when the proteins were incubated with protein kinase in the presence of ^{32}P-γ-ATP. Dependence of the reaction on cyclic AMP was also clear. Incorporation of phosphate into myelin basic protein was confirmed with gel electrophoresis followed by autoradiography.

The labelled myelin basic protein was hydrolysed in 6 N HCl at 100° C for 4 h to examine into which amino acid residue phosphate was incorporated. The hydrolysate was applied to high voltage paper electrophoresis, and labelled phosphoserine and phosphothreonine were detected. The ratio of phosphorylated serine and threonine residues was 7:9, whereas histone was phosphorylated only at a seryl residue. The phosphorylation of myelin basic protein was inhibited by a modulator of cyclic AMP-dependent protein kinase, as was the phosphorylation of histone[7,8].

TABLE 1 Comparison of phosphorylation of various substrates by cyclic AMP-dependent protein kinase from bovine brain.

Substrate	Amount (μg)	Protein kinase activity (pmol per 5 min per 4 μg enzyme) Without cyclic AMP	With 1 μM cyclic AMP
Histone mixture	200	8.7	79.9
Lysine-rich histone	200	6.3	36.7
Slightly lysine-rich histone	200	4.1	31.7
Myelin basic protein	200	2.4	22.5
Arginine-rich histone	200	2.3	18.9
Casein	500	1.2	6.0
Protamine	500	2.4	4.3
Phosvitin	500	0.5	0.6
Bovine serum albumin	500	0.7	0.8

The phosphorylation of myelin basic protein was proportional to the time of incubation. After 24 h a plateau was reached, with 3.8 mol of phosphate incorporated into the protein molecule, indicating that the protein has four residues which accept phosphate. The extensively phosphorylated protein migrated more slowly, as was expected.

It seemed necessary to prove the *in vitro* phosphorylation of myelin basic protein under more physiological conditions. The myelin fraction was prepared from rat brains according to the method of Uyemura *et al.*[9] and was incubated in the presence of ^{32}P-γ-ATP. Phosphorylation of myelin basic protein by endogenous protein kinase contained in the myelin fraction was demonstrated. Incorporation of phosphate was examined by hydrochloric acid extraction of the basic protein followed by disc gel electrophoresis. Autoradiography and determination of radioactivity in slices of the gel verified the phosphorylation of the basic protein.

We then performed an *in vivo* experiment. We injected 6.1 μCi ^{32}P-orthophosphate into the ventricle of rat brain by the method of Hayden *et al.*[10] The brains were dissected 30 min after the injection and myelin basic protein was purified. Incorporation of labelled phosphate into the protein was demonstrated by autoradiography of disc gel electrophoresis.

The final proof was obtained by the demonstration of the presence of phosphoseryl and phosphothreonyl groups in native myelin basic protein from bovine brain. The contents of the two amino acid residues of the protein was determined by acid hydrolysis and electrophoretic separation of the phosphoamino acids followed by amino acid analysis. Each molecule of bovine myelin basic protein contains 0.07 mol of phosphoserine and 0.09 mol of phosphothreonine residues. The value was calibrated by a partial loss of the phosphoamino acids at acid hydrolysis.

So it is clear that myelin basic protein is present in the brain in a partially phosphorylated form, and we think that the protein is in a dynamic state between phosphorylated and dephosphorylated forms. Methylation and glucosylation of myelin basic protein have been already established. Until now myelin has been considered only as a stable component of neurones, but its function should be reconsidered.

In vitro phosphorylation of myelin basic protein was reported by Dr Carnegie after this study was completed at the 4th International Meeting of the International Society of Neurochemisry. Details of these experiments will be described elsewhere.

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[1] Greengard, P., and Kuo, J. F., in *Role of Cyclic AMP in Cell Function* (edit. by Greengard, P., and Costa, E.), 287 (Raven, New York, 1970).
[2] Carnegie, P. R., *Biochem. J.*, **123**, 57 (1971).
[3] Eylar, E. H., Brostoff, S., Hashim, G., Caccam, J., and Burnett, P., *J. biol. Chem.*, **246**, 5770 (1971).
[4] Miyamoto, E., Petzold, G. L., Kuo, J. F., and Greengard, P., *J. biol. Chem.*, **248**, 179 (1973).
[5] Oshiro, Y., and Eylar, E. H., *Archs Biochem. Biophys.*, **138**, 392 (1970).
[6] Reisfeld, R., Lewis, U., and Williams, D., *Nature*, **195**, 281 (1962).

[7] Donnelly, T. E., jun., Kuo, J. F., Reyes, P. L., Liu, Y. P., and Greengard, P., *J. biol. Chem.*, **248**, 190 (1973).
[8] Donnelly, T. E., jun., Kuo, J. F., Miyamoto, E., and Greengard, P., *J. biol. Chem.*, **248**, 190 (1973).
[9] Uyemura, K., Tobari, C., Hirano, S., and Tsukada, Y., *J. Neurochem.*, **19**, 2607 (1972).
[10] Hayden, J. F., Johnson, L. R., and Maickel, R. P., *Life Sci.*, **5**, 1509 (1966).

The neurogenic and myogenic hypotheses in human (Duchenne) muscular dystrophy

X-LINKED recessive (Duchenne) muscular dystrophy (DMD) is the most common, consistent, and disastrous form of the diseases in man. Evidence that it is neurogenic[1] is open to criticism[2,3]. Though an apparently similar condition in mice, often used as a model, may be neurogenic[4], its different serology[5] and mode of inheritance[6] suggest a fundamental dissimilarity. The following observations on the human disease bring new evidence to bear on the issue.

In mammalian skeletal muscle, each fibre has near its mid-point a single endplate supplied by a single nerve fibre; more than one endplate is a rarity[7–9]. The axon of each lower motor neurone branches repeatedly to supply in this way very many muscle fibres[10] often widely scattered through the muscle[11,12].

If DMD were neurogenic and mediated by the lower motor neurone, then in afflicted males the dystrophic X chromosome in every neurone should render all the musculature dystrophic, as in fact it is[13]. Likewise, in carriers of DMD, the normal early random inactivation as stable[14] sex chromatin of one X chromosome in every female somatic cell[15], including neurones[16], would give an individually proportioned mosaic

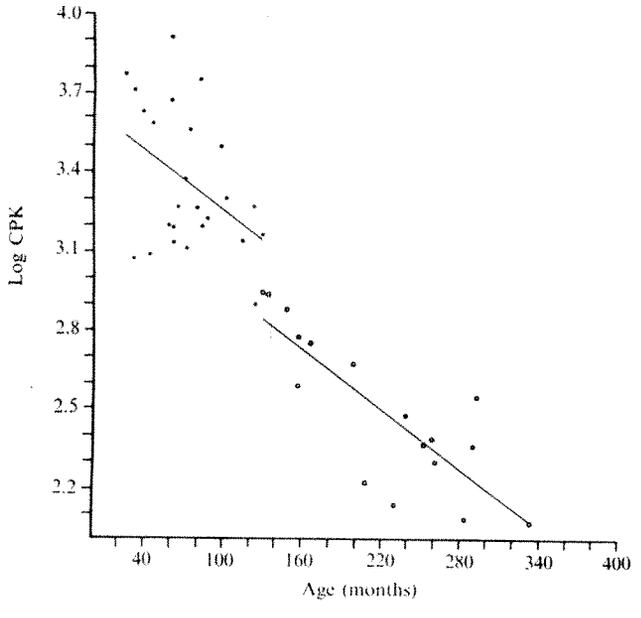


FIG. 1 Linear regressions of log(CPK) on age in sera from boys with clinically classical DMD, showing intercept and slope in equations providing exponential decay rate constant Kd and halflife $t_{1/2}$ of CPK values. Serum CPK throughout is assayed enzymatically[19] and expressed in International Units as mU ml^{-1} at 25° C, with male and female normal upper limits of 58.1 and 37.7 mU ml^{-1} respectively. ●, DMD ambulant: $n = 25$; $y = 3.61886 - 0.003787x$, ($t = -2.22477$ on 33 d.f.; $P < 0.05$), Kd = 0.00872 per month, $t_{1/2} = 79.49036$ months (6.6242 yr); ○, DMD wheelchair: $n = 17$, $y = 3.318915 - 0.0037813x$ ($t = -5.70201$ on 15 d.f.; $P < 0.001$), Kd = 0.008707 per month, $t_{1/2} = 79.61014$ months (6.6342 yr).

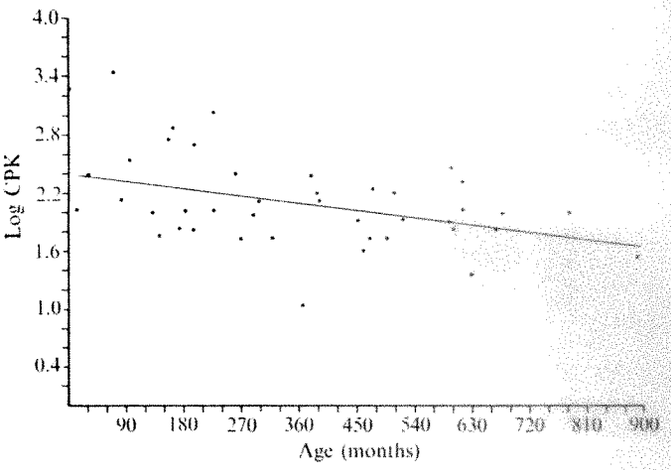


FIG. 2 Linear regression of log(CPK) on age in sera from female DMD carriers positively identified genetically and biochemically[21]. $n = 41$, $y = 2.398989 - 0.000848x$ $t = -2.87954$ on 39 d.f.; $P < 0.01$), Kd = 0.001953 per month, $t_{1/2} = 354.9886$ months (29.5824 yr).

of dystrophic motor neurones governed by the dystrophic X chromosome and healthy neurones by the normal X chromosome. Muscle fibres innervated by the former would then be wholly dystrophic, decaying as quickly as in afflicted males, but healthy if innervated by the latter. Thus carrier muscle should show a dual population of fibres, with clear-cut distinctions between them evident at biopsy. No such distinctions are seen, however. Instead, diffuse general changes are specifically noted[17], and recent electron microscopic findings seem to show all carriers affected[18]. Nor, as we now show, is there biochemical evidence for the expected rapid decay of a population of dystrophic fibres.

In DMD, the easily-measured[19] serum elevations of the muscle-specific enzyme creatine phosphokinase (CPK) due to the disease decline with age as the muscle mass diminishes, and covariance analysis of the data in Fig. 1 shows a constant process in which this exponential rate of decay of CPK is not significantly different between ambulant and wheelchair cases ($F = 0.0004$, 1 and 38 d.f.), with a joint estimate of slope of 0.00379 thus defining the disease. This slope gives estimated intercepts of 3.618 and 3.320 respectively, with a significant difference of about 0.3 ($F = 5.36$. 1 and 39 d.f., $P < 0.05$) implying on taking antilogs that CPK elevations are almost halved on transition from ambulation to the wheelchair. A third determinant is thus apparent, so that CPK in DMD specifically measures active dystrophic muscle mass, and may precisely assess prospective DMD therapy if activity and mass remain little changed over a short period.

In DMD the lifespan rarely exceeds 25 yr, but is apparently normal in carriers. Then if DMD were neurogenic, carriers should show initially a similar steep slope during decay of the dystrophic fibres, becoming horizontal later as in normal women[19]. Figure 2, however, shows a much gentler slope throughout, with covariance analysis giving no significant difference in the slopes before and after 30 yr. Its exponential rate of decline may be due to variable local anatomical disadvantages causing successive disappearance of a small number of fibres throughout life, occasionally evident at biopsy[17]. Its slightness is emphasised by consecutive CPK analyses over 3–7 yr in 22 carriers giving individual slopes neither significantly different from zero nor correlating significantly with initial age; clearly so small an interval with such a slope gives little information. In older carriers, moreover, derivation of probable earlier values from present CPK elevations gives reasonable results (obviously) if the

slope for DMD carriers is used, but unacceptably high values if that for DMD ambulant is used, indicating that wholly dystrophic fibres cannot account for present persistent increases.

Further, the collateral reinnervation by other motor axon sprouts found in denervated muscle fibres[20] has now been described in the fragmenting fibres of DMD[3], particularly in the evolving disease but not in the preclinical or terminal stages, with either minimal alteration or excessive damage. Then on the neurogenic view, if dystrophic carrier fibres decayed as quickly as in DMD itself, proportional reinnervation by adjacent normal axon sprouts and restoration towards normal of some dystrophic fibres should subtract them even more quickly, with a correspondingly more rapid decline of CPK than in DMD. Clearly this does not occur.

These findings and the wide range of sustained manifestation in DMD carriers, from undetectable to markedly abnormal[21], are readily explained by random fusion during early growth of mononucleate myoblasts from clones with one or other X chromosome already inactivated, to give a dual population of nuclei, in different proportions for different individuals, in the mature multinucleated muscle cell[17]. In contrast, a neurogenic view based on orthodox carrier anatomy does not show how the commanding influence of a dystrophic lower motor neurone may be so extensively moderated by a healthy upper motor neurone and other connections, or *vice versa*, to give so wide a range of individually homogeneous carrier manifestation. Nor does it show how in DMD the invariable myocardial involvement, eventually lethal in patients and generally detectable even in carriers[22], occurs without comparable innervation; no such involvement is seen[22] in the putatively neurogenic[23] limb girdle type of muscular dystrophy. The myogenic view of DMD, however, does explain these observations, and if accepted in carriers applies equally to the disease itself in males.

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1 McComas A. J., Sica, R. E. P., and Currie, S., *Nature*, **226**, 1263–1264 (1970).
2 Scarpalezos, S., and Panayiotopoulos, C. P., *Lancet*, **ii**, 458 (1973).
3 Desmedt, J. E., and Borenstein, S., *Nature*, **246**, 500–501 (1973).
4 Hironaka, T., and Miyata, Y., *Nature new Biol.*, **244**, 221–223 (1973).
5 Schapira, F., and Dreyfus, J.-C., *Enzymol. biol. clin.*, **3**, 53–57 (1963).
6 Stevens, L. C., Russell, E. S., and Southard, J. L., *Proc. Soc. exp. Biol. Med.*, **95**, 161–164 (1957).
7 Schwarzacher, H. G., *Acta. anat., Basel*, **30**, 758–774 (1957).
8 Guth, L., *Physiol. Rev.*, **48**, 645–687 (1968).
9 Le Gros Clark, W. E., *The Tissues of the Body* (Clarendon Press, Oxford, 1971).
10 Feinstein, B., Lindegård, B., Nyman, E., and Wohlfart, G., *Acta anat., Basel*, **23**, 127–142 (1955).
11 *Gray's Anatomy* (edit. by Warwick, R., and Williams, P. L.) 482 (Longman, Edinburgh, 1973).
12 Edström, L., and Kugelberg, E., *J. Neurol. Neurosurg. Psychiat.*, **31**, 424–433 (1968).
13 Bonsett, C. A., *Neurology, Minneap.*, **13**, 728–738 (1963).
14 Migeon, B. R., *Nature*, **239**, 87–89 (1972).
15 Lyon, M. F., *Nature*, **190**, 372–373 (1961).
16 Moore, K. L., in *The Sex Chromatin* (edit. by Moore, K. L.) 19 (W. B. Saunders Co., 1966).
17 Emery, A. E. H., *J. med. Genet.*, **2**, 1–7 (1965).
18 Afifi, A. K., Bergman, R. A., and Zellweger, H., *J. Neurol. Neurosurg. Psychiat.*, **36**, 643–650 (1973).
19 Sweetin, J. C., and Thomson, W. H. S., *Clin. chim. Acta*, **48**, 49–63 (1973).
20 Wohlfart, G., *Neurology, Minneap.*, **8**, 175–180 (1958).
21 Thomson, W. H. S., *Clin. chim. Acta*, **26**, 207–221 (1969).
22 Emery, A. E. H., *Br. med. J.*, **2**, 418–420 (1969).
23 Sica, R. E. P., and McComas, A. J., *J. Neurol. Neurosurg. Psychiat.*, **34**, 469–474 (1971).

Filaments and ribbons in vertebrate smooth muscle

Since Lowry *et al.*[1] found X-ray evidence for the presence of longitudinally ordered myosin in vertebrate smooth muscle there has been much debate on the precise form in which it is present. Small *et al.*[2,3] have found well ordered and well preserved broad ribbon-shaped elements (80 Å wide and 200–1100 Å broad) in micrographs of vertebrate smooth muscle fixed after relaxation in isotonic and hypertonic salines. They believe that these ribbons are the myosin contractile elements and support this view with the observation that the meridional X-ray reflection of 143 Å (indicative of an ordered myosin structure) could arise from such ribbons.

Choi[4], the first to observe thick filaments in vertebrate smooth muscles, found narrow ribbon-shaped structures in his preparations and so did Fawcett[5]. Many others, however, reported thick filaments in their preparations, either ribbon shaped, polygonal, or circular in cross section[6–14].

Vertebrate smooth muscle myosin is very labile and its demonstration in fixed preparations depends on preparative procedures[13,15], therefore contradictory results were not unexpected and the importance of carrying out all X ray and electron microscopic work in conditions which correspond as nearly as possible to those *in vivo* must be emphasised. We think for the following reasons that work at low temperatures is unsatisfactory, and report studies on muscles relaxed at 37° C.

Lowy *et al.*[1] showed that the 143 Å reflection is best observed in muscles relaxed by cooling, and attributed the increase in intensity of the 143 Å reflection when the muscle is cooled as due only to suppression of movement of cross-bridges upon relaxation[19] but at 0° C, changes in ionic content take place[16] and in particular Ca ions are taken up by the tissue[17] and the myosin appears to have undergone configurational changes[13]. Furthermore, we observe in the electron microscope that when taenia coli is equilibrated in ice-cold isotonic Bülbring-Golenhofen saline[18], as used by Lowy and also by Small, for different periods of time (30 min and 2, 4, 6 and 24 h) and the muscle is then fixed in 2.5% Taab glutaraldehyde buffered with 0.1 M cacodylate buffer at *p*H 7.0, the shape of the thick filaments varies with the period of equilibrium. After 30 min and 2, 4 or 6 h the thick filaments have mixed shapes, narrow ribbons, polygonal or circular as commonly seen with this method of fixation. After 24 h the thick filaments are mainly ribbon shaped (some quite broad ribbons can be observed—up to 770 Å wide) and some cell shrinkage has often occurred.

Like Small *et al.*[2,3] we observe mostly ribbon-shaped thick filaments in taenia coli fixed with acrolein[20] after equilibrating the muscle for only 2 h in ice-cold saline before fixation, probably because acrolein penetrates more rapidly than glutaraldehyde (Fig. 1).

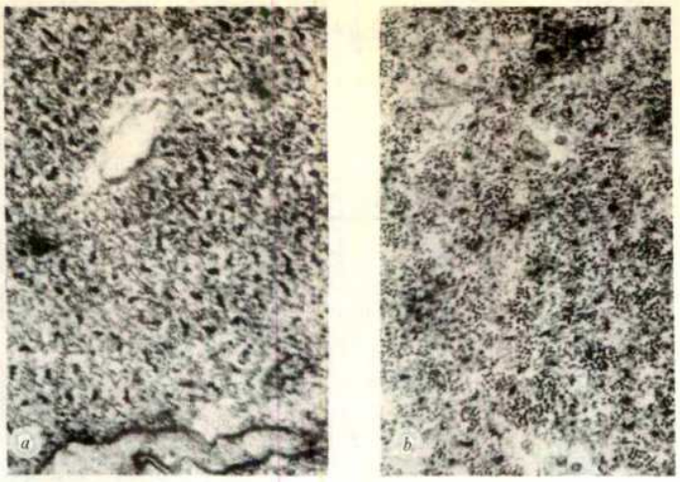


FIG. 1 *a*, Guinea pig taenia coli muscle equilibrated for 2 h in oxygenated Bülbring Golenhofen saline at 4° C. Primary fixation in acrolein[20]. Sucrose was omitted from the fixative. Fixation was followed by an overnight wash in 0.1 M cacodylate buffer, *p*H 7.0, with additional 7% sucrose. Post fixation was in 1% osmium tetroxide dissolved in the washing solution. The muscle was under load throughout equilibration and fixation. Transverse section: note ribbons 80 Å by 320–560 Å (×50,000). *b*, Guinea pig taenia coli muscle equilibrated for 1 h in oxygenated Bülbring–Golenhofen saline at 37° C. The muscle was then relaxed by the addition of xylocaine to the muscle bath (final concentration of xylocaine 1.5 mM). For the primary fixation 5% glutaraldehyde was added to the muscle bath. The muscle was under isometric tension and the tension was recorded throughout equilibration, the addition of xylocaine and primary fixation. Fixation was followed by an overnight wash in 0.1 M cacodylate buffer, *p*H 7.0, with additional 7% sucrose. Post fixation was in 1% osmium tetroxide dissolved in the washing solution. In this transverse section the thick filaments have ribbon shaped, polygonal and circular profiles. 80 Å by 160–320 Å (×50,000).

The size of the ribbons and the degree of shrinkage are very much enhanced if the muscles are equilibrated in cold hypertonic salines and indeed the 143 Å X-ray reflection is usually stronger and much clearer in a hypertonic solution at 4° C than it is in an isotonic solution (Fig. 2).

Also, if a muscle equilibrated for 24 h in the cold is transferred to an oxygenated isotonic saline at 37° C and the tension is recorded, it takes at least 30–40 min for the reappearance of activity and tension development, probably the time necessary for the ion concentration to return to normal and the ribbons to regain their normal shape.

We think therefore that the muscle undergoes considerable changes when it is cooled, and muscles should be studied at normal physiological temperatures (37° C).

Lowy *et al.*[19] relaxed muscles at 37° C by removing calcium from the bathing solution, but such treatment does not make the 143 Å reflection stronger than that of a contracting muscle unless the bathing solution is also made hypertonic (a treatment which enhances the reflection in the cold). We decided to work on muscles equilibrated in an isotonic saline at 37° C and to relax the muscle with a drug if one could be found which did not affect the contractile mechanism of the muscle. The anaesthetic xylocaine (suggested to us by Dr Kiernan of the Health Sciences Centre, University of Western Ontario, Canada) proved satisfactory. When the drug was added to the organ bath in a concentration of approximately 1.5 mM the muscle lost all tension and activity, and showed no sign of activity or development of tension for periods lasting as long as 24 h. If after 24 h the xylocaine was washed away, the muscle recovered normal activity within minutes, suggesting that only the membrane had been affected by the drug. In addition, P. J. Goodford and G. S. Wootton measured the wet weights and the extracellular space of muscles equilibrated in isotonic saline at 37° C with or without the addition of xylocaine and calculated from these measurements that although the cell volume decreased slowly in the control muscles, it stayed unchanged after a slight initial shrinkage in the xylocaine treated tissue. Therefore, in the xylocaine treated material, there is not likely to be an associated effect on the structure of the thick filaments which could affect the X-ray or electron microscope observations.

X-ray diffraction patterns were taken of taenia coli from adult guinea pigs equilibrated at 37° C in oxygenated Bülbring–Golenhofen saline after the muscle had been relaxed by the addition of xylocaine. The X-ray patterns were taken with a mirror monochromator camera on an Elliott G×13 X-ray generator; the tension of the muscle was monitored throughout. At 37° C the 143 Å reflection was not visible in xylocaine relaxed muscles although the actin equatorial reflections and the collagen reflections were good, so we should have seen the 143 Å reflection had it been present: one muscle was cooled to 4° C and the 143 Å reflection did then become visible.

Electron microscope observations were carried out on taenia coli from young guinea pigs (weighing less than 300 g), the muscles were treated in the same way as those used for X-ray observations and when they had relaxed completely after addition of the xylocaine they were fixed by addition of 2.5% Taab glutaraldehyde to the organ bath. The primary fixation which lasted for 1.5 h was carried out at 37° C, washing, post fixation and dehydration proceeded as previously described[14]. Thick filaments were present in many of the cells, but were not always numerous enough to account for all the myosin present in the cells. In cross section an assortment of narrow ribbons, polygonal and circular shapes were observed. (Fig. 1*B*). It has so far not been possible to observe a preponderence of narrow ribbons in cells from muscles after equilibration at 37° C.

Thus, in conditions in which the muscles can contract, when ionic and temperature conditions are appropriate for contraction we can find no X ray or electron microscopic evidence for the presence of broad ribbons.

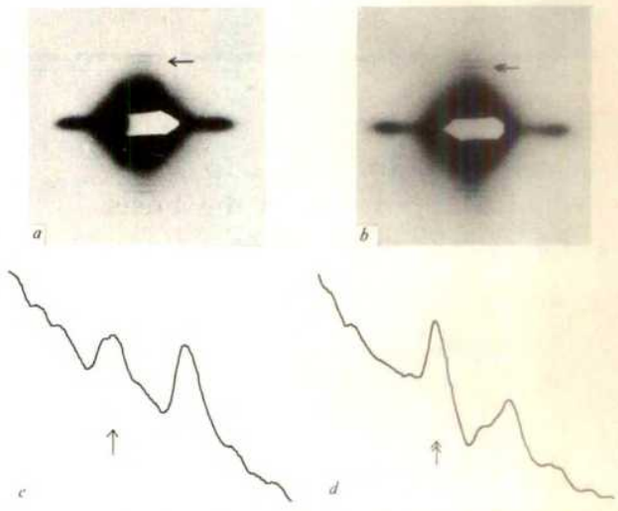


FIG. 2 *a*, X-ray diffraction pattern of a muscle in Bülbring-Golenhofer saline at 5° C; *b*, densitometer trace of part of the meridian of pattern *a*, showing the 143 Å reflection (arrowed) weaker than the adjacent collagen reflection; *c*, X-ray diffraction pattern and *d*, densitometer trace of a muscle in a hypertonic (480 mOsmole) saline solution at 8° C. The 143 Å reflection (arrowed) is stronger than the adjacent collagen reflection. The actin equatorial reflection is much sharper and corresponds to a smaller separation of actin filaments than in the normal saline solution.

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1 Lowy, J., Poulsen, F. R., Vibert, P. J., *Nature*, **225**, 1053 (1970).
2 Lowy, J., and Small, J. V., *Nature*, **227**, 46 (1970).
3 Small, J. V., and Squire, J. M., *J. molec. Biol.*, **67**, 117 (1972).
4 Choi, J. K., *Proc. 5th Int. Conf. Electron Microscopy* (Philadelphia M9, 1962).
5 Fawcett, D. W., *Atlas of Fine Structure, The Cell: Its Organelles and Inclusions*, 243 (Saunders, Philadelphia, 1966).
6 Nonomura, Y., *J. Cell Biol.*, **39**, 741 (1968).
7 Rice, R. V., Moses, J. A., McManus, G. M., Brady, A. C., and Blasik, L. M., *J. Cell Biol.*, **47**, 183 (1970).
8 Heumann, H., *Cytobiologie*, **3**, part 2, 259 (1971).
9 Garamvölgyi, N., Vizi, E. S., and Knoll, J., *J. Ultrastruct. Res.*, **34**, 135 (1971).
10 Somlyo, A. P., Devine, C. E., Somlyó, A. V. and North, S. R., *J. Cell Biol.*, **51**, 722 (1971).
11 Somlyo, A. P., Somlyo, A. V., Devine, C. E. and Rice, R. V., *Nature new Biol.*, **231**, 243 (1971).
12 Cooke, P. H., and Fay, F. S., *Expl Cell Res.*, **71**, 265 (1972).
13 Shoenberg, C. F., *Phil. Trans. R. Soc.*, **263**, 265 (1973).
14 Shoenberg, C. F., Goodford, P. J., Wolowyk, M. W. and Wootton, G. S., *J. Mechanochem. Cell Motility*, **2**, 69 (1973).
15 Needham, D. M., and Shoenberg, C. F., *Proc. R. Soc.*, **B160**, 517 (1964).
16 Freeman-Narrod, M., and Goodford, P. J., *J. Physiol., Lond.*, **163**, 399 (1962).
17 Bauer, H., Goodford, P. J., and Hüter, J., *J. Physiol., Lond.*, **176**, 163 (1965).
18 Bülbring, E., and Golenhofen, K., *J. Physiol., Lond.*, **193**, 213 (1967).
19 Lowy, J., Vibert, P. J., Haselgrove, J. C., and Poulsen, F. R., *Phil. Trans. R. Soc.*, **B265**, 191 (1973).
20 Robison, W. G., and Lipton, B. H., *J. Cell Biol.*, **43**, 117a (1969).

Production of antibodies to tetrahydrocannabinol as the basis for its radioimmunoassay

TETRAHYDROCANNABINOL (THC) is the main pharmacologically active constituent of cannabis. A sensitive method for its measurement in plasma would be expected to have important clinical, experimental, and forensic uses. Brief descriptions of the production of antibodies to THC have appeared in the past[1–3]. In one case[2], the antibodies were used to bind THC and its derivatives on a quantitative basis involving measurement of the inhibition of antibody fluorescence quenching or enhancement of hapten fluorescence when bound to antibody.

Here we use radioactively-labelled THC to detect the presence of antibodies. Each of six sheep was immunised with a different conjugate of THC and bovine serum albumin (BSA). Five milligrams of conjugate were dissolved in 2 ml of sterile water and emulsified with 4 ml of Freund's complete adjuvant. The mixture was injected intramuscularly into six sites in the medial surface of the thighs of a sheep. Thereafter animals were injected with 2.5 mg of conjugate once a month and bled 7–10 d after each injection. One sheep produced an antiserum capable of binding ^{3}H-THC and this material was investigated further. The conjugate which provoked antibody production was produced by preparation of the chlorocarbonate derivative of THC with phosgene and subsequent coupling of the derivative to BSA by the Schotten-Baumann reaction[4]. By the inclusion of a small amount of ^{3}H-THC in the process, it was shown that an average of 12 haptens were conjugated to each BSA molecule.

^{3}H-THC (2 μCi μg^{-1}) was used to assess the binding capacity of the sheep antisera. One nanogram of labelled THC was incubated for 1 h in a mixture containing a 1:12 dilution of antiserum. The diluent buffer was 0.04 M phosphate, *p*H 7.4, containing 0.15 M sodium chloride and 0.2% bovine gammaglobulin. Separation of the free and antibody-bound fractions of the tracer was effected with 5% uncoated charcoal (UCC)[5]. Aliquots of the supernatant, containing antibody-bound tracer, were taken for β-counting (Table 1).

TABLE 1 Assay protocol

	100%	Zero	Test
Diluent buffer	0.5	0.4	0.4
Normal serum (1:2)	—	0.1	—
Antiserum (1:2)	—	—	0.1
^{3}H-THC (1 ng)	0.1	0.1	0.1
UCC (5%)	—	0.2	0.2

Values indicate volumes in ml. Sera were diluted with diluent buffer. ^{3}H-THC was stored in dioxane and an aliquot diluted with diluent buffer before use. The incubation time was 1 h at 4°C. UCC was 5% charcoal in phosphate-buffered saline.

Table 2 shows the degree of binding of ^{3}H-THC tracer exhibited by 1:12 dilutions of sera collected after successive booster immunisations. The results are expressed as the % of tracer present in the antibody-bound fraction (% bound). With serum taken following the third boost, the decrease in tracer binding produced by increasing amounts of unlabelled THC was measured. Figure 1 shows the calibration curve obtained with a 1:12 dilution of antiserum. The

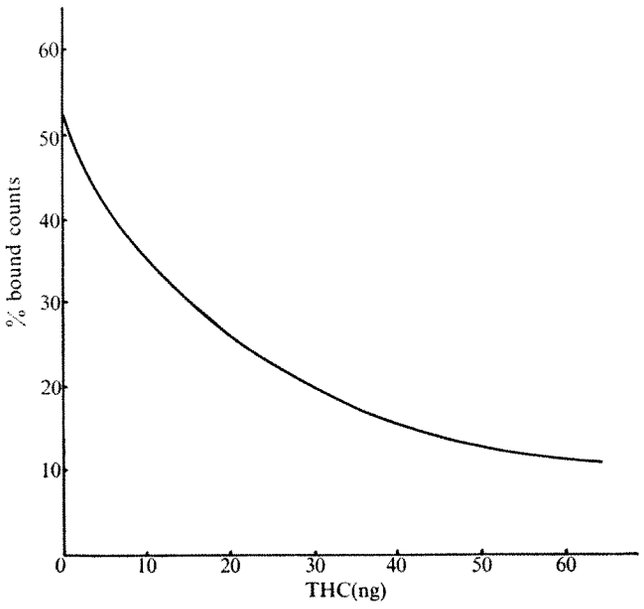


FIG. 1 Inhibition of tracer antibody binding by the addition of increasing amounts of THC to the assay system. The antiserum was used at a 1:12 final dilution and was that obtained following the third booster immunisation. Labelled and unlabelled THC were added simultaneously to the assay system and the incubation time was 1 h at 4° C.

TABLE 2 The ability to bind tracer of 1:12 final dilutions of the sera taken following monthly booster immunisations

Booster	% bound counts
1st	15.2
2nd	21.0
3rd	51.5

smallest amount of unlabelled THC producing measurable inhibition of ^{3}H-THC tracer binding was 2 ng. With higher specific activity tracer, it should be possible to lower the detection limit appreciably. Such a tracer would allow smaller amounts to be used in an incubation system containing less antiserum, and could constitute the basis for an immunological assay for THC with a sensitivity equal to or greater than those of physicochemical methods already in use[6].

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1 Gross, S. J., Grant, J. D., Bennett, R., Wong, R., and Lomax, P., *Steroids*, **18**, 555 (1971).
2 Grant, J. D., Gross, S. J., Lomax, P., and Wong, R., *Nature new Biol.*, **236**, 216 (1972).
3 Levine, L., *Pharmac. Rev.*, **25**, 293 (1973).
4 Erlanger, B. F., Borek, F., Beiser, S. M., and Lieberman, S., *J. biol. Chem.*, **228**, 713 (1957).
5 Binoux, M. A., and Odell, W. J., *J. clin. Endocr. Metab.*, **36**, 303 (1973).
6 Agurell, S., Gustafsson, B., Holmstedt, B., Leander, K., Lindgren, J.-E., Nilsson, I., Sandberg, F., and Asberg, M., *J. Pharm. Pharmac.*, **25**, 554 (1973).

Structural analysis of human proparathyroid hormone by a new microsequencing approach

RECENT evidence indicates that the synthesis of several polypeptide hormones, including parathyroid hormone, involves the production of precursor molecules larger than their corresponding hormones[1–5]. The hormones are generated intracellularly by specific enzymatic cleavage. This process may represent an important control mechanism for hormonal synthesis, cellular storage and eventual secretion of the hormone. Thus complete structural characterisation of both precursor and product molecules for further peptide hormones in several species is clearly desirable. The precursor forms are often present in extremely low concentrations in glandular tissue, however, rendering difficult their isolation and sequence analysis by conventional chemical methods.

We describe here a microsequencing procedure based on automated Edman degradation of internally labelled polypeptides isolated in trace quantities from *in vitro* tissue incubation systems. We used this method, which is about 10,000 times more sensitive than the conventional technique for determination of amino acid sequences[6], to confirm the structure of bovine proparathyroid hormone[7], and then to determine the structure of human proparathyroid hormone.

The bovine and human prohormones of parathyroid hormone were biosynthesised *in vitro* in tissue slices from bovine and human glands. During this process, radioactive lysine, serine, valine, and arginine were incorporated into the prohormone sequences (see Fig. 1 legend for details). These amino acids were chosen because they constitute the residues of the known bovine prohormone-specific hexapeptide. The prohormone fractions were isolated as described previously[1] and sequentially degraded in the protein sequenator.

The results of the sequence analyses are shown in Fig. 1. The sequence of the amino terminal portion of bovine prohormone is the same as that determined by conventional techniques[7]; human proparathyroid hormone contains an amino terminal hexapeptide sequence identical to that of the bovine prohormone. At each cycle of the degradation where an incorporated radioactive residue was released from the prohormone, a clearly detectable, five to ten-fold increase in radioactivity above background was observed. The identity of each residue was confirmed by thin-layer chromatography of the radioactive amino acids removed at each degradation cycle. Approximately 85% of the radioactivity in each aliquot comigrated with the appropriate amino acid–phenylthiohydantoin standards.

We used two controls on the accuracy of the sequence analysis by the radioactive method. First, the analyses were continued for at least twelve cycles to overlap into the bovine and human parathyroid hormone structures already determined by conventional techniques[8–11], where the respective radioactive amino acids were detected in their proper positions (Fig. 1). Second, each cycle of the degradation was monitored for completeness of reaction by using gas–liquid chromatography[12] to measure the molar yield of each residue released from sperm whale apomyoglobin, which was used as a carrier protein in the sequence analyses. By this method each degradation could be shown to have a repetitive yield in the range of 92–94%[13].

Since the additional amino acid residues present in the prohormone-specific sequence are not required for the expression of hormonal activity, one might expect, as with bovine and human proinsulin[4], more amino acid mutations in the prohormone-specific peptide than in the active region or secreted form of the hormone itself. If, however, the proparathyroid hormone-specific sequence, which is identical in human and bovine proparathyroid hormones, is found in other species too, this may reflect constraints in sequence variation which are important for the efficient transformation of prohormone to hormone. Since the sequence of the biologically active region of human parathyroid hormone (first thirty-four residues) is known[10–11], a synthetic peptide[14–15] can now be prepared that comprises the active portion of the human hormone with the additional prohormone-specific hexapeptide added to its amino terminal end. With this peptide, it should be possible to develop a radioimmunoassay for detection of the human prohormone, by techniques used previously with the bovine prohormone[16]. Such an assay should help to delineate the physiological importance of proparathyroid hormone in man.

This highly sensitive microsequencing method represents the culmination of efforts in our laboratory and elsewhere to reduce significantly the quantity of protein or polypeptide needed for structural studies. Conventional non-radioactive Edman methods require at least 100 nmol of starting protein to accomplish a twenty-cycle degradation[13]. Our earlier use of a radioactive coupling reagent, ^{35}S-phenylisothiocyanate, for the automated Edman degradation, reduced the required quantity of protein to 5 nmol[17]. Recently we developed another microsequencing method, the degradation of radioiodinated proteins. The method is useful in determining the position of tyrosine and histidine residues in the sequence of polypeptides, available in only picomole quantities[13]. With the degradation of internally labelled proteins described here,

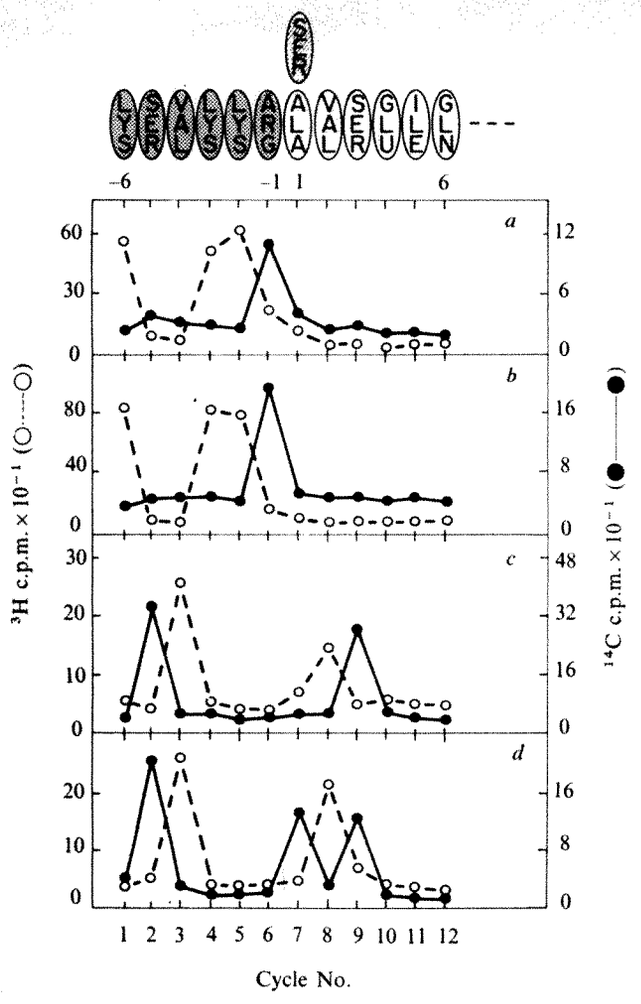


FIG. 1 Sequence analysis of radiolabelled human and bovine proparathyroid hormone. Bovine parathyroid glands were obtained from freshly slaughtered calves, and human parathyroid adenomas came from patients with hyperparathyroidism at the time of operation. Aliquots of about 100 mg of the tissues were incubated for 1 h at 37° C in 2 ml of Earle's balanced salt solution supplemented with 5% foetal calf serum, essential and non-essential amino acids (5 mm) and either a mixture of ^{3}H-lysine (26.8 Ci mmol^{-1}) and ^{14}C-arginine (0.31 Ci mmol^{-1}) or ^{3}H-valine (4.8 Ci mmol^{-1}) and ^{14}C-serine (0.15 Ci mmol^{-1}). The radiolabelled bovine and human prohormones were isolated from the tissue by extraction in 8 M urea–0.2 N HCl and electrophoresis on polyacrylamide gels as described previously[1]. Approximately 0.5 μg (50 pmol) of each of the specifically labelled prohormones containing about 20,000 c.p.m. ^{3}H and 5,000 c.p.m. ^{14}C were isolated by this procedure. The radiolabelled prohormones were dissolved in 0.2 ml of 20% acetic acid, to which 2 mg of sperm whale apomyoglobin was added as carrier protein during automated Edman degradation[6,20]. The prohormones were sequentially degraded for twelve cycles in the Beckman 890 C sequencer using a standard protein program as described by Edman and Begg[6]. A 20 nmol sample of the appropriate unlabelled phenylthiohydantoin amino acids (lysine and arginine or, alternatively, serine and valine) were added to each tube of the fraction collector before the start of degradation to protect against absorptive losses of the trace amounts of labelled amino acids. At the completion of the twelve-cycle degradation, aliquots were taken from each fraction collected and were counted directly in Liquiflour (NEN) in a Packard dual channel spectrometer at a setting which allowed independent measurement of ^{3}H and ^{14}C in the same vial. The remainder of the sample was allocated for thin-layer and gas-liquid chromatography. At each step in the degradation, where a peak of radioactivity appeared, the phenylthiohydantoin derivative of the labelled amino acid was identified by chromatography on silica gel thin-layer plates using solvent systems described previously[6,13]. Unlabelled phenylthiohydantoins were used as markers for thin-layer chromatography. The amino acid phenylthiohydantoins were scraped from the plate and counted for ^{14}C or ^{3}H activity at each successive cycle of the degradation. Release of radioactive amino acids (vertical axes) was plotted against cycle of the degradation (lower horizontal axis). At the top of the figure, in ovals, is the known amino terminal sequence of bovine proparathyroid hormone. The shaded ovals represent the hexapeptide sequence, specific to the prohormone numbered −6 to −1, with the amino terminal bovine hormone sequence beginning at position 1. The human prohormone has an identical amino-terminal sequence with the bovine species with the exception of position 1, where a serine (cross-hatched oval above the bovine sequence) represents a human specific mutation in parathyroid hormone. *a*, bovine ^{3}H-lysine and ^{14}C-arginine; *b*, human ^{3}H-lysine and ^{14}C-arginine; *c*, bovine ^{3}H-valine and ^{14}C-serine; *d*, human ^{3}H-valine and ^{14}C-serine.

sequence analysis involving all amino acids is possible with picomole quantities of polypeptide. Analyses reported here were carried out on 10 pmol of proparathyroid hormone.

Certain issues concerning the use of a carrier protein to protect trace quantities of polypeptide from chemical and mechanical losses during automated Edman degradation have been clarified[18]. The use of such carriers to achieve protected degradation is essential for the success of micromethods. In our earlier work polypeptides to be sequenced were not radioactive; rather, increased sensitivity was achieved with ^{35}S-phenylisothiocyanate. In this method, synthetic polypeptide carriers containing non-natural amino acids are necessary to avoid interference with the identification of the unknown sequence. In our new method internal labelling through biosynthesis enabled us to use as carrier a natural protein of known sequence; thereby conventional sequence analysis of the carrier protein provides a valuable monitor of the efficiency of each step of degradation of the trace, radiolabelled peptide.

Microsequencing methods involving radioactive amino acids promise to be generally applicable to the determination of the primary structure of trace quantities of other biologically important peptides. The work described here, as well as a study describing the sequence positions of a single radioactive residue (leucine) in a precursor to an immunoglobulin chain[19], suggests that the internal labelling method can be expanded to include the use of all twenty natural amino acids as radioactive precursors in the *in vitro* biosynthesis of proteins of unknown sequence. Smithies *et al.* have used such an approach for amino terminal sequence analysis of immunoglobulin chains (personal communication). Where the approach through biosynthesis is not feasible, peptides could readily be labelled with tritium to high specific activities and sequenced[20]. In some circumstances, sequence analysis of polypeptides bearing radioactive side chain prosthetic groups may be informative. These techniques that can be used to label polypeptides internally at high specific activity, coupled with the use of natural or synthetic carrier molecules, should extend the applicability of automated Edman degradation to sequence analyses of polypeptides of biological interest that are available only in trace quantities. Such advances, coupled with improvements in speed and efficiency of peptide synthesis[21], represent an amplification essential to biochemical and physiological investigation of biologically interesting polypeptides since milligram or even gram amounts of pure polypeptides can be synthesised chemically, even though they cannot be obtained from natural sources.

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sequence information for human prohormone using similar methods[22]. Earlier, at the Keating symposium in September 1973, Cohn *et al.* presented preliminary data indicating that positions 1, 4 and 5 were likely to be lysine in the human prohormone structure.

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1 Kemper, B., Habener, J. F., Potts, jun., J. T., and Rich, A., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 643 (1972).
2 Habener, J. F., Kemper, B., Potts, jun., J. T., and Rich, A., *Science*, **178**, 630–633 (1972).
3 Cohn, D. V., MacGregor, R. R., Chu, L. L. H., Kimmel, J. R., and Hamilton, J. W., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1521 (1972).
4 Steiner, D. F., and Oyer, P. E., *Proc. natn. Acad. Sci. U.S.A.*, **57**, 473 (1967).
5 Tager, H. S., and Steiner, D. F., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2321 (1973).
6 Edman, P., and Begg, G., *Eur. J. Biochem.*, **1**, 80 (1967).
7 Hamilton, J. W., Niall, H. D., Keutmann, H. T., Potts, jun., J. T., and Cohn, D. V., *Fedn. Proc.*, **32**, 315 (1973).
8 Niall, H. D., Keutmann, H. T., Sauer, R. T., Hogan, M. L., Dawson, B. F., Aurbach, G. D., and Potts, jun., J. T., *Z. physiol. Chem.*, **351**, 1586 (1970).
9 Brewer, Jr., H. B., and Ronan, R., *Proc. natn. Acad. Sci. U.S.A.*, **67**, 1862 (1970).
10 Brewer, jun., H. B., Fairwell, T., Ronan, R., Sizemore, G. W., and Arnaud, C. D., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3585 (1972).
11 Niall, H. D., Sauer, R. T., Jacobs, J. W., Keutmann, H. T., Segre, G. V., O'Riordan, J. L. H., Aurbach, G. D., and Potts, jun., J. T., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 384 (1974).
12 Pisano, J. J., and Bronzert, Jr., T. J., *J. biol. Chem.*, **244**, 5597 (1969).
13 Niall, H. B., *Methods in Enzymology*, **27**, 942 (1973).
14 Tregear, G. W., van Rietschoten, J., Greene, E., Keutmann, H. T., Niall, H. D., Reit, B., Parsons, J. A., and Potts, jun., J. T., *Endocrinology*, **93**, 1349 (1973).
15 Merrifield, R. B., *J. Am. chem.*, **85**, 2149 (1963).
16 Habener, J. F., Tregear, G. W., Stevens, T. D., Dee, P. C., and Potts, jun., J. T., *Endocr. Res. Commun.* (in the press).
17 Jacobs, J. W., Sauer, R. T., Niall, H. D., Keutmann, H. T., O'Riordan, J. L. H., Aurbach, G. D., and Potts, jun, J. T., *Fedn. Proc.*, **32**, 2445 (1973).
18 Niall, H. D., Jacobs, J. W., van Rietschoten, J., and Tregear, G. W., *FEBS Lett.* (in the press).
19 Schechter, I., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2256 (1973).
20 Hembree, C. W., Ehrenkaufer, R. E., Lieberman, S., and Wolfe, A. P., *J. biol. Chem.*, **248**, 5532 (1973).
21 Tregear, G. W., van Rietschoten, J. Greene, E., Keutmann, H. T., Niall, H. D., Parsons, J. A., and Potts, jun., J. T., in *Endocrinology 1973* (edit, by Taylor, S.). (Heinemann, London, in the press).
22 Cohn, D. V., MacGregor, R. R., Chu, L. L. H., Huang, J., Anast, C. T., and Hamilton, J. W., *Am. J. Med.* (in the press).

Effects of previous exposure on olfactory discrimination in *Acomys cahirinus*

Experiments with chicks[1–3], rats[4,5], and rhesus monkeys[6] indicate that previous exposure to a visual pattern facilitates subsequent discrimination learning involving that pattern and a novel visual stimulus. This facilitation of discrimination learning as a function of previous exposure has been found regardless of whether the exposure stimulus was the reinforced or the non-reinforced pattern in the subsequent discrimination task.

By comparison with animals exposed to only one of the visual discriminanda before testing, those exposed to both stimuli generally take considerably more trials to master a discrimination task[2,4]. Studies with monkeys and chicks indicate that animals that were familiar with both stimuli actually did less well on a discrimination task with those stimuli than did control animals for whom both of the stimuli were novel[2,7]. For both hooded and albino rats, however, subjects familiar with both visual discriminanda learned a discrimination task faster than did control animals[4,8,9].

In general, such studies have consistently been concerned with visual stimuli; even though vision is not the dominant sense in the rat which is often used as a test species. Here we report a first attempt to investigate the effects of previous exposure to one or both discriminanda upon subsequent discrimination of olfactory stimuli. The subject species, *Acomys cahirinus* (spiny mouse) is a nocturnal murid rodent little used in laboratory experimentation.

Individual adult male spiny mice were randomly assigned to one of three treatment conditions ($n = 8$ per condition) which varied as a function of whether the animals were exposed to one, both, or neither of the olfactory stimuli for 7 d before the start of testing. The two olfactory stimuli, ethyl benzoate (EB) and acetophenone (AC) have similar diffusion rates and have been effectively used in previous studies with rats[10], guinea pigs[11,12], and garter snakes[13]. The odourants were dropped onto cotton wool inside small plastic cannisters with holes drilled through the cap. Two of these cannisters were placed in the cage of each subject on the first day of exposure training and remained for 7 consecutive days. The cannisters in the cages of the control (C) animals remained empty throughout the training period; for the Experimental II (E-II) animals, one cannister contained AC and the second EB; the cannisters in the cages of the Experimental I (E-I) mice both contained the same odour (either AC or EB), which was randomly determined for each E-I subject. Mice were housed in four separate rooms as a function of the training odour(s) to which they were exposed.

Each animal was given a single 10 min habituation period in the olfactory discrimination apparatus (a T maze consisting of a runway with a short stem) on the first and second days of stimulus exposure. On days 3–7 of exposure, each mouse was given four pretraining trials during which he was placed into one end of the runway and allowed to run to the opposite end to obtain a small bit of apple. Discrimination trials began on day 8.

During discrimination testing, the two odours were blown into opposite ends of the runway, and two small food cups containing a piece of apple as a reward were placed into the runway—one at each end. The apple in the non-reinforced (incorrect) end was inaccessible behind a mesh screen. This procedure was adopted to equate for the presence of apple odour in each end of the runway. To obtain the apple reinforcement, the mouse had to approach the appropriate odour of the two being emitted into the T maze. A correction procedure was used[14] whereby incorrect trials were repeated until the correct choice was made. Subjects were run for a total of four reinforced trials on each day until reaching a criterion of 8/9 correct consecutive responses—or until reaching a maximum of 50 reinforced trials. Throughout the training and discrimination testing, the apparatus was washed, rinsed and dried after testing each animal on each day.

For both the E-II and C conditions, four animals were reinforced for running to AC and four for running to EB. For each of the E-I animals, the designation of reinforced compared with non-reinforced odour was randomly determined. Thus, five E-I mice were reinforced for running towards the previous exposure odour, while for the remaining three E-I animals, the exposure odour was the negative (non-reinforced)

TABLE 1 Performance on olfactory discrimination test as a function of previous exposure to the test odour(s).

	Treatment condition		
	Control (C) $n = 8$	Experimental I (E-I) $n = 8$	Experimental II (E-II) $n = 8$
Mean reinforced trials to criterion	25.25	12.87	34.12
s. d.	11.22	11.04	10.27
		$H = 10.65$, $P < .01$	
Mean errors to criterion	15.9	5.0	21.5
s. d.	9.67	8.19	11.52
		$H = 10.305$, $P < .01$	

stimulus. The left/right positions of positive and negative odours was randomly determined each day for each subject, with the limitation that each odour would be introduced from each end for two trials per d.

Table 1 includes a summary of the results of the olfactory discrimination tests. Differences between the three conditions on the number of trials to criterion and errors to criterion were analysed with the Kruskal-Wallis test. As indicated in Table 1, significant differences ($P < .01$) were found between the treatment conditions on both dependent measures. Following the significant overall H tests, all possible paired comparisons for both dependent variables were tested using the Mann-Whitney U test. The results of these *post mortem* comparisons indicate that for both the number of reinforced trials to criterion and errors to criterion, the E-1 condition had significantly lower scores than either the E-II or C conditions. When comparing trials to criterion for the E-I compared with C conditions, $U = 7$, $P = 0.006$ (2-tailed test) and for the E-I : E-II comparison, $U = 7$, $P = 0.006$. On the errors to criterion measure, the E-I : C comparison yielded $U = 7.5$, $P < .01$ and for the E-I : E-II comparison, $U = 5.5$, $P < .005$. No significant differences were obtained between the E-II compared with C conditions on either of the measures of discrimination learning (for trials to criterion, $U = 17$, $P = 0.13$; and for errors to criterion, $U = 22.5$, $P > 0.30$).

The behaviour of the E-I animals run to the exposure odour did not differ greatly from those for whom the exposure odour was the negative stimulus during discrimination testing; and both of these subgroups differed noticeably from the E-II and C conditions. The mean trials to criterion value was 15 for E-I animals for whom the exposure odour was the correct stimulus during testing, and 9.3 for those E-I animals for which the exposure odour was incorrect during subsequent testing. Mean errors to criterion values for these two subgroups of the E-I condition were 6.2 and 3 respectively.

These results indicate that spiny mice will learn to discriminate between a familiar and a novel odour faster than they will learn to discriminate between two unfamiliar odours. As animals in the E-I condition showed more rapid learning than did subjects of the other two conditions—regardless of whether the exposure odour was the rewarded or the unrewarded stimulus—this enhanced performance as a function of familiarity with one of the discriminanda cannot be due to previous attachment to the rewarded odour *per se*. These results tend to generally corroborate and extend those experiments in which previous exposure to one visual pattern has been found to facilitate subsequent discrimination between that pattern and a novel visual pattern[1,4–6].

The performance of the spiny mice exposed to one of the olfactory discriminanda (E-I) was superior to that of conspecifics who had been exposed to both discriminanda before discrimination testing. Those animals for whom both odours were familiar, performed roughly comparably to the controls for whom both odours were novel. As we have mentioned however, earlier studies in which visual stimuli were used revealed that rhesus monkeys and chicks who were familiar with both discriminanda were inferior to controls[7,2], while albino and hooded rats, for whom both visual discriminanda were familiar, learned a visual discrimination faster than did controls[4,8]. As pointed out by Chantrey[2], the visual stimuli used in these studies were probably more relevant to the diurnal rhesus monkeys and chicks than to nocturnal rodent species. On the other hand, olfaction (the sense under study here) seems to be the primary sense used by nocturnal rodents, including spiny mice. The relatively rapid acquisition of the olfactory discrimination test shown by the mice in the present study no doubt reflects the value of this capacity to nocturnal rodent species in coping with their environment. It may therefore be the case that as the value of any sense increases for the species in question, previous exposure to both discriminada to be subsequently discriminated may tend to have little effect, or to interfere with acquisition—rather than facilitate subsequent discrimination between these stimuli. It must be emphasised, of course, that this is at best a very tentative *post hoc* hypothesis. A series of parametric studies using stimuli within several senses and with the same subject species would be required to test this hypothesis effectively.

Bateson and Chantrey[7,2] interpret the poor performance of chicks and monkeys on a discrimination task in which both visual stimuli are familiar as being a result of the subjects classifying the stimuli together during the exposure period and subsequently having difficulty 'declassifying' them. In the present study, although the spiny mice that were exposed to both discriminanda did not differ significantly from the controls, they were inferior to those animals exposed to only one of the odours before testing (E-I). Therefore, it would seem that relative to the E-I condition, the E-II animals had trouble declassifying the two odours as a unitary stimulus complex as a function of their previous exposure; while the relatively poor performance of the control animals was a function of the total novelty of both discriminanda.

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1 Bateson, P. P. G., *Nature*, **202**, 421–422 (1964).
2 Chantrey, D. F., *J. comp. physiol. Psychol.*, **81**, 256–261 (1972).
3 Kovach, J. K., Fabricius, E., and Falt, L., *J. comp. physiol. Psychol.*, **61**, 449–454 (1966).
4 Forgus, R. H., *J. comp. physiol. Psychol.*, **51**, 75–78 (1958a).
5 Forgus, R. H., *J. comp. physiol. Psychol.*, **51**, 588–591 (1958b).
6 Bateson, P. P. G., in *Experience and Capacity* (edit. by Kimble, D. P.), 146 NY Academy of Science, New York, 1968).
7 Bateson, P. P. G., and Chantrey, D. F., *Nature*, **237**, 173–174 (1972).
8 Gibson, E. J., and Walk, R. D., *J. comp. physiol. Psychol.*, **49**, 239–242 (1956).
9 Gibson, E. J., Walk, R. D., Pick, H. L., and Tighe, T. J., *J. comp. physiol. Psychol.*, **51**, 584–587 (1958).
10 Marr, J. N., and Lilliston, L., *Behaviour*, **33**, 277–282 (1970).
11 Carter, C. S., and Marr, J. N., *Anim. Behav.*, **18**, 238–244 (1970).
12 Porter, R. H., Fullerton, C., and Berryman, J. C., *Z. Tierpsychol.*, **32**, 489–495 (1973).
13 Porter, R. H., and Czaplicki, J. A., *Physiol. Behav.*, **12**, 75–77 (1974).
14 Sutherland, N. S., and Mackintosh, N. J., *Mechanisms of Animal Discrimination Learning*, (Academic Press, New York, 1971).

Role of C3 in *in vitro* lymphocyte cooperation

COMPLEMENT is one of the best known effector systems of immunological reactions, and has also recently been shown to participate in non-immunological inflammation[1], and blood clotting[2–4]. Complement arises early in both phylogeny[5] and ontogeny[6], and the concept[7] that it may in addition play a part in the induction of specific antibody production is of practical and theoretical interest. The complement C3-cleaving protein (CoF) of cobra venom and other C3-reactive agents have been shown in mice to inhibit the induction of thymus-dependent but not thymus-independent antibody responses *in vivo* (refs 7 and 8 and M.B.P., unpublished). Evidence was obtained which related suppression of antibody production to cleavage of C3, rather than to other possible effects of the C3-reactive agents, such as antigenic competition (refs 7 and 8 and M.B.P., unpublished). Interpretation of the data in terms of the role of C3 in the antibody response is difficult, however, especially as fluid phase C3 fragments (C3b and C3c) 'block' B lymphocyte C3 receptors and also suppress antigen-induced *in vitro* human lymphocyte transformation (refs 7 and 8 and M.B.P., and A. E. Butterworth, unpublished).

The requirement for C3 in cooperative antibody production was therefore studied *in vitro* where it is possible to exclude such effects as antigenic competition, and to differentiate between depletion of functional C3 and the generation of C3 fragments. The presence of murine C3 in the supernatant of mouse spleen cell cultures was first demonstrated and the effects on antibody responses of these cultures of isolated specific anti-mouse C3 antibodies were then examined.

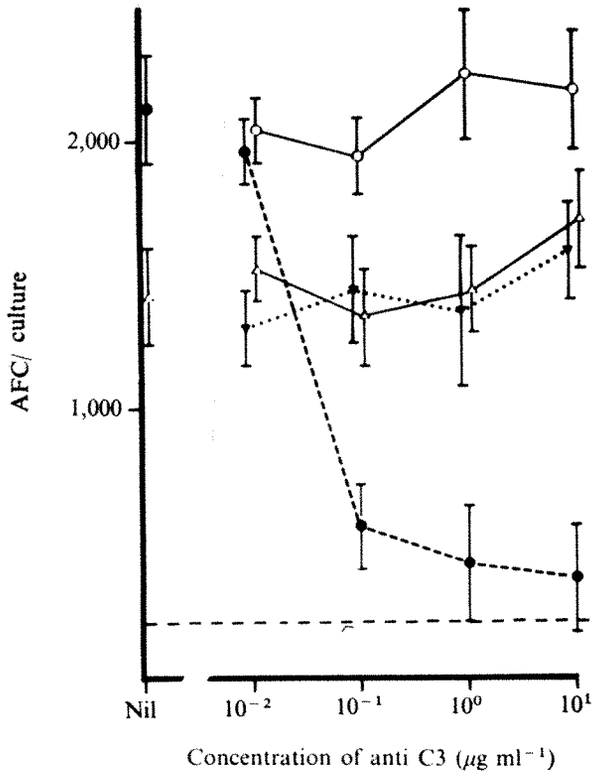


FIG. 1 Effect of anti-C3 on the response of TNP-KLH primed mouse spleen cells *in vitro*. ●, Response to TNP-KLH in the presence of anti-C3; ○, response to TNP-KLH in the presence of control IgG_1; ▼, response to DNP-POL in the presence of anti-C3; △, response to DNP-POL in the presence of control IgG_1; ----, background. Results from one seven experiments, using batch A. Values are metric means of four cultures ± s.e.m. IgM response measured at day 4.

TABLE 1 Response to DNP-POL in serum-free medium.

Cells cultured	Antigen (μg ml^{-1})	Antibody (μg ml^{-1})	Anti-DNP response (AFC per culture ± s.e.)
Normal spleen*	Nil	Nil	55 ± 28†
Normal spleen*	DNP-POL[1]	Nil	612 ± 124
Normal spleen*	DPN-POL[1]	10^{1} anti-C3	568 ± 86
Normal spleen*	DPN-POL[1]	10^{0} anti-C3	597 ± 104
Normal spleen*	DPN-POL[1]	10^{-1} anti-C3	728 ± 131
Normal spleen*	DPN-POL[1]	10^{1} IgG_1	636 ± 154
Normal spleen*	DNP-POL[1]	10^{0} IgG_1	532 ± 87

* 1.5×10^7 unprimed CBA spleen cells were cultured for 4 d in serum-free medium.

† Values are arithmetic means ± s.e. mean of four cultures per group.

CBA/H mice between 60 and 196-d-old, and bred at either University College, or at the Imperial Cancer Research Fund Laboratory, London, were used throughout. Some mice were injected intraperitoneally three times at 2 week intervals with 100 μg trinitrophenylated keyhole limpet haemocyanin (TNP-KLH) or KLH absorbed on bentonite[9]. They were used for culture 4 to 12 weeks later. TNP-KLH with 800 TNP groups per 8×10^6 daltons and dinitrophenylated-polymeric flagellin (DNP-POL) with 1.7 DNP groups per 40,000 daltons, prepared as described previously[10] were used. Tissue culture was performed in Marbrook/Diener flasks[11,12] using Eagle's minimal essential medium supplemented with sodium bicarbonate and 5% foetal calf serum (Biocult Laboratories, Glasgow). In some experiments, foetal calf serum was absent. Antibody-forming cells (AFC) were counted by the technique of Cunningham and Szenberg[13]. Anti-DNP AFC were assayed by using SRC coated with a dinitrophenylated rabbit anti-SRC Fab' fragment (DNP-Fab')[14]. Only the IgM response is reported here as the IgG response were relatively low.

Isolated anti-C3 antibodies were prepared from sheep anti-mouse C3 serum by elution from Sepharose C3 (ref. 8). The anti-mouse C3 serum was raised in sheep by a modification of the method of Mardiney and Müller-Eberhard[8,15]. It was monospecific for C3, did not cross react with free or fixed bovine C3 from foetal calf serum, nor with either DNP-POL or TNP-KLH. The anti-C3 activity was estimated by agglutination of 1% sheep erythrocytes alexinated with mouse complement[8]; the protein concentrations and titres of the two preparations used in the present experiment were 90 μg ml^{-1}, 1/512 (Batch A) 170 μg ml^{-1}, 1/256 (Batch B).

Using a radioimmunoassay system (M. B. P., and D. V. Wilson, unpublished) to test for the presence of murine C3 in the supernatant of *in vitro* cultures of mouse spleen cells, it was found that the supernatants after 3.7 d culture of 1.5×10^7 spleen cells ml^{-1} regularly contained 0.3–0.6 μg ml^{-1} of murine C3, assuming that normal mouse serum used as reference standard contains 1.0–2.0 ml C3 ml^{-1}.

The effect of isolated anti-mouse C3 antibodies on the thymus-dependent response (to TNP-KLH) and the thymus-independent response (to DNP-POL) of spleen cells from mice primed to TNP-KLH was investigated. Seven experiments, the results of one of which is shown in Fig. 1, gave the same result. Anti-mouse C3 suppressed the response to an optimal dose of TNP-KLH (10^{-2} μg ml^{-1}) but did not affect that to an optimal dose of DNP-POL (10^{-1} μg ml^{-1}). The IgG_1 fractions of the same anti-mouse C3 serum depleted of anti-C3 activity did not suppress the response to either antigen; nor did anti BSA antiserum at various concentrations, which would also induce the presence of immune complexes in the cultures (results not shown). The maximum suppression obtained in different experiments varied from 100% (response of background or less) to 70% or so. Preliminary studies indicate that the least suppression occurred with spleen cells taken from mice cultured

relatively soon after priming. Analogous experiments were performed with spleen cells from mice primed with carrier only, that is, KLH (Fig. 2). Again the response to TNP-KLH 10^{-1} μg ml^{-1} was readily suppressed by anti-C3 while that to DNP-POL (1 μg ml^{-1}) was not.

The absence of suppression by anti-C3 of the response to DNP-POL was studied further, by using suboptimal concentrations of antigen. Responses to DNP-POL concentrations of 10^{-3} to 10 μg ml^{-1} were not affected by anti-C3 (10 μg ml^{-1}) (Fig. 3). It has been suggested that B cell activation *in vitro* may be dependent on bovine C3 provided by the foetal calf serum[13]. The sheep anti-mouse C3 which suppressed cooperative responses did not react with bovine C3, however, and unprimed spleen cells responded to DNP-POL in serum-free medium, even in the presence of anti-C3 (Table 1).

These results show that a thymus-dependent antibody response is suppressed by as little as 10^{-1} μg ml^{-1} of isolated anti-mouse C3 antibodies, a concentration comparable with that of murine C3 present in the culture supernates. In contrast, the thymus-independent response to DNP-POL was unaffected by as much as 10 μg ml^{-1} of anti-C3 even when suboptimal doses of antigen (Fig. 3) or serum-free cultures (Table 1) were used. Cooperative antibody production, such as induced by TNP-KLH, which requires the participation of three cell types namely T and B lymphocytes and macrophages[17,18] thus requires C3, whereas no such requirement was demonstrable in a non-cooperative response induced by DNP-POL which only involves B cells[19]. Both B cells[20] and macrophages[21] have surface receptors, albeit of different types, for fixed C3, and it is therefore possible that the role of C3 in lymphocyte cooperation may be expressed at either the B cell level, the macrophage level

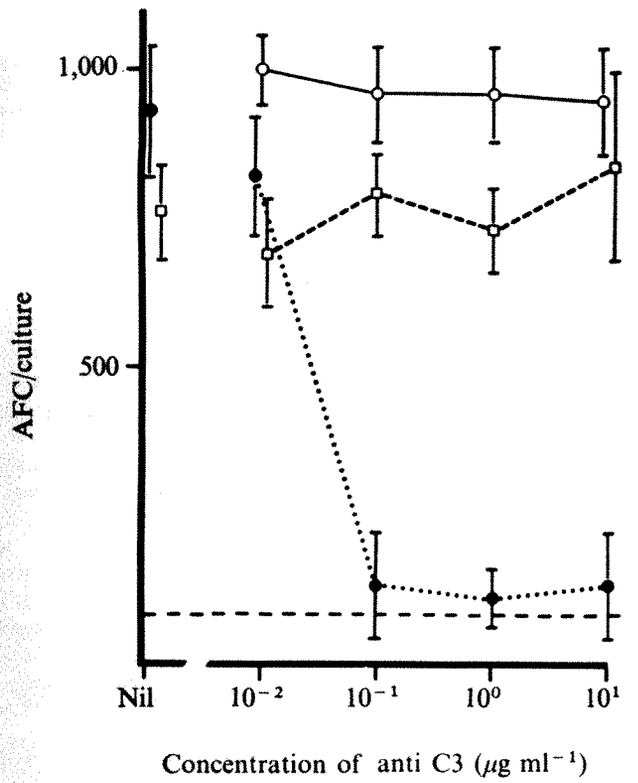


FIG. 2 Effect of anti-C3 on the response of KLH-primed mouse spleen cells *in vitro*. ●, Response to TNP-KLH in the presence of anti-C3; ○, response to TNP-KLH in the presence of control IgG$_1$; □, response to DNP-POL in the presence of anti-C3; ----, background. Results taken from one of six similar experiments using batch B of anti-C3. Values are metric means of four cultures ± s.e.m.

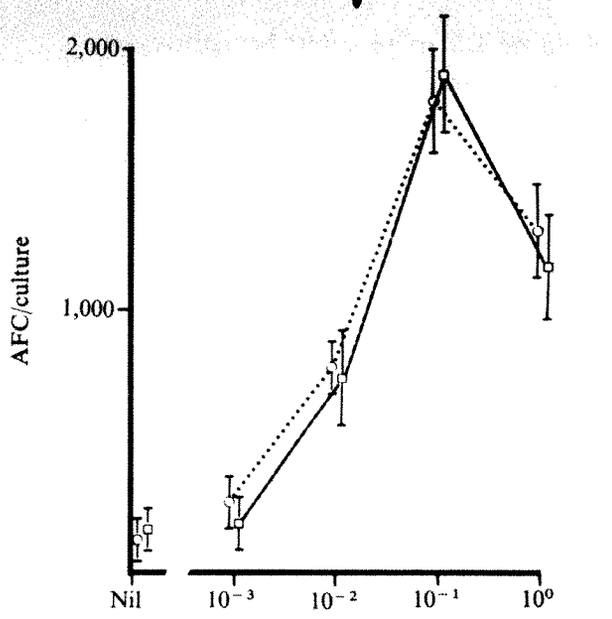


FIG. 3 Effect of anti-C3 on the response of spleen cells to DNP-POL *in vitro*. ○, Response to DNP-POL in presence of anti-C3; □, response to DNP-POL in presence of control IgG$_1$. Results taken from one out of three such experiments, using batch B of anti-C3. Values are metric means of four cultures ± s.e.m.

or both. For example, fixed C3 interacting with cell surface C3 receptors might assist in T-dependent presentation of antigen to B cells[7,8]. The contrasting suggestion[16] that interaction of C3 with B cell C3 receptors is an obligatory signal for triggering of all B cells by either mitogens or antigens is not supported by this or other published work[7,22]. The recent observation of a difference between the B cell precursors responsive to DNP-POL and TNP-KLH, particularly in primed mice[23], might provide an alternative explanation of the present data if the difference could be correlated with heterogeneity of lymphocyte C3 receptors. A combination of cell separation and tissue culture techniques may help to clarify the role of C3 in antibody production.

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1 Hill, P. J., and Ward, P. A., *J. exp. Med.*, **134**, 885 (1971).
2 Taylor, F. B., and Müller-Eberhard, H. J., *J. clin. Invest.*, **49**, 2068 (1970).
3 Zimmerman, T. S., Arroyave, C. M., and Müller-Eberhard, H. J., *J. exp. Med.*, **134**, 1591 (1971).
4 Zimmerman, T. S., and Müller-Eberhard, H. J., *J. exp. Med.*, **134**, 1601 (1971).
5 Gigli, I., and Austen, K. F., *A. Rev. Microbiol.*, **25**, 309 (1971).
6 Solomon, J. B., *Foetal and Neonatal Immunology* (North Holland Publishing Co., Amsterdam, 1971).
7 Pepys, M. B., *Nature new Biol.*, **237**, 157 (1972).
8 Pepys, M. B., *thesis*, Univ. Cambridge (1973).

[9] Bullock, W. W., and Rittenberg, M. B., *Immunochemistry*, **7**, 310 (1970).
[10] Feldmann, M., *J. exp. Med.*, **135**, 735 (1971).
[11] Marbrook, J., *Lancet*, **ii**, 1279 (1967).
[12] Dlener, E., and Armstrong, W. D., *Lancet*, **ii**, 1281 (1967).
[13] Cunningham, A., and Szenberg, A., *Immunology*, **14**, 599 (1968).
[14] Strausbach, P., Sulica, A., and Givol, D., *Nature*, **220**, 927 (1970).
[15] Mardiney, M. R., and Müller-Eberhard, H. J., *J. Immun.*, **94**, 877 (1965).
[16] Dukor, P., and Hartmann, K. U., *Cell. Immun.*, **7**, 349 (1973).
[17] Feldmann, M., *J. exp. Med.*, **136**, 737 (1972).
[18] Feldmann, M., *J. exp. Med.*, **135**, 1049 (1972).
[19] Feldmann, M., *Eur. J. Immun.*, **2**, 130 (1972).
[20] Nussenzweig, V., Bianco, C., Dukor, P., and Eden, A., *Prog. Immun.*, **1**, 73 (1971).
[21] Mantovani, B., Rabinovitch, M., and Nussenzweig, V., *J. exp. Med.*, **135**, 780 (1972).
[22] Janossy, G., Humphrey, J. H., Pepys, M. B., and Greaves, M. F., *Nature new Biol.*, **245**, 108 (1973).
[23] Gorczynski, R. M., and Feldmann, M., *Eur. J. Immun.*, (in the press).

Lymphocytes from human newborns abrogate mitosis of their mother's lymphocytes

THE conceptus is not automatically rejected by its mother as an allograft even though it differs genetically and therefore antigenically[1–3], perhaps in part because foetal tissues are segregated from immunologically competent maternal cells. For example, the surface of trophoblastic cells is partially covered by highly sulphated acid mucoprotein and the trophoblasts themselves have few if any transplantation antigens[3–8]. Also, blocking factors may prevent the mother from immunologically damaging her foetus(es). For example, pregnant mice have a serum factor which blocks their lymphocytes from inhibiting the growth of antigenically foreign mouse embryonic cells[9]; similar results have been observed with human materials[10,11]. We now report a further possible mechanism by which the newborn actively suppresses mitosis of maternal and other foreign lymphocytes.

Blood was obtained from the umbilical cords of male singleton newborns after delivery but before expulsion of the placenta, from their mothers' arm veins within 1 h of delivery and from nonpregnant adult females and adult males (ages 21-43, all healthy). Lymphocytes were prepared primarily from blood defibrinated by glass beads and purified by Ficoll-Hypaque gradient centrifugation[12], and on occasion passage through glass wool columns. Using Türk's reagent, lymphocyte purity was 95% or better as judged by light microscopy. Equal numbers of living lymphocytes from each mother and her newborn (1–2 × 10^6 from each) were cultured in 5 ml of RPMI 1640 media supplemented with 20% foetal calf serum, L-glutamine and antibiotics. Cultures were set up in parallel with and without phytohaemagglutinin P (PHA-P) because most cells needed PHA to go into mitosis. Adult and newborn lymphocytes cultured separately with similar amounts of PHA served as controls. We used 50 μg of PHA per ml of medium, previously determined to be an optimal dose for both types of lymphocyte. After 60–72 h incubation at 37° C in 5% CO_2 and 95% air, cultures were treated with colchicine, collected and prepared as described before[13]. Slides were stained with 0.15% quinacrine hydrochloride, washed, mounted in modified McIlwaine's citric acid buffer[14], examined by fluorescent microscopy and photographed, after which the karyotype was constructed. This technique marks the Y chromosome specifically. Under the culture conditions used, cells from mothers survived as well as cells from the newborns, as judged by trypan blue dye exclusion.

TABLE 1 Results of mixing lymphocytes from human newborns with lymphocytes from their mothers

Case identification	PHA-P (50 μg ml^{-1})	% of metaphase cells having a Y chromosome
E.B.	+	100
J.E.	+	100
J.S.	+	100
V.V.	+	100
A.M.	+	99
A.M.	0	98
V.G.	+	100
V.G.	0	100
Expected result	+ or 0	50

Equal numbers of lymphocytes (1 to 2 × 10^6) from the mother and her male newborn were mixed in culture. After 3 d incubation the cells were treated with colchincine, collected and stained with quinacrine hydrochloride (to mark the Y chromosome). Usually 100 cells in metaphase were examined from each culture for the presence or absence of the Y chromosome. Lymphocytes were refined by Ficoll-Hypaque gradient centrifugation and, on occasion, by passage through glass wool columns. Under the culture conditions used, lymphocytes from the mother survived as well as lymphocytes from the newborns.

TABLE 2 Results of mixing lymphocytes from human adults with lymphocytes from human newborns

Case identification	Mixed lymphocytes male	female	PHA-P (50 μg ml^{-1})	% of metaphase cells having Y chromosome
A.M. + A.M.	Newborn	His mother	+	99
A.M. + A.M.	Newborn	His mother	0	98
B.M. + M.C.	Newborn	Alien mother	+	90
B.C. + M.M.	Newborn	Alien mother	+	93
B.S. + A.J.	Newborn	Alien mother	+	90
B.C. + R.D.	Newborn	Nonpregnant adult	0	92
V.S. + B.C.	Newborn	Nonpregnant adult	+	88
B.J. + C.H.	Adult	Nonpregnant adult	+	54
B.J. + M.W.	Adult	Nonpregnant adult	0	60
M.O. + C.H.	Adult	Nonpregnant adult	+	53
Expected result	1:1 Mixture		+ or 0	50

Equal numbers of lymphocytes (1 to 2 × 10^6) from male newborns or male adults and lymphocytes from adult females were mixed in culture. After 3 d incubation, the cells were treated with colchicine, collected and stained with quinacrine hydrochloride (to mark the Y chromosome). At least fifty cells in metaphase were examined from each culture and the percent having Y chromosome calculated. Under the culture conditions used, lymphocytes from males or females survived equally well.

Table 1 shows that after 3 d culture of a mixture of lymphocytes most cells undergoing mitosis were from the newborn. With or without PHA, 98% or more of the total cultured peripheral lymphocytes in mitosis were the newborn's (as determined by the presence of the Y chromosome), whereas 2% or less were from the mother.

In further experiments the chromosome labelling technique was used to study the mixture of lymphocytes from newborns and adult lymphocytes other than those of their mother. In all cases, lymphocytes from male newborns inhibited mitosis of lymphocytes from unrelated mothers and nonpregnant adult females (Table 2). In contrast, lymphocytes of adult males failed to inhibit mitosis of lymphocytes from adult females. Viability of cultured lymphocytes from adults and newborns was again similar in each experiment. We were unable to assess the mixing of lymphocytes from newborn females with their mothers' lymphocytes because of similarities in chromosome markers.

These results clearly show that lymphocytes from human male newborns effectively inhibit division of their mothers'

lymphocytes *in vitro*; however, lymphocytes obtained from the mother and cultured separately were able to enter mitosis. Preliminary experiments suggest that a soluble factor released from the newborn's lymphocytes may be in part responsible for inhibition of mitosis. Further, this inhibition is a general phenomenon, in that lymphocytes from newborns also block division of lymphocytes from unrelated mothers and other adult females. In contrast, lymphocytes from adult males failed to abrogate mitosis of lymphocytes from adult females, suggesting that (1) the Y chromosome per se is not important in this inhibition and (2) the inhibitory effect is a property of the lymphocytes of newborns. No attempts were made to assess the suppression of maternal response by transplantation antigens. Others have shown that lymphocytes of both the newborn and its mother respond less to mitogen stimulation when mixed together in a unidirectional mixed culture relative to paternal or other third party cells[15,16]. Experiments to be reported elsewhere show that lymphocytes from the mother are not killed when mixed only with lymphocytes from her newborn.

Our results suggest that any lymphocytes from the mother coming into contact with the foetus by transplacental passage are actively prevented from dividing. Such a process could protect foetal tissue from aggressor lymphocytes, prevent foetal rejection and explain the experimental difficulty in causing a graft *versus* host reaction in the foetus by cell transfer[1]. While lymphocytes from human male newborns can inhibit mitosis of lymphocytes from their mothers, one cannot conclude from *in vitro* data that this is the sole reason why antigenically different embryos are not injured *in vivo*.

Finally, our results offer an explanation for earlier observations that immune responses were difficult to elicit in newborn rabbits after adoptive transfer of sensitised homologous adult lymphocytes, whereas such adult lymphocytes mounted sizable antibody responses after transfer to X-irradiated homologous adult rabbits[17,18]. Perhaps lymphocytes from the newborn rabbits prevented potentially capable transferred cells from forming antibody. We are investigating whether lymphocytes from newborns can turn off a humoral response by preventing the division of primed immunocompetent cells and whether such lymphocytes are T suppressor cells.

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1 Beer, A. E., and Billingham, R. E., in *Adv. Immunol.* (edit. by Dixon, F., and Kunkel, H.), **14**, 1 (Academic Press, New York, 1971).
2 Billingham, R. E., *New Engl. J. Med.*, **270**, 667–672 (1964).
3 Currie, G. A., *Proc. R. Soc. Med.*, **61**, 1206 (1968).
4 Edidin, M., in *Transplantation Antigens, Markers of Biological Individuality* (edit. by Kahan, B. D., and Reisfeld, R. A.), **75** (Academic Press, New York, 1972).
5 Bradbury, S., Billington, W. D., Kirby, D. R. S., and Williams, E. A., *Am. J. Obst. Gyn.*, **104**, 416–418 (1969).
6 Currie, G. A., van Doornick, W., and Bagshawe, K. D., *Nature*, **219**, 191–192 (1968).
7 Currie, G. A., and Bagshawe, K. D., *Lancet*, **i**, 708–710 (1967).
8 Tai, C., and Halasz, N. A., *Science*, **158**, 125–126 (1967).
9 Hellström, K. E., Hellström, I., and Braun, J., *Nature*, **224**, 914–915 (1969).
10 Leventhal, B. G., Buell, D. N., Yankee, R., Rogentine, G. N., and Terasaki, P., in *Proc. Fifth Leukocyte Culture Conference*, **473** (Academic Press, New York, 1970).
11 Kasakura, S., *J. Immun.*, **107**, 1296–1301 (1971).
12 Mendelsohn, J., Skinner, A., and Kornfeld, S., *J. Clin. Invest.*, **50**, 818–826 (1971).
13 Moorhead, P. S., Nowell, P. C., Mellman, W. J., Battips, D. M., and Hungerford, D. A., *Expl Cell Res.*, **20**, 613–616 (1960).
14 Khudr, G., and Benirschke, K., *Am. J. Obst. Gyn.*, **110**, 1091–1095 (1971).
15 Ceppellini, R., Bonnard, G., Coppo, F., Miggiano, V., Pospisilm, C., and Pellegrino, M., *Transplant. Proc.*, **3**, 58–63 (1971).
16 Bonnard, G., and Lemos, L., *Transplant. Proc.*, **4**, 177–180 (1972).
17 Dixon, F. J., and Weigle, W. O., *J. exp. Med.*, **105**, 75–83 (1957).
18 Dixon, F. J., and Weigle, W. O., *J. exp. Med.*, **110**, 139–146 (1959).

Effects of barbiturate withdrawal on audiogenic seizure susceptibility in BALB/c mice

BARBITURATES are commonly used to prevent epileptic seizures[1], but it has also been observed that susceptibility to 'spontaneous' seizure in both normal humans and animals is increased during abrupt withdrawal from chronically administered barbiturates and other central nervous system depressants[2]. Here we show that barbiturate withdrawal is highly effective in producing audiogenic seizure susceptibility in a strain of mice which are normally resistant to seizure.

In addition, we present evidence that the barbiturate withdrawal effect is not an age-dependent phenomenon and further suggest that this drug-induced susceptibility to seizure shares features in common with the seizure susceptibility that can be induced in these same mice following a reduction of auditory input.

Audiogenic seizures, that is seizures driven by a loud sound, can be produced in normally seizure-resistant mice by 'priming' them with an intense acoustic stimulus a few days before testing for audiogenic seizures[3–5]. These primed animals show a severe loss in cochlear sensitivity indicative of cochlear damage. Saunders *et al.*[6] proposed an auditory deprivation hypothesis to account for the apparent paradox that deafened mice over-react to a loud sound. Essentially, this hypothesis suggested that priming caused damage to the cochlea which in turn effectively reduced auditory input to higher auditory structures. As a result of this disuse, the unused structures became supersensitive so that on re-exposure to a loud sound, the supersensitive structures were excited and a seizure precipitated. Gates, Chen and Bock[7] and Chen, Gates and Bock[8] later confirmed that auditory deprivation was the important condition for the development of supersensitivity.

Careful destruction of the tympanic membrane produces a reduction of input to the cochlea (approximately 40 dB) and would not seem to produce cochlear damage. There is, however, the chance that secondary infection could be introduced into the middle ear and then to the cochlea, but this did not seem to be the case with our animals. McGinn *et al.*[9] have subsequently confirmed our finding that a reduction of auditory input, by means of ear plugs, is also effective in producing audiogenic seizure-prone mice. Willott and Henry[10] report that preliminary histology from primed mice localises damage in the outer hair cells of the cochlea. Although we have no histological evidence of hair cell damage from our BALB/c mice following priming, there is no reason to believe that priming does not produce the same effect as in C57BL/6J mice. The 'poor' cochlear microphonic obtained from BALB/c mice after priming is indicative of hair cell damage.

Disuse in the nervous system, regardless of how it is produced, seems to be the condition responsible for an increase in nervous system excitability[11]. In view of the evidence

Table 1 Effects of barbiturate withdrawal on seizure susceptibility in non-susceptible BALB/c mice as a function of age

Group	Age	No.	Incidence of seizure pattern* Wild running	Clonic	Tonic	Death
Barbiturate	36 ± 3	10	10	10	9	0
Saline	36 ± 3	6	1	0	0	0
Barbiturate	270 – 275	8	8	8	5	0
Saline	270 – 275	11	1	1	1	0

* Fisher exact probability tests were used to compare the barbiturate and saline control mice for each stage of the seizure pattern. Exact probability values are given in the text.

which suggests that barbiturate anaesthetics produce a general reduction in excitability in at least some parts of the auditory pathway[12] and as a result of the suggestion put forward by Jaffe and Sharpless[13] (and others) that pharmacological disuse supersensitivity resembles other types of disuse supersensitivities, we decided to examine the effect of barbiturate withdrawal on susceptibility of BALB/c mice to audiogenic seizure.

Twenty-one mice from eight litters were divided into two groups (split litters) at 36 ± 3 d of age. This age group was the closest we could get to the immature mouse without introducing other difficulties. Each of the 14 experimental mice was given an intragastric dose of 1.8 mg (approximately 60 mg kg^{-1}) of sodium phenobarbitone dissolved in 0.3 ml of water twice daily at 12 h intervals for 5 d. The other group of seven mice was also given an intragastric dose of isotonic saline of the same volume on the same regimen. The barbiturate was given orally in both age groups. Four experimental and one control animal died during the treatment period. (We were concerned that interference with growth, through food deprivation, might introduce a confounding factor into the experiment but by lowering the dosage and increasing the time over which the drug is administered another problem is created—achieving the effect within the desired age group.)

Thirty hours after the last dose of phenobarbitone had been given to the experimental group, all mice from both groups were tested for susceptibility to audiogenic seizure by exposing each mouse to the sound of an intense electric bell (131 dB relatively to 0.0002 dyne cm^{-2}) inside a circular, sound-insulated chamber for 60 s or until seizure occurred. The types of seizure reactions were noted in those mice which convulsed and the results are shown in Table 1.

All the animals that had been withdrawn from phenobarbitone had florid seizures, but only one of the control animals showed the first stage of the audiogenic seizure sequence; that is, wild running without subsequent seizure. Fisher exact probability tests on incidence of each level of seizure behaviour reveal significant differences between the drug and saline control groups (wild running, $P = 0.0014$; clonic seizure, $P = 0.00012$; tonic seizure, $P = 0.0009$). Barbiturate withdrawal is clearly effective in inducing seizure susceptibility in these normally seizure-resistant mice.

Production of audiogenic seizure susceptibility in resistant mice by previous auditory priming is often regarded as being a developmental phenomenon; that is, the mice can only be made susceptible to seizure if priming occurs during a sensitive period when the mice are still undergoing neural maturation. Gates and Chen[14], however, have recently shown that seizure susceptibility can be achieved in adult mice if the intensity of the priming stimulus is greater than that used to prime younger animals. In view of this finding we thought it useful to examine the effects of barbiturate withdrawal on seizure susceptibility in mature mice.

Twenty-three mice from five litters were divided into two groups (split litters) of 12 and 11 respectively at 270–275 d of age. Each of the 12 experimental mice was given a 60 mg kg^{-1} intragastric dose of sodium phenobarbitone, dissolved in 0.3 ml of water, twice a day for 4.5 d and the 11 control animals were given an intragastric dose of 0.3 ml of isotonic saline on the same regimen. Thirty-four hours after the last dose of barbiturate had been administered, both groups were tested for susceptibility to audiogenic seizure using the method described previously.

The results of testing are shown in Table 1. Four of the animals in the test group died during barbiturate habituation. All of the remaining animals in this group exhibited audiogenic seizures during withdrawal and only one of the 12 control animals showed seizure behaviour. Fisher exact probability tests show that there are significant differences between the groups in incidences of wild running ($P = 0.0001$), clonic seizure ($P = 0.0001$) and tonic seizure ($P = 0.041$). Barbiturate withdrawal is therefore also an effective method for inducing seizure susceptibility in mature mice.

BALB/c mice are genetically resistant to audiogenic seizure. As we have already pointed out, however, susceptibility to seizure may be induced in these mice by exposure to either a loud sound or by depriving them of auditory input several days before the test. Fuller and Wimer[15] and Freund and Walker[16] have also shown that ethanol withdrawal is effective in inducing seizure susceptibility. Although all these methods of producing seizure susceptibility seem to be unrelated, they all share the common property of producing a depression of normal central nervous system activity. Priming produces cochlear damage which reduces input to higher auditory structures while auditory deprivation, by means of tympanic membrane destruction, has a similar effect. Phenobarbital and ethanol are both central nervous system depressants producing a reduction or depression of normal nervous activity, The major effect of reducing activity in the nervous system is that unused structures become supersensitive or over-reactive to sensory stimulation[2,17]. On exposure to a loud sound, the supersensitive structures are driven by auditory input, either directly or indirectly, and a seizure is precipitated. The audiogenic seizure is merely the behavioural manifestation of this underlying supersensitive state.

The significance of our experiment is that we have been able to induce seizure susceptibility by pharmacological means in a strain of mice which can be made susceptible to audiogenic seizure by means of auditory disuse. The fact that auditory stimulation is effective in driving a seizure during barbiturate withdrawal indicates that such a model might be useful in determining whether or not the different methods of disuse share a common or similar mechanism of supersensitivity. Our model is easily prepared and tested and lends itself to a wide variety of biochemical manipulations. These results also prompt speculation about 'spontaneous' seizures which occur in other animals during barbiturate withdrawal; such seizures may be induced by sensory stimulation.

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1 Browning, R. A., and Maynert, E. W., in *Antiepileptic Drugs* (edit. by Woodbury, T. M., Penry, J. K., and Schmidt, R. P.), 345 (Raven, New York, 1972).
2 Wikler, A., and Essig, C. F., *Epilepsy mod. Probl. Pharmacopsychiat.*, 4, 170 (1970).

[3] Huff, S. D., and Fuller, J. L., *Science*, **144**, 304 (1964).
[4] Fuller, J. L., and Collins, R. L., *Devl. Psychobiol.*, **1**, 185 (1968).
[5] Iurrian, W. C., and Fink, G. B., *Devl. Psychobiol.*, **1**, 230 (1968).
[6] Saunders, J. C., Bock, G. R., Chen, C.-S., and Gates, G. R., *Expl Neurol.*, **36**, 426 (1972).
[7] Gates, G. R., Chen, C.-S., and Bock, G. R., *Expl Neurol.*, **38**, 488 (1973).
[8] Chen, C.-S., Gates, G. R., and Bock, G. R., *Expl Neurol.*, **39**, 277 (1973).
[9] McGinn, M. D., Willott, J. F., and Henry, K. R., *Nature new Biol.*, **244**, 255 (1973).
[10] Willott, J. F., and Henry, K. R., *J. comp. physiol. Psychol.*, **86**, 1 (1974).
[11] Sharpless, S. K., in *Basic Mechanisms of the Epilepsies* (edit. by Jasper, H. H., Ward, A. A., and Pope, A.), 329 (Little, Brown, Boston, 1969).
[12] Bock, G. R., *PhD Thesis*, (Monash University, Australia 1973).
[13] Jaffe, J. H., and Sharpless, S. K., *Res. Publs Ass. Res. nerv. ment. Dis.*, **47**, 226 (1967).
[14] Gates, G. R., and Chen, C. S., *Expl Neurol.*, **41**, 457 (1973).
[15] Fuller, J. L., and Wimer, R. E., in *Biology of the Laboratory Mouse* (edit. by Green, E. L.), 609 (McGraw-Hill, New York, 1966).
[16] Freund, G., and Walker, D. W., *Psychopharmacologia Berlin*, **22**, 45 (1971).
[17] Saunders, J. C., Bock, G. R., James, R., and Chen, C.-S., *Expl Neurol.*, **37**, 388 (1972).

Correlation between euploid structural chromosome rearrangements and mental subnormality in humans

For some time cytogeneticists have been concerned with the question of whether structural chromosome rearrangements in humans, while apparently balanced, can have deleterious effects, either physical or mental. I wish to report data which suggest that the latter at least is possible.

Between 1959 and 1972 the chromosomes of blood leukocytes of 33,533 people were examined in the Medical Research Council Clinical and Population Cytogenetics Unit in Edinburgh, and ninety-four euploid structural rearrangements of the autosomes were found, apparently of independent origin. These consisted of thirty-eight Robertsonian translocations, forty-seven reciprocal translocations and nine pericentric inversions. The chromosomes of both parents of forty-six of the subjects were studied and thirty-four of the rearrangements were found to be familial, sixteen being paternal and eighteen maternal in origin, while twelve seemed to have arisen *de novo* and are referred to here as mutants (Table 1). Blood groups and red cell and serum enzymes were studied in all the mutants and their parents and there was no evidence that the legal parents were not the biological parents.

When these data were being compiled the proportion of individuals who were mutants for a euploid chromosome rearrangement and who were also severely mentally retarded seemed to exceed chance expectation. To test this the data have been examined according to the method of ascertainment (see Tables 2 and 3 for details). At the time the original 33,533 blood samples were obtained the reason for the cytogenetic observation, that is, the method of ascertainment, was recorded for each patient. On the basis of this record the patients were divided into three groups: (*a*) those where the ascertainment was 'mental subnormality excluding Down's syndrome', (*b*) those where the ascertainment was 'consecutive or random liveborn babies', and (*c*) the remainder (Table 2). As the proportion of mutants in categories (*b*) and (*c*) was not significantly different ($P < 0.05$—Fisher's exact test) the groups were combined and the proportion of mutants in the mental subnormality group was compared with the proportion among the remainder. The proportion of mutants among the mentally subnormal was significantly greater than the proportion among the other individuals ($P < 0.01$—Fisher's exact test).

While 100% of the members of subnormal group were retarded, most being institutionalised and severely subnormal, several individuals in the other two groups must also have been mentally subnormal although only a very small proportion was likely to be severely retarded. In an attempt to see whether mutants in the other two groups were associated with mental subnormality, the method of discovery and clinical condition of the mutants in these classes were reviewed (Table 3).

Five of the seven mutants whose discovery was independent of any investigation of mental retardation were found during a survey of consecutive liveborn hospital births. One of the remaining mutants, who had a chromosome rearrangement t(3:20), (Table 3) was found during a survey of individuals occupationally exposed to ionising radiations, while the other, who had a rearrangement t(14q15q), was discovered during the investigation of a family in which a t(6p14p; 6q14q) rearrangement was segregating and it was shown that the t(14q15q) in the son must have been maternal in origin. It seems probable, especially as a break at or near the centromere of a chromosome 14 is involved in both translocations, that one rearrangement influenced the occurrence of the other[1].

All the newborn mutants appeared normal at birth, with the exception of laxity of the right hip in one baby (No. 173/71). This baby required hospitalisation at the age of 1 month because of myoclonic fits; however these were not observed in hospital and her EEG was normal. She is now well and at the age of 2 yr development seems to be normal. One child, 177/69, developed myoclonic epilepsy at the age of 3 months with a typical hypsarrhythmia pattern in his EEG. The fits were extremely difficult to control with medication and there was progressive mental deterioration until sudden death at the age of 3.5 yr. The remaining three mutant babies appear developmentally normal and the two mutant adults are physically and intellectually unremarkable.

Table 1 Euploid structural rearrangement of the autosomes

Type of rearrangement	Total No. observed	Total-where both parents examined	Mutant	Inherited Father	Inherited Mother
Robertsonian translocation	38	19	3	10	6
Reciprocal translocation	47	21	9	4	8
Inversion	9	6	0	2	4
Total	94	46	12	16	18

Table 2 Euploid structural rearrangements by method of ascertainment

Method of ascertainment	No. of individuals	Euploid rearrangements No.	Both parents examined	No. of mutants
Mental subnormality (excluding Down's Syndrome)	2,426	12	7	5
Newborn survey	12,295	20	17	5
Other	18,812	62	22	2
Total	33,533	94	46	12

TABLE 3 Mutants ascertained independently of mental subnormality

Patient No.	Karyotype	Method of ascertainment	Phenotype
173/71	45, XX, t(14q21q)	Survey of consecutive liveborn babies	Laxity of right hip at birth. Physically and developmentally normal at 2 yr
26/69	46, XY, t(Cp−; Cq+)	Survey of consecutive liveborn babies	Normal at birth and reported by medically qualified parents to be normal in all respects at the age of 1 yr
177/69	46, XY, t(5; 11) (p11; p15)	Survey of consecutive liveborn babies	Normal at birth. Myoclonic epilepsy at age 3 months, progressive mental deterioration till death at age 3.5 yr
190/72	46, XX, t(2; 10) (q11; q22)	Survey of consecutive liveborn babies	Physically and developmentally normal at birth and at age 1 yr
231/72	46, XX, t(1; 15) (q2; q15 or 21 or 22)	Survey of consecutive liveborn babies	Physically and developmentally normal at birth and at age 6 months
228/72	45, XY, t(14q15q)	Son of chromosomally abnormal parent	Physically and mentally normal 27-yr-old unmarried male
14/71	45, XY, t(3; 20) (q29; p11)	Survey of males occupationally exposed to ionising radiation	Physically and mentally normal 47-yr-old male, with two children, both with a normal karyotype

TABLE 4 Published surveys of mentally subnormal individuals

Reference	No. of individuals examined (excluding Down's syndrome)	No. of euploid rearrangements	Both parents examined	No. of mutants
2	50	1	1	0
3	50	2	1	0
4	264	2	1	0
5	83	2	2	2
6	44	0	–	–
7	85	0	–	–
8	537	1	0	–
9	1,000	4	0	–
10	54	0	0	–
Total	2,167	12	5	2

Very few chromosome surveys of mentally subnormal individuals who do not have Down's syndrome have been published and, in almost every case where data are available, the individuals examined represent a highly selected subgroup of the mentally subnormal. The results of nine published surveys are given in Table 4 and while 12 of the 2,167 people examined were reported to have a euploid structural rearrangement, in only five were both parents examined. Two of these were found to be mutants and while these data are small, they agree with the observation that there is an excess of mutant euploid structural rearrangement among the mentally subnormal.

A deleterious effect of apparently balanced translocations in the heterozygous state has, as far as I am aware, no reported parallel in the animal kingdom. This may be because an experiment designed to detect such an effect would be virtually impossible with most laboratory animals. The phenomena may be widespread and many apparently balanced autosomal exchanges may have a deleterious effect in the heterozygous state on the cell and/or organism carrying them. The mechanism for such an effect could be either (*a*) that the rearrangement is in reality unbalanced and carries a small deletion below the level of cytological detectability; or (*b*) that chromosome breakage and exchange results in a gene mutation at one or both the break points; or (*c*) that certain rearrangements are associated with a position effect detrimental to their carriers.

Whatever the mechanism responsible, the data presented here suggest that the possession of a *de novo* euploid structural rearrangement of the autosomes is, in some cases, associated with and presumably causal to, severe mental retardation. However the follow-up of a large series of newborn babies who are mutants for a euploid structural rearrangement will be necessary before a precise prognosis for mental retardation can be made. More intensive study of mutant individuals might well enable us to determine not only the proportion of euploid mutations associated with a phenotypic effect but also the mechanism by which the effect is produced.

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1 Jacobs, P. A., Buckton, K. E., Christie, S., Newton, M., and Matthew, D., *J. med. Genet.* **11**, (in the press).
2 Summitt R. L., *J. Pediat.*, **74**, 58 (1969).
3 Daly, R. F., *J. Pediat.*, **77**, 444 (1970).
4 Chen, A. T. L., Sergovich, F. R., McKim, J. S., Barr, M. L., and Gruber, D., *J. Pediat.*, **76**, 393 (1970).
5 Thorburn M. J., and Martin, P. A., *J. med. Genet.*, **8**, 59 (1971).
6 Brogger, A., Morh, J., Wehn, M., and Vislie, H., *Int. Copenhagen Cong. Scientific Study of Mental Retardation*, 128 (1964).
7 Yanagisawa, S., *Acta Paediat. Jap.*, **10**, 30 (1968).
8 Iivanainen, M., and Gripenberg, U., *Acta Neurol. scand.*, **43**, Suppl. 31, 53 (1967).
9 Breg, W. R., Miller, D. A., Allerdice, P. W., and Miller, O. J., *Am. J. Dis. Childhood*, **123**, 561 (1972).
10 Lubs, H. A., and Lubs, M. L., *Nobel Symposium*, **23**, 241 (Academic Press, New York, 1973).

Negative outcome of a blind assessment of the association between spray adhesive exposure and human chromosome breakage

THE recent claim of an association of exposure to spray adhesives with a rate of chromosome breakage five to six times that in control subjects, and the report of birth defects in two children of exposed parents led the United States Federal Consumer Product Safety Commission, in August 1973, to ban abruptly the sale of these products. They were recalled from the market and a widely publicised nationwide warning urged all people exposed, particularly pregnant women, to seek chromosome studies. This resulted in widespread popular concern. Cytogeneticists throughout the country received many requests for advice from exposed women concerning termination of pregnancy and future genetic risks. Evaluation of these requests was particularly difficult because there had been no formal publication of the original data at the time of the warning. (And as of January 15, 1974 none has appeared.) The only information released to investigators who queried the Product Safety Commission directly was a copy of a letter from J. R. Seely

TABLE 1 Chromosome breakage in individuals exposed to spray adhesives and in matched controls

	Study A		Study B		Both studies together	
	Exposed	Controls	Exposed	Controls	Exposed	Controls
No. of subjects	11	11	4	4	*	*
No. of metaphases per subject	~36	~36	72	72	*	*
Total No. of metaphases studied	397	398	288	288	685	686
Frequency (and %) of cells with evidence for breakage	7 (1.8%)	3 (0.8%)	2 (0.7%)	4 (1.4%)	9 (1.3%)	7 (1.0%)
	$P = 0.34$ ($\chi_c^2 = 0.92$)		$P = 0.68$ (Fisher's exact test, two-tailed)		$P = 0.81$ ($\chi_c^2 = 0.06$)	

Phytohaemagglutinin stimulated peripheral blood was used; the total time in culture was 72 h because in all experiments collections at 48 h revealed growth too sparse to provide adequate numbers for counting. (The Oklahoma report also used cells cultured for 72 h.) Our procedure included treatment with hypotonic KCl, air drying of cell preparations dropped onto cold water-wet slides from a distance of 18 inches, and staining with carbol fuchsin. Total time in colcemid (0.5 $\mu g\ ml^{-1}$) was 2 h. Unstained areas in chromatids, no matter how large, were considered gaps not breaks, unless there was a clear discontinuity of the chromatid as well as nonalignment of the chromatid axis. Cells with gaps only were not scored as having evidence for chromosome breakage. Study *A* lasted for 2 weeks, and one batch of media was used. Exposed and control subjects could not always be set up on the same day. Only metaphases with at least forty-four centromeres were scored. In the exposed group there was one cell with a dicentric chromosome and six others, each with an acentric fragment. The dicentric and one fragment occurred in the same individual. Otherwise no more than one break occurred in any individual studied. In the controls there were two cells with isochromatid breaks and one with an acentric fragment. (There was also one dicentric in a control cell with only forty-one centromeres, and hence not scored.) In study *B*, blood from each exposed individual and matched control of a pair was drawn, cultured and studied at the same time. The specimens were coded at the time of sampling, and the code was not broken until after analysis. There was an interval of about 1 month between the sampling of first and last pairs. Individuals who were resampled from the original eleven included three of the four with heavy exposure (the fourth was unavailable) and one individual chosen randomly from the three with intermediate exposure. (The group resampled included the exposed individual in the first study with the dicentric and an acentric fragment. In none of the controls resampled had a break been found in any cell examined in study *A*.) In study *B*, two cells, each with an acentric fragment, were found in those exposed and one cell with a dicentric and three others with an acentric fragment were found in the controls.

* Seven pairs were studied once, (~36 metaphases from each subject), and four pairs twice (~108 metaphases from each subject). See text and footnote above.

at the University of Oklahoma who had made the original claim.

This evidence offered in support of the association we found suggestive, but hardly convincing, for the studies did not involve a blind assessment of chromosome breakage. The claimed relationship of birth defects to chromosome breaks and spray adhesive exposure was obscure moreover, for one of the two malformed children with alleged breaks had been conceived several months after the parents ceased use of the spray. In addition, because many of those exposed knew each other, even if there were an increased rate of chromosome breakage, this might have been caused by some other shared environmental factor.

We undertook a study of the problem in September 1973. Eleven individuals with histories of recent exposure to spray adhesives were matched with controls of the same age and sex. Blood specimens were obtained from each, coded, and set up in culture (Table 1). Approximately thirty-six metaphase plates were examined from each individual, providing a total of about 400 cells for analysis from each group. (The anticipated background rate of cells with chromosome breakage in adult controls from earlier studies was about 1.2%. Therefore, should equal rates at this level have been found in both groups, this observation would have excluded, with 95% confidence, a two-fold or greater increase over expected control rate.) When the code was broken, the results (study *A*, Table 1) revealed no significant difference between the two groups. In fact the observed frequency of breaks in the exposed subjects excluded with 95% confidence a rate five times or greater that observed in the controls, which had been the order of magnitude of the increase reported by the Oklahoma investigator. We then repeated the study on four of the original eleven subjects studied who were among those with the heaviest exposure (study *B*, Table 1). The controls were the same. Seventy-two metaphases from each individual were examined. Again there was no statistically significant difference between exposed and control subjects, and the results in the exposed excluded with 95% confidence a rate twice or more that observed in the controls.

No experiment can ever prove negative results, that is the absence of a difference between exposed and controls. The results can only put boundaries on the confidence limits of the observed rates in a particular group of people with a particular history of exposure. Our observations, however, provide no grounds to reject the null hypothesis. That is, on the basis of our results, we believe there is no reason now to accept a purported association of significant magnitude between chromosome breakage and exposure to spray adhesives.

(While our manuscript was in preparation the Consumer Product Safety Commission announced the ban would be lifted[1]. It has been reported that a blind reevaluation of the original slides failed to confirm the initial interpretation[2], and that other investigators, as well as ourselves, could not confirm the original report[2].)

This episode raises questions as to what evidence of chromosome breakage in humans should be grounds for action that may provoke unnecessary public alarm and adverse economic effects.

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1 Dunn, S. E., *Federal Register*, **39**, No. 19, January 28, 3582–3 (1974).
2 *Med. World News*, **15**, February 15, 17 (1974).

Linkage between immune response potential to DNA and X chromosome

INTEREST in the regulation of the immune response to nucleic acids stems largely from the spontaneous occurrence of anti-nucleic acid antibodies in the sera of patients with systemic lupus erythematosus[1–4]. Genetic factors seem to play a role in this disease, which is associated with certain human transplantation antigens[5]. Spontaneous production of antibodies to different forms of nucleic acids has been observed in some mouse strains, and is suggested to be correlated with autoimmune diseases[1–4,6–8]. It is of interest to find out whether the response potential of different mouse strains to nucleic acids, on active immunisation, is related to the spontaneous occurrence of antibodies and/or to autoimmune-like diseases.

We have previously reported that various mouse strains differ in their ability to respond to denatured DNA[9]. Thus, DBA/2 mice were found to be low responders to denatured DNA, whereas SJL/J mice were high responders to this immunogen. The SJL mice are of special interest, as they develop spontaneous reticulum cell neoplasms which resemble Hodgkin's disease in man[10], as well as some immunological abnormalities including the production of anti-nuclear antibodies[7,11]. It was found that the low responsiveness of DBA/2 mice to denatured DNA, is a result of their inability to form antibodies of the IgG class. SJL mice produced anti-denatured DNA antibodies of both 7S and 19S classes, whereas DBA/2 mice elicited only 19S antibodies[9].

Here we demonstrate that the ability to respond to denatured DNA is genetically regulated by an X-linked gene carried on the X chromosome.

SJL mice, DBA/2 mice, their (SJL × DBA/2)F1 hybrids and the backcrosses of the SJL and DBA/2 females with F1 males, namely, SJL × F1 (SJL × DBA/2) and DBA/2 × F1 (SJL × DBA/2), were injected intraperitoneally with 150 μg denatured DNA in a complex with equal weight of methylated bovine serum albumin (MBSA).

The mice were immunised twice within a 3 week interval. The first immunisation was given in complete Freund's adjuvant, whereas in the second one the denatured DNA-MBSA complex was given in phosphate-buffered saline (0.01 M sodium phospate buffer, *p*H 7.0, 0.15 M NaCl). The mice were bled 10 d after the second injection, and their sera were tested for anti-DNA titres by the antigen binding capacity assay, using ^{14}C-DNA, as previously described[9]. In addition, mice of the backcross DBA/2 × F1 (SJL × DBA/2) were assayed for their H-2 antigenic specificities. The animals were typed by the haemagglutination method[12,13], using anti-H-$2^{s/s}$ and anti-H-$2^{d/d}$ alloantisera[13,14].

The immune response to DNA of the animals described above, after the second injection, is demonstrated in Fig. 1. As was shown earlier[9], the SJL mice are high responders, whereas DBA/2 mice are low responders. The F1 hybrids are intermediate responders. The backcross animals, DBA/2 × F1 (SJL × DBA/2) and SJL × F1 (SJL × DBA/2), segregate in their response as expected from the response potential of the parental strains. Thus, the ability to respond to denatured DNA is genetically controlled.

Typing of the backcross mice DBA/2 × F1 (SJL × DBA/2) for their major histocompatibility (H-2) specificity indicated that the capacity to respond to denatured DNA is not linked to H-2, as the H-2^s antigenic specificity, donated by the SJL high responder strain, was detected in low and high responder animals. The results obtained with these backcross mice, however, demonstrate that the gene which regulates the response potential to DNA is on the X chromosome. All the males of the DBA/2 × F1 (SJL × DBA/2) backcross were low responders, whereas all the females responded well (Fig. 1). These results could be expected if the response to DNA is controlled by a dominant X-linked gene. All the males of this backcross possess the X_{low} allele donated by the DBA/2 low responder females. On the other hand, all the females carry one X_{high} allele donated by the parental males F1 (SJL × DBA/2). These F1 parental males carry the X_{high} allele, as they are offspring of a cross between SJL high responder females (X_{high} X_{high}) and DBA/2 low responder males (X_{low} Y). Mice of the backcross SJL × F1 (SJL × DBA/2) were good antibody producers (Fig. 1), as expected, as all of them carry at least one X_{high} allele. Thus, the females of this backcross are X_{high} X_{high} and the males are X_{high} Y.

Analysis of the antibody classes produced by mice of the backcross DBA/2 × F1 (SJL × DBA/2) indicated that a poor responsiveness to DNA is associated with inability to elicit antibodies of the IgG class, as was previously shown for the DBA/2 low responder parental strain[9]. Thus, the low responder males of this backcross produced only 19S antibodies, whereas in the sera of the high responder females, antibodies of both 19S and 7S were present.

The immune response to another nucleic acid, namely, double-stranded polyinosinic-polycytidylic acid, (poly(I)·poly(C)), was also found to be controlled by a gene on the X chromosome[15]. It has been suggested that poly(I)·poly(C) is a thymus-independent antigen, as neonatally thymectomised mice produced antibodies to this antigen[16]. The immune response to type III pneumococcal polysaccharide, which is a thymus-independent antigen, is also linked with the X chromosome[17]. Is the response potential to denatured DNA thymus independent, and if so, is an association between the

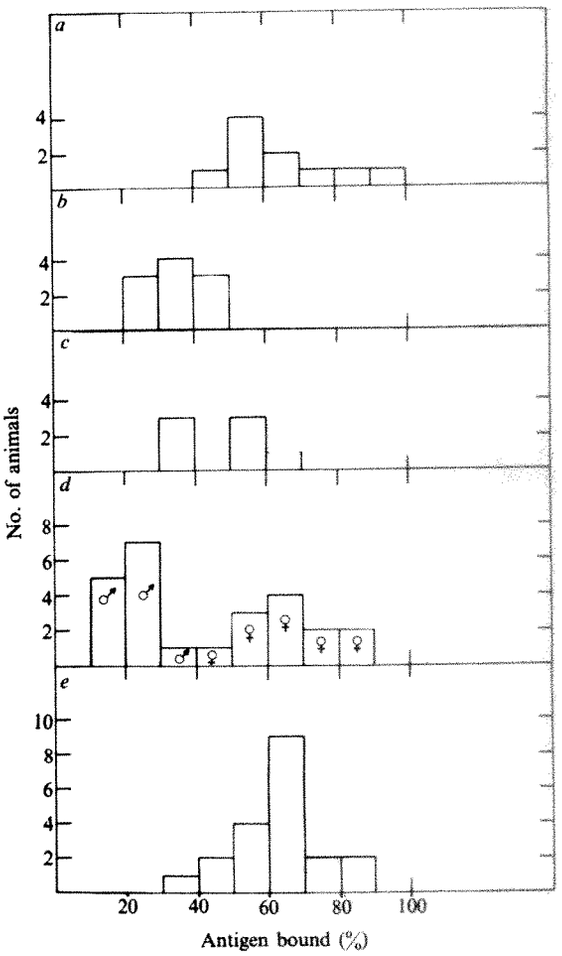


FIG. 1 The antibody response of mice immunised with denatured DNA-MBSA and assayed with ^{14}C-DNA. The horizontal axis plots the % antigen bound in the antigen-binding assay, while the vertical axis plots the number of animals falling into a given percentile for antigen bound values. 10 μl of each antiserum were assayed. *a*, SJL; *b*, DBA/2; *c*, (SJL × DBA/2) F1; *d*, DBA/2 × F1 (SJL × DBA/2); *e*, SJL × F1 (SJL × DBA/2). The sex of the animals is shown in *d*.

gene(s) controlling the response to thymus-independent immunogens and the X chromosome a general phenomenon?

An efficient immune response to denatured DNA could be obtained only when the immunogen was complexed with MBSA. Strain-dependent differences in the response potential to some synthetic polypeptides[18,19] have been shown to be obliterated when the immunogens were injected as complexes with MBSA. In the case of denatured DNA, strain differences in the ability to produce antibodies were observed following immunisation with complexes of denatured DNA–MBSA.

Here we have demonstrated a linkage between the ability of mice to respond to denatured DNA upon active immunisation, and the X chromosome. Such a linkage may contribute to the elucidation of the phenomenon of autoimmune diseases, especially if a similar association should also be found between spontaneous antibodies to DNA and the sex chromosome. This experimental approach may be of relevance for the understanding of human immune disorders, as the incidence of some autoimmune diseases in man seems to be associated with sex[20,21].

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1 Thoburn, R., Koffler, D., and Kunkel, H. G., *Proc. Soc. exp. Biol. Med.*, **136**, 711–714 (1971).
2 Levine, L., and Stollar, B. D., *Prog. Allergy*, **12**, 161–191 (1968).
3 Koffler, D., Carr, R., Agnello, V., Thoburn, R., and Kunkel, H. G., *J. exp. Med.*, **134**, 294–312 (1971).
4 Steinberg, A. D., Baron, S., and Talal, N., *Proc. natn. Acad. Sci. U.S.A.*, **63**, 1102–1107 (1969).
5 Grumet, F. C., Coukell, A., Bodmer, J. G., Bodmer, W. F., and McDevitt, H. O., *New Engl. J. Med.*, **285**, 193–196 (1971).
6 Norin, L. C., and Holmes, M. C., *J. Immun.*, **93**, 148–154 (1964).
7 Ben-Yaakov, M., and Haran-Ghera, N., *Israel J. med. Sci.*, **8**, 666 (1972).
8 Teague, P. O., Friou, G. J., and Myers, L. L., *J. Immun.*, **101**, 791–798 (1968).
9 Stollar, B. D., Fuchs, S., and Mozes, E., *J. Immun.*, **111**, 121–129 (1973).
10 Murphy, E. D., *37th A. Rep.*, 33 (The Jackson Laboratory, Bar Harbor, Maine, 1964–65).
11 Wanebo, H. J., Gallmeier, W. M., Boyse, E. A., and Old, L. I., *Science*, **154**, 901–903 (1966).
12 Stimpfling, J. H., *Transplant. Bull.*, **27**, 109–111 (1961).
13 Maron, E., Scher, H. I., Mozes, E., Arnon, R., and Sela, M., *J. Immun.*, **111**, 101–105 (1973).
14 Stimpfling, J. H., Boyse, E. A., and Mishell, R., *Meth. med. Res.*, **10**, 18–21 (1964).
15 Scher, I., Frantz, M. M., and Steinberg, A. D., *J. Immun.*, **110**, 1396–1401 (1973).
16 Steinberg, A. D., Law, L. W., and Talal, N., *Arthritis Rheum.*, **13**, 369–377 (1970).
17 Amsbaugh, D. F., Hansen, C. T., Prescott, B., Stashak, P. W., Barthold, D. R., and Baker, P. J., *J. exp. Med.*, **136**, 931–949 (1972).
18 Green, I., Paul, W. E., and Benacerraf, B., *J. exp. Med.*, **123**, 859–879 (1966).
19 McDevitt, H. O., *J. Immun.*, **100**, 485–492 (1968).
20 Mackay, I. R., and Burnet, F. M., *Autoimmune Diseases*, 71–104 (Thomas, Springfield, Illinois, 1963).
21 Christian, C. L., in *Immunological Diseases*, (edit. by Samter, M., and Alexander, H. L.) 725–736 (J. and A. Churchill, London, 1965).

Lymphocyte separation from blood

MUCH attention has been focussed on the role of lymphocytes in the immune response of the tumour-bearing host. To study the cytotoxic effects of lymphocytes from rabbits bearing VX2 tumours towards the tumour cells *in vitro*, a suitable method for obtaining lymphocytes from peripheral blood was sought. With the recognition that both T and B lymphocyte populations are heterogeneous, and have different properties and functions which modulate each other's activities[1], the question of cell yield assumes importance in lymphocyte preparations. The method of choice should also be simple and rapid.

Several methods have been described in the literature for preparing comparatively pure lymphocyte suspensions from peripheral blood. For separation of lymphocytes from granulocytes, columns of cotton wool or glass beads have been widely used[2–4]. This method is rather lengthy and is difficult to carry out in sterile conditions. The major disadvantage is that erythrocytes are not efficiently retained by the columns, and the effluents always contain a similar or higher number of erythrocytes as lymphocytes. The yield of lymphocytes recovered by this method is usually small (about 50%). Purification of lymphocytes from blood by either Triosil (Isopaque)-Ficoll or carbonyl iron powder seemed to be simple and less time consuming, and these two methods were examined for preparing lymphocytes from rabbit blood.

Böyum[5] devised the Triosil-Ficoll method for separating lymphocytes from human blood. His lymphocyte preparation usually contained about 80% lymphocytes, 18% monocytes and 6% erythrocytes with a lymphocyte yield of greater than 95%. Harris and Ukaejiofo[6] using the same method, obtained on average 70% of the available lymphocytes. Using this Triosil-Ficoll method, we obtained lymphocyte yield from human blood of between 30% and 40% and from rabbit blood a yield of between 6% and 50%. Attempts to obtain higher yields were not successful. In addition, the purity of the lymphocyte preparations was not satisfactory.

Several groups of workers have used the carbonyl iron method for preparing lymphocyte suspensions[7–9]. These workers do not state the actual yield of lymphocytes recovered per ml of blood, although from the results of Coulson and Chalmers[7] it can be calculated that their yield for human blood was 25–50%. A lymphocyte yield from rabbit blood of greater than 70% (average 74%) has been consistently obtained by us with this technique. The yield of lymphocytes obtained from human blood, however, was always about 40%. The carbonyl iron method is simple and the entire purification procedure requires less than 2 h.

Venous blood (10 ml) was placed in a siliconised universal bottle containing 200 units of phenol-free heparin (Heparin BP (Mucous), Boots Pure Drug Co. Ltd., Nottingham, England) in 1 ml of saline. To this, 3.3 ml of sterile 1% methyl cellulose (viscosity 15 centipoise, Standard Grade, Dow Chemical Co.) in saline containing 120 mg of sterile carbonyl iron powder 3μm size (SF, GAF, Great Britain Ltd., Manchester) was added. The universal bottle containing the blood–methyl cellulose–iron mixture was incubated at 37° C for 30 min and was gently shaken at 5 min intervals. At the end of 30 min incubation, the contents of the bottle were transferred to another siliconised universal bottle which was left on a magnet at 37°C for 30 min. Red cells, granulocytes and monocytes settle with the iron particles. The supernatant, containing mainly lymphocytes and some iron particles, was pipetted off into a fresh universal bottle using a sterile Nylon Cannula (Portex Ltd., Kent, England) attached to a 1 ml syringe. The red cell pellet was spun at low speed (1000 r.p.m.) for 30 s using an MSE bench centrifuge. The supernatant was carefully pipetted off as above and added to the previous supernatant. The lymphocytes from the combined supernatants were centrifuged (2000 r.p.m. for 5 min on MSE

bench centrifuge), and washed three times with warm Waymouth medium (Medium MB 752/1, Difco Laboratories). During this washing procedure, the lymphocytes are freed from residual iron particles by resuspending the cells in the medium without disturbing the iron deposit, and decanting to a fresh siliconised universal glass bottle. Finally, the lymphocytes were suspended in Waymouth medium containing 5% bovine serum albumin previously warmed to room temperature. The differential count was performed using white blood cell (WBC) fluid (1% acetic acid in saline with two drops of methyl blue).

The lymphocyte preparation from rabbit blood contains over 95% lymphocytes compared with 50–60% lymphocytes in the blood before separation. One or two erythrocytes are usually present for every five lymphocytes, as observed by Giemsa staining. The preparation of human blood obtained by this method contains 50–60% lymphocytes compared to 30–40% lymphocytes in the original blood. Usually one or two erythrocytes are present for every lymphocyte in the suspension.

Lymphocytes prepared as above from rabbits bearing VX2 tumours are currently being investigated for cytotoxic activity towards the tumour cells previously labelled by ^{125}I-iododeoxyuridine by *in vitro* or *in vitro* methods.

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1 Raff, M. C., *Nature*, **242**, 19 (1973).
2 Rabinowitz, Y., *Blood*, **23**, 811 (1964).
3 Lamvik, J. O., *Acta Haemat.*, **35**, 294 (1966).
4 Scholar, E. M., and Calabresi, P., *Cancer Res.*, **33**, 94 (1973).
5 Böyum, A., *Scand. J. clin. Lab. Invest.*, **21**, suppl. 97, 77 (1968).
6 Harris, R., and Ukaejiofo, E. O., *Lancet*, **ii**, 327 (1969).
7 Coulson, A. S., and Chalmers, D. G., *Immunology*, **12**, 417 (1967).
8 Pentycross, C. R., *J. clin. Path.*, **21**, 175 (1968).
9 Catalona, W. J., Sample, W. F., and Chrelien, P. B., *Cancer*, **1**, 65 (1973).

T lymphocyte requirement for MSV tumour prevention or regression

Adult mice injected with murine sarcoma virus Moloney isolate (M-MSV) develop sarcomas which have a high incidence of spontaneous regression. As tumour cells are strongly antigenic and release virus continuously, it is generally agreed that tumour regression is mediated by a host immune reaction[1]. In fact, M-MSV induces a progressively growing tumour in newborn or adult mice immunologically depressed by X-irradiation, anti-lymphocyte serum or neonatal thymectomy[2–4]. It is still not clear, however, if humoral or cellular immunity is mainly responsible for M-MSV tumour regression[5–8]. Both bone marrow-derived (B) and thymus-derived (T) lymphocytes have been shown to possess a killer effect *in vitro* against target cells bearing Moloney virus specified cell surface antigens[9,10]. Here we report preliminary data which indicate that T lymphocytes are necessary for spontaneous M-MSV tumour regression.

CBA mice of both sexes were thymectomised at 2 months of age. Two weeks later they were both irradiated with 850 rad and injected intravenously with 5×10^6 syngeneic bone marrow cells. These mice were then considered 'deprived' for T cell functions. A cell-free tumour extract of M-MSV, maintained by serial *in vivo* passage in 1–2-week-old CBA or BALB/c mice, was prepared as previously reported[8]. Three to four weeks after irradiation, deprived mice were injected intramuscularly in the left thigh with 0.05 ml of this tumour extract diluted to 10^{-2} g equivalent. Control CBA mice were similarly treated. All mice were inspected daily for tumour development and growth.

As shown in Table 1, 27 of 30 deprived mice (90%) developed tumours with a mean latent period of 17 d; moreover, 24 of these 27 mice died with progressive tumours 2–3 months following M-MSV injection. No tumours were observed in M-MSV-injected control mice. These results indicate that the deficiency of the T cell population is responsible for the induction and progression of tumours in the deprived mice.

To better evaluate whether restoration of T cell function could reverse the oncogenic response, deprived mice were grafted under the kidney capsule with neonatal thymus lobes, either from CBA or CBA/H-T6T6 donors, at different time intervals before or after M-MSV injection. These mice were considered 'reconstituted' for T cell function. Reconstitution was also evaluated in mice grafted with CBA/H-T6T6 thymuses, by means of karyotypic analyses of peripheral blood lymphocytes after PHA stimulation[11]. As seen in Table 2, mice receiving the thymus graft 30 d before M-MSV injection did not develop tumours while mice which were grafted 1, 5 or 25 d after M-MSV injection presented a tumour incidence of 87.5, 69 and 100%, respectively, with a latency of 15–19 d. Furthermore, the mice reconstituted 1 or 5 d following M-MSV injection presented 100% regression while the mice grafted with a thymus 25 d after M-MSV injection presented tumours which progressed and killed the host.

It has been reported that following thymus grafting in deprived mice, a gradual restoration of T cell functions occurs and is accomplished in about 30 d (ref. 12). Consequently, our results may easily be explained in terms of complete or partial restoration of T-cell activity at the time of M-MSV injection. Thus, the mice grafted before M-MSV injection were as immunocompetent as the controls and did not develop tumours. Mice grafted 1 or 5 d after M-MSV injection present tumours but all of them regress, most likely because of a gradual increase in the T cell population during tumour growth. In this regard, the time needed for regression was greater (31 d) for mice grafted on the fifth day than that needed for mice grafted on the first day following M-MSV injection (13 d). Finally, thymus grafting 25 d after M-MSV does not affect the tumour because the neoplasm has probably reached a very advanced stage.

Table 1 Tumour induction by M-MSV in normal and deprived CBA mice

	Total No. mice	No. mice with tumour (%)*	No. mice with regressed tumour (%)†	No. mice dead with tumour (%)‡
Normal	30	0 (0)	0 (0)	0 (0)
Deprived	30	27 (90)	3 (11)	24 (89)

* % of mice with tumour evaluated from total number of mice.
† % of mice with regressed tumour calculated from number of mice developing tumour.
‡ % of mice dead with tumour calculated from number of mice developing tumour.

TABLE 2 Tumour induction in CBA mice thymus-grafted at different intervals before or after M-MSV injection

Time of thymus graft (d)	Total No. mice	No. mice with tumour (%)*	No. mice with regressed tumour (%)†	No. mice dead with tumour (%)‡
−30	14	0 (0)	0 (0)	0 (0)
+ 1	8	7 (87.5)	7 (100)	0 (0)
+ 5	13	9 (69.2)	9 (100)	0 (0)
+25	6	6 (100)	0 (0)	6 (100)

* % of mice with tumour evaluated from total number of mice.
† % of mice with regressed tumour calculated from number of mice developing tumour.
‡ % of mice dead with tumour calculated from number of mice developing tumour.

TABLE 3 M-MSV neutralisation titres* of sera from normal, deprived and reconstituted mice at different days following M-MSV injection

	Days from M-MSV Injection		
	15	20	60
Normal	8	16	32
Deprived	4	8	16
Reconstituted	—	16	32

Pools of sera inactivated at 56°C for 30 min, were incubated at various dilutions for 60 min at room temperature with 30–40 focus forming units (f.f.u.) of M-MSV. The mixtures of sera and M-MSV were then assayed for focus formation on DEAE-dextran treated 3T3FL mouse cells plated the day before at concentration of 10^5 in 60 mm plastic petri dishes. The cultures, in duplicate, were grown in Dulbecco's medium supplemented with 10% inactivated and filtered foetal calf serum and antibiotics. Foci were scored on the fifth day.

* Reciprocal of serum dilution that resulted in a 50% reduction of focus formation is given.

As there is evidence indicating that humoral antibodies may interfere with M-MSV tumour induction and progression[5,7,13], it was considered worthwhile to study whether deprived and reconstituted mice could produce virus-neutralising antibodies. Mice, normal, deprived and reconstituted before M-MSV, were bled at various intervals following M-MSV injection. Their sera were assayed by the *in vitro* focus reduction method on 3T3 FL cells[14]. As shown in Table 3, both deprived and reconstituted mice produced neutralising antibody, although the former had a somewhat lower titre. This last finding could be due to the decrease of antibody in free form, which is usually observed in the serum of mice with progressing tumours[6]. Alternatively, since both 19S and 7S immunoglobulins are responsible for virus neutralisation[15], it might be that the capability to synthesise 7S antibodies, which is a function of T and B cell cooperation[16], is reduced or absent in deprived mice. Obviously, the fact that deprived mice produce virus-neutralising antibody does not exclude the possibility that their serum contains also antibody with an enhancing or blocking effect, although these antibodies are mainly of the 7S type[17]. The importance of T cells in restraining M-MSV oncogenicity clearly emerges in the experimental model studied. However, whether T cells operate directly by a cytotoxic effect on tumour cells or through cooperation with B cells resulting in more efficient antibody production, remains to be elucidated.

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[1] Fefer, A., McCoy, J. L., and Glynn, J. P., *Cancer Res.*, **27**, 962 (1967).
[2] Fefer, A., McCoy, J. L., and Glynn, J. P., *Cancer Res.*, **27**, 1626 (1967).
[3] Law, L. W., Ting, R. C., and Allison, A. C., *Nature*, **220**, 611 (1968).
[4] Law, L. W., Ting, R. C., and Stanton, M. F., *J. natn. Cancer Inst.*, **40**, 1101 (1968).
[5] Bubenik, J., Turano, A., and Fadda, G., *Int. J. Cancer*, **4**, 648 (1969).
[6] Pearson, G. R., Redmon, L. W., and Bass, L. R., *Cancer Res.*, **33**, 171 (1973).
[7] Fefer, A., *Cancer Res.*, **29**, 2177 (1969).
[8] Chieco-Bianchi, L., Pennelli, N., Collavo, D., and Tridente, G., in *Morphological and Functional Aspects of Immunity* (edit. by Lindahl-Kiessling, Alm and Hanna), 155 (Plenum Press, New York-London, 1971).
[9] Lamon, E. W., Skurzak, H. M., and Klein, E., *Int. J. Cancer*, **10**, 581 (1972).
[10] Leclerc, J. C., Gomard, E., Plata, F., and Levy, J. P., *Int. J. Cancer*, **11**, 426 (1973).
[11] Doenhoff, M. J., Davies, A. J. S., Leuchars, E., and Wallis, V., *Proc. R. Soc. Lond.*, **B76**, 69 (1970).
[12] Davies, A. J. S., *Transpln. Rev.*, **1**, 43 (1969).
[13] Chieco-Bianchi, L., Collavo, D., Biasi, G., and Colombatti, A., *Br. J. Cancer*, **28**, 238 (1973).
[14] Hartley, J. W., and Rowe, W. P., *Proc. natn. Acad. Sci., U.S.A.*, **55**, 780 (1966).
[15] Lamon, E. W., Klein, E., Andersson, B., Fenyo, E. M., and Skurzak, H. M., *Int. J. Cancer*, **12**, 637 (1973).
[16] Miller, J. F. A. P., in *Contemporary Topics in Immunobiology* (edit. by Davies and Carter), 151 (Plenum Press, New York–London, 1973).
[17] Hellstrom, I., and Hellstrom, K. E., *Int. J. Cancer*, **4**, 587 (1969).

Induced redistribution of membrane particles, anionic sites and con A receptors in *Entamoeba histolytica*

THE extent to which the distribution of antigens and other receptors at the cell surface is determined by structural components within the plasma membrane is largely unknown. In erythrocyte ghost membranes, freeze-fracture and freeze-etch studies have demonstrated that structural components, 'membrane intercalated particles', are the exclusive sites at the surface which bear A and B antigens[1,2], wheat germ agglutinin[3], influenza virus[3], con A receptors (Pinto da Silva and Nicolson, unpublished), and anionic sites[4]. It is clear, however, that conclusions derived from the study of this membrane system cannot be freely extrapolated to living cells where the relationship cell surface-membrane structure may be more complex. We report a study of this relationship on the plasma membrane of living *Entamoeba histolytica* cells in conditions which induce a redistribution of membrane intercalated particles, anionic sites or con A receptors.

Trophozoites of *E. histolytica*, a pathogenic strain cultured under axenic conditions[5], were washed with phosphate-buffered saline (PBS). Aggregation of membrane particles was induced by gradual impregnation with 25% glycerol in PBS, incubation for 30 min at 34° C and 30 min more at

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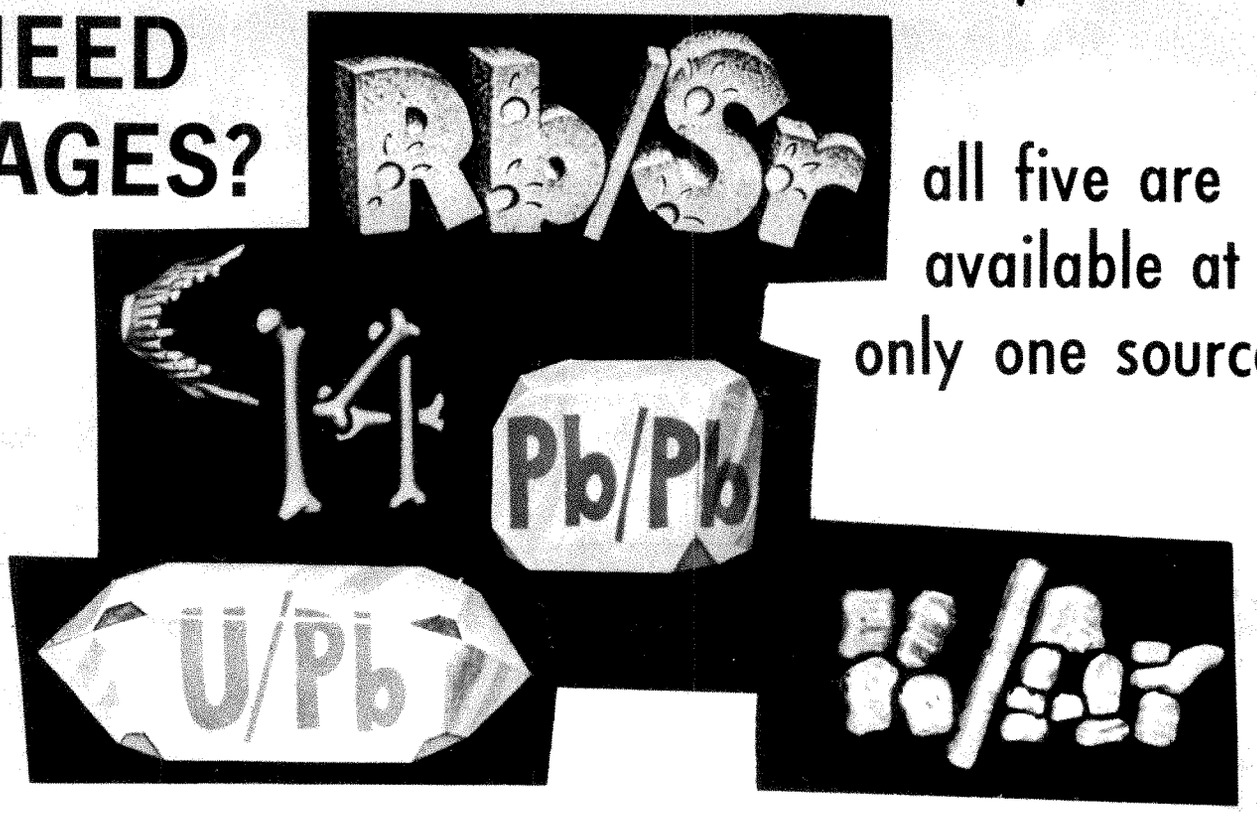
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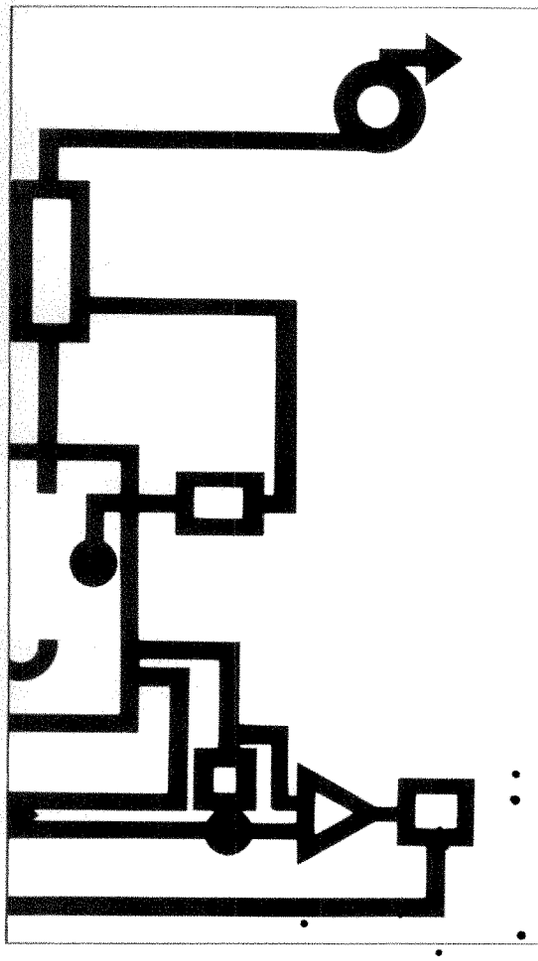
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Introduction to Space Charge Electron Optics

G. A. Nagy and M. Szilagyi

This introduction to space charge electron optics is an updated translation of an original Hungarian publication which presents to the West for the first time a comprehensive survey of the vast work carried out in this field in the USSR and Eastern Europe. It is however more than just a summary of literature as it also contains the solution of many problems and so points the way to future developments. £9.00

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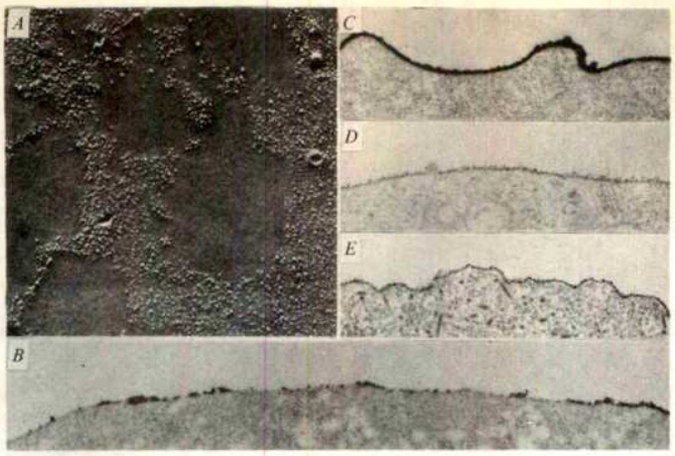


FIG. 1 Membrane intercalated particles, anionic sites and con A receptors in *E. histolytica* plasma membranes. *A*, Freeze fracture; *B*, colloidal iron at *p*H 1.8; *C*, con A–peroxidase; *D*, ferritin at *p*H 4.0; *E*, colloidal iron, prefixed cell. *A*, and *D*, 33,000; *B*, *C* and *E* × 16,500.

24° C. Cell viability of glycerol impregnated cells as determined by exclusion of trypan blue remained over 75%, dead cells being easily distinguished in thin sections or in freeze-fracture replicas. For freeze fracture, cells were rapidly frozen in the liquid phase of partially solidified Freon 22, and fractured at −100° C in a Balzers 300 apparatus equipped with a turbomolecular pump. The ultrastructural distribution of acidic sites ionised at *p*H 1.8 was determined by labelling the cells with colloidal iron[6]. Negatively charged sites ionised at *p*H 4.0 were labelled with positively charged ferritin, obtained by dialysis at *p*H 4.0, that is, below its isoelectric point[7]. Con A receptors were detected by the con A–peroxidase reaction[8].

To alter the distribution of structural components within the plane of the plasma membrane, living cells were incubated in glycerol, which results in aggregation of the membrane intercalated particles revealed by freeze fracture[9]. In our experimental conditions, we find that glycerol-induced aggregation of membrane particles in *E. histolytica* (Fig. 1*A*) is more prominent than that previously reported for lymphoid cells[9,10]. Glutaraldehyde prefixation completely prevents glycerol-induced aggregation of membrane particles. In consequence, the aggregated pattern of membrane particle distribution which is observed on the fracture faces of plasma membranes is clearly distinguished from the non-aggregated pattern observed in cells fixed before glycerol impregnation. As can be seen in other cells systems, detailed examination of uncontaminated replicas of prefixed cells demonstrates, under favourable shadowing conditions, the existence of an extremely heterogeneous particle population where all transitions ranging from a slight rugosity—here termed 'subparticle'—to a definite membrane particle can be observed. This renders difficult the unequivocal identification of membrane particles. Aggregation of membrane particles induced by glycerol, however, provides a clear distinction between smooth and particulate regions of membrane fracture faces, because both particles and subparticles coaggregate into a clearly delimited network, which leaves in between regions denuded of any rugosity. The proportion of membrane area which is occupied by aggregates of particles and subparticles varies, even within a given cell, from 30 to 70%.

Ultrastructural observation of glycerol-pretreated cells demonstrates a patchy distribution of acidic sites labelled by colloidal iron at *p*H 1.8 (Fig. 1*B*). This constrasts sharply with the continuous and uniform distribution of both con A receptors (Fig. 1*C*) and acidic sites ionised at *p*H 4.0 (Fig. 1*D*). In control cells prefixed in glutaraldehyde, uniform labelling over the entire cell surface is observed with all three reactions, as is illustrated in Fig. 1*E* for colloidal iron. Cells treated with ferritin, at *p*H 7.0, remain unlabelled.

In view of the report that con A induces aggregation of membrane particles in plasmocytoma cells[11], and the high agglutinability of pathogenic strains of *E. histolytica* relative to strains isolated from asymptomatic carriers[12], we studied the effect of con A on the distribution of membrane particles in pathogenic HK9 trophozoites. In our experimental conditions (5, 10, 20, or 100 μg ml^{-1} con A, 30 min, 34° C) the lectin does not cause any apparent alteration in the distribution of membrane particles. In addition, treatment of cells with con A does not result in redistribution of membrane receptors detected with the peroxidase–benzidine reaction. When cells are fixed after con A–peroxidase treatment, however, the electron-dense precipitate is confined to a cell region containing the uropod, in a manner analogous to 'cap' formation in lymphocytes[13], as will be reported in detail elsewhere. Freeze-fracture examination of these cells does not reveal a corresponding accumulation of membrane particles.

Our results demonstrate that glycerol treatment of *E. histolytica* cells results in marked aggregation of the membrane intercalated particles and in patchy distribution of acidic sites labelled by colloidal iron at *p*H 1.8, that glycerol induced aggregation of membrane particles is not accompanied by a redistribution of acidic sites ionised at *p*H 4.0 or of con A receptors and that con A receptors may redistribute independently of the membrane particles.

Our observations indicate that the cell surface–membrane structure relationship in *E. histolytica* cells is not straightforward. Although it remains possible that anionic sites labelled at *p*H 1.8 may correspond to the membrane intercalated particles, no such direct relationship exists between either acidic sites ionised at *p*H 4.0 or con A receptors and the membrane intercalated particles.

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1 Pinto da Silva, P., Branton, D., and Douglas, S. D., *Nature*, **232**, 194 (1971).
2 Pinto da Silva, P., *thesis*, (University California, Berkeley, 1971).
3 Tillack, T. W., Scott, R. E., and Marchesi, V. T., *J. exp. Med.*, **135**, 1209 (1972).
4 Pinto, da Silva, P., Moss, P. S., and Fudenberg, H. H., *Expl. Cell Res.*, **81**, 127 (1973).
5 Diamond, L. S., *J. Parasitol.*, **54**, 715 (1968).
6 Gasic, G., Berwick, L., and Sorrentino, M., *Lab. Invest.*, **18**, 63 (1968).
7 Greer, M. H., and Baker, R. F., *Proc. 7th Int. Cong. Electron Microscopy*, **3**, 31 (Grenoble, 1970).
8 Bernhard, W., and Avrameas, S., *Expl Cell Res.*, **64**, 232 (1971).
9 McIntyre, J. A., Gilula, N. B., and Karnovsky, M. J., *J. Cell Biol.*, **59**, 208a (1973).
10 Mandel, T. E., *Nature new Biol.*, **239**, 112 (1972).
11 Benedetti, E. L., Dunia, I., and Diawara, A., *Eur. J. Cancer*, **9**, 263 (1973).
12 Martínez-Palomo, A., González-Robles, A., and de la Torre, M., *Nature new Biol.*, **245**, 186 (1973).
13 Taylor, R. B., Duffus, W. P. H., Raff, M. C., and de Petris, S., *Nature new Biol.*, **233**, 225 (1971).

Platelet secretion induced by divalent cation ionophores

SECRETION by platelets is stimulated by agents of diverse structure such as thrombin, collagen and adrenaline; the substances secreted include adenine nucleotides, 5-hydroxytryptamine and calcium[1]. In many cells secretion seems to be mediated by the influx of Ca^{2+} (ref. 2), but this mechanism has not been demonstrated for all secretory systems and, in fact, no extracellular calcium is required for secretion by platelets. From kinetic studies of thrombin-stimulated secretion of ATP and calcium[3,4] we proposed that stimulus-secretion coupling in platelets is mediated by intracellular calcium. We have now tested this hypothesis with the divalent cation ionophores X-537A (Lasalocid, Hoffman-La-Roche) and A23187 (Lilly). These antibiotics increase the permeability of membranes to divalent cations and can perturb intracellular cation gradients[5–10]. The probable mechanism involves formation of lipophilic chelate compounds[10]. If secretion by platelets were mediated by the intracellular release of stored calcium (or some other divalent cation) the ionophores should disrupt the intracellular ion gradients and trigger secretion. This was the observed result.

Secretion was observed as the release of ATP, which was continuously measured by the firefly luminescence method[4]. The experiments were performed in the absence of extracellular Ca^{2+}, but addition of 1 mM Ca^{2+} or Ca^{2+} chelators did not affect the reactions. Both ionophores cause release of ATP with a time course that is nearly identical to that of thrombin-induced secretion (Fig. 1). The dependence on concentration of ionophore (Fig. 2) is consistent with other studies that show that A23187 is more effective as a calcium ionophore than is X537A (refs 5 and 7). Since the rate constant for release (Fig. 1) and the amount released (Fig. 2) are the same whether induced by thrombin or ionophores, the final steps in the multistep stimulus-secretion mechanism must be the same. In addition, no further ATP can be released by thrombin after treatment with ionophores and, conversely, ionophores do not release additional ATP from thrombin-treated platelets.

X537A and A23187 also cause release of calcium. We attempted to follow the kinetics of this release by the murexide dye binding technique[3] but this method gave spurious results in this system. The apparent yield of calcium estimated

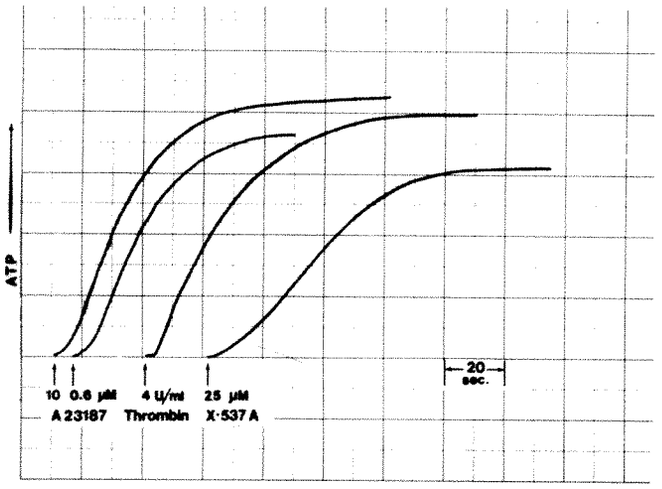


FIG. 1 Thrombin-induced and ionophore-induced secretion of ATP by washed human platelets. The preparation of platelets has been described before and ATP secretion was measured by the firefly luminescence procedure under conditions identical to those in ref. 4. A23187 was added as 5 μl of an ethanol solution and X537A as 5 μl of a dimethylformamide solution. Addition of solvents alone had no effect. Thrombin was purified by the method of Glover and Shaw[11] and had a specific activity of 1800 to 2000 U mg^{-1}.

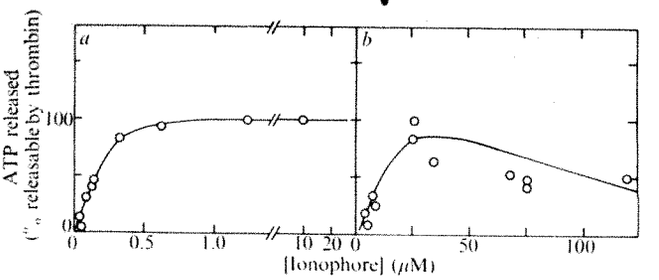


FIG. 2 Dependence of ATP secretion on concentration of ionophore. Yields are shown as percentage of ATP released by 4 U ml^{-1} of thrombin from the same platelet preparation. *a*, A23187; *b*, X537A.

from change in optical density of murexide was about twice the amount directly measured by atomic absorption. This error is probably due to binding of murexide to the membrane, a less polar environment. (We have measured large spectral shifts in murexide and calcium-murexide in non-polar solvents such as acetonitrile–water mixtures.) Atomic absorption measurements indicated that after 2 min exposure to ionophore nearly all the platelet calcium was recovered in the supernatant.

Interpretation of these results requires consideration of the known biological activity of these ionophores[5–10]. X537A causes release of accumulated Ca^{2+} from isolated sarcoplasmic reticulum vesicles and also induces contraction in intact muscle preparations[8,9]. Both compounds have also been shown to cause secretion of histamine from mast cells, but only in the presence of extracellular calcium[7]. There is no evidence that ionophores are capable of transporting ATP, and although X537A has been found to change the permeability of cells to monovalent cations or amines[3,5,7], no such effect has been shown for A23187. In this regard, we also found that the K^+ ionophore valinomycin did not cause secretion by platelets. Thus the most likely effect of the ionophores in these experiments is to disrupt divalent cation gradients. Since this must involve intracellular ion, the results are consistent with the hypothesis of an internal calcium flux triggering secretion in platelets. We favour calcium as the active ion (ionophores also transport Mg^{2+}) because of the well known role of extracellular calcium in secretion processes[2]. We therefore propose as a tentative model that there is an intracellular store of calcium analogous to sarcoplasmic reticulum in muscle. It is this calcium that triggers secretion. Thrombin causes release of the calcium by a series of steps following binding to the membrane. Ionophores by-pass the thrombin steps (just as they by-pass electrical stimulation in muscle preparations) and directly release the trigger calcium which causes secretion in the normal way.

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1 Mustard, J. F., and Packham, M. A., *Pharmac. Rev.*, **22**, 97 (1970).
2 Rubin, R. P., *Pharmac. Rev.*, **22**, 389 (1970).
3 Detwiler, T. C., and Feinman, R. D., *Biochemistry*, **12**, 282 (1973).
4 Detwiler, T. C., and Feinman, R. D., *Biochemistry*, **12**, 2462 (1973).
5 Pressman, B. C., *Fed. Proc.*, **32**, 1698 (1973).

[6] Reed, P. W., and Lardy, H. A., *J. biol. Chem.*, **247**, 6970 (1972).
[7] Foreman, J. C., Mongar, J. L., and Gomperts, B. D., *Nature*, **245**, 249 (1973).
[8] Scarpa, A., and Inesi, G., *FEBS Lett.*, **22**, 273 (1972).
[9] Levy, J. V., Cohen, J. A., and Inesi, G., *Nature*, **242**, 461 (1973).
[10] Johnson, S. M., Herrin, J., Liu, S. J., and Paul, I. C., *J. Am. chem. Soc.*, **92**, 4428 (1970).
[11] Glover, G., and Shaw, E., *J. biol. Chem.*, **246**, 4594 (1971).

Urinary excretion of carnitine in progressive muscular dystrophy

ABNORMAL fat metabolism in the muscles of patients with progressive muscular dystrophy is one possible explanation for the aetiology of this genetic disease but the nature of this abnormality, if any, is not known. In dystrophic mice, the skeletal muscles affected are defective in oxidising fatty acids, but can increase synthesis[1–3]. In this disease, carnitine might be expected to be involved in the impaired fatty acid metabolism, because it is of major importance in the oxidation of long-chain fatty acids as it transports them to the mitochondrial sites where they are oxidised[4–6]. If there were a carnitine deficiency, fatty acid oxidation would be impaired, resulting in an excessive synthesis of triglyceride from unoxidised substrate. To examine whether carnitine is involved in the pathogenesis of progressive muscular dystrophy, urinary excretion of carnitine was estimated in patients suffering from generalised muscular atrophy of various aetiologies.

Twenty-four hour samples of urine were collected from 54 patients; 39 with Duchenne-type dystrophy, 4 with facioscapulohumeral dystrophy, 1 with lime-girdle dystrophy and 6 with spinal progressive muscular atrophy and other diseases. As control subjects, 121 healthy men and 101 healthy women, aged from 8 to 45 yr, were studied. Carnitine was isolated from urine by Amberlite-120 (H^+) ion-exchange column chromatography and was determined by a modification of Friedman's method[7].

The results are summarised in Table 1. In normal controls, there is an obvious difference between men and women, men usually having a higher excretion rate than women. The results also indicate low carnitine excretion in all 39 boys and men with Duchenne-type dystrophy as compared with the control subjects ($P < 0.001$) and it is evident that carnitine excretion is significantly lower in all patients compared with controls, matched for sex and age. In these patients, no significant correlation was found between carnitine excretion and the severity or the duration of the disease, and the level of serum creatine phosphokinase. Carnitine excretions of the patients with facioscapulohumeral or lime-girdle dystrophy are within normal range. Six patients with spinal progressive muscular atrophy have high excretions.

TABLE 1 Urinary excretion of carnitine in healthy control subjects and in patients with neuromuscular diseases

	No. of cases	Carnitine excretion (mg d^{-1})
Normal control male	121	57.7 ± 9.6
female	101	44.1 ± 2.9
Duchenne-type dystrophy	39	30.9 ± 3.2
Facioscapulohumeral dystrophy	4	59.9 60.5 61.8 63.4
Lime-girdle dystrophy	1	44.7
Myotonic dystrophy	2	29.2 58.6
Spinal progressive muscular atrophy	6	63.4 65.5 71.5 92.6 103.0 148.8
Amyotrophic lateral sclerosis	1	71.6
General muscle cramp[8]	1	36.8

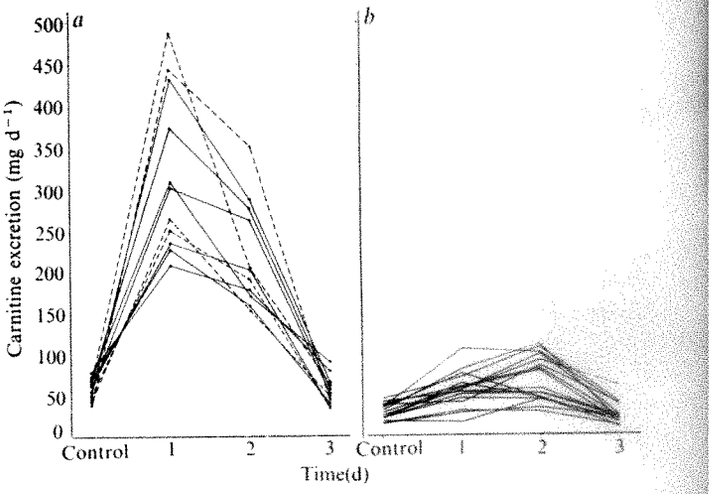


FIG. 1 Changes of urinary excretion of carnitine in patients with Duchenne-type muscular dystrophy and in control subjects before and after injection of synthetic β^{1-24}ACTH-Z. Synthetic β^{1-24}ACTH-Z 1 mg was injected intramuscularly at 0800 h on the first day of the experiment and then urine collections were made consecutively for 3 d. *a*, Normal control; ——, men; – – –, women; *b*, Duchenne-type dystrophy.

Figure 1 illustrates the effect of synthetic β^{1-24} ACTH-Z on urinary excretion of carnitine in the control subjects and in the patients with Duchenne-type dystrophy. ACTH-Z injection stimulated carnitine excretion in the controls, but not in the patients. In other diseases, spinal progressive muscular atrophy and general muscle cramp[8], ACTH-Z injection did increase urinary excretion to the same extent as that in the controls. ACTH has been known to have some lipolytic activity, but the mechanism whereby it promotes cranitine excretion remains to be elucidated.

Engel and Angelini[9] demonstrated in a myopathy with excessive lipid that the patient's muscle homogenates had an impaired ability to oxidise long-chain fatty acids because of the low levels of carnitine and that the changes favouring oxidation might occur after addition of carnitine to the homogenates.

The results reported here suggest that a similar deficiency state of carnitine exists in the muscles of Duchene-type dystrophy but direct evidence is lacking. Further investigations are required to draw any definite conclusion and such studies are now in progress in our laboratory.

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[1] Susheela, A. K., *Clinica chim. Acta*, **22**, 219 (1968).
[2] Lin, C. H., Hudson, A. J., and Strickland, K. P., *Life Sci.*, **8**, 21 (1969).
[3] Strickland, K. P., Lin, C. H., and Hudson, A. J., in *Muscle Disease*, (edit. by Walton, J. N., *et al.*) 273 (Excerpta Medica, Amsterdam, 1970).
[4] Fritz, I. B., in *Advances in Lipid Research*, (edit. by Paoletti, R., and Kritchevsky, D.) 285 (Academic Press, New York, 1963).
[5] Fritz, I. B., in *Cellular Compartmentalization and Control of Fatty Acid Metabolism*, (edit. by Gran, F. C.) 39 (Academic Press, New York, 1968).

[6] Bressler, R., in *Comprehensive Biochemistry*, (edit. by Florkin, M., and Stotz, E. H.) **18**, 331 (Elsevier Publishing Co., New York, 1970).
[7] Friedman, S., *Archs Biochem. Biophys.*, **75**, 24 (1958).
[8] Satoyoshi, E., and Yamada, K., *Archs Neurol.*, Chicago, **16**, 254 (1967).
[9] Engel, A., and Angelini, C., *Science*, **179**, 899 (1973).

Early hominid ulna from the Omo basin, Ethiopia

A NEARLY complete right ulna (Loc 40–19) was recovered by G. Eck in 1971 in Member E of the Shungura Formation in the lower Omo basin of Southern Ethiopia. It was found in a locality within unit E5 which immediately underlies Tuff F, the K–Ar age for which is 2.04 Myr[1–4]. This locality has yielded other vertebrate fossils but no further hominid remains in spite of intensive searching. The lower limb of fossil hominids is much better represented than the upper and the remains that do exist are mostly fragmentary. This is the first early hominid forearm bone to be recovered in a state of completeness that will allow locomotor inferences, based on pelvic and lower limb material, to be correlated with good evidence from the upper limb.

The specimen (see Fig. 1) has been damaged both before and after fossilisation and the surface bone has many linear cracks, some of which are more than 1 mm wide and are filled with matrix. The shaft is broken in five places and at the distal three sites it has been possible to reassemble the fragments.

The ulna shaft is long and attenuated. It is curved, convex posteriorly, throughout its length. The sinuous curvature that is seen from below in modern human ulnae is scarcely detectable. The shaft is triangular in section; distally the angles become less well defined. The interosseus border forms an irregular, low crest, which fades out at the level of the bony markings for pronator quadratus. It is unlike the accentuated crest of modern human bones.

The rounded medial border forms the blunt apex of the triangular shaft section. It begins as a low ridge from the medial side of the subcutaneous border of the olecranon and continues distally to a point just proximal to where the interosseus crest is no longer palpable.

The posterior border begins as a crest at the apex of the subcutaneous area of the olecranon. The height of the crest diminishes distally, and it is impalpable in the distal third of the shaft. The border is convex laterally near its proximal end but straightens distally.

The anterior surface of the shaft lies between the medial and interosseus borders. It is damaged at the junction of its proximal and middle thirds; some surface bone is missing and some flakes of bone are depressed below the surface; this area probably included the site of the nutrient foramen. There are also cracks, parallel to the long axis of the shaft, probably produced by antero-posterior crushing of the shaft. The effect of these has been to accentuate the prominence of the medial border. The proximal end of the anterior surface is separated by an irregular ridge of bone from the triangular-shaped area just distal to the radial notch. This rough surfaced area is only very slightly hollowed, in contrast to the marked hollowing for the radial tuberosity in modern human bones.

The postero-medial surface is bounded by the posterior and medial borders. It is curved convexly in its long axis and the distal end follows the spiralling of the shaft.

The postero-lateral surface lies between the interosseus and posterior borders and is the least extensive of the three surfaces. Proximally it is flat from side to side and becomes more convex distally. It is marked by three oblique lines probably related to the attachments of the extensor and abductor muscles. A low ridge, accentuated by linear cracks in the bone, runs along the long axis of this surface, presumably marking the attachment of the deep extensor aponeurosis.

The distal third of the shaft is more cylindrical in section. It is distinguished by a prominent curved crest of bone which runs between the anterior and postero-medial aspects, marking the origin of the pronator quadratus.

The distal end of the specimen is expanded. It includes most of the head, but the posterior aspect of the shaft and the styloid process are missing. The head bears a convex articular surface on its antero-lateral side for articulation with the radius, and a much smaller, less distinct, distally directed articular surface. These surfaces, seen from below, are crescentic.

The proximal end of the ulna bears two processes, the coronoid and olecranon, and two articular areas, the trochlear and radial notches. Only the olecranon process has escaped significant damage.

The proximal surface of the olecranon is marked, from above downwards, by synovial reflection, the triceps bursa and the triceps insertion. A crest separates the olecranon from the rough triangular, subcutaneous area of the proximal end of the ulnar shaft. The subcutaneous area is in line with the posterior border of the shaft; viewed from the side, it does not project posteriorly as it does in some non-hominid ulnae. At the medial border of the olecranon process is a pronounced tubercle for the origin of flexor carpi ulnaris and for the insertion of the posterior fibres of the medial ligament of the elbow point. Distal to the tubercle is a strongly concave area for the origin of flexor digitorum

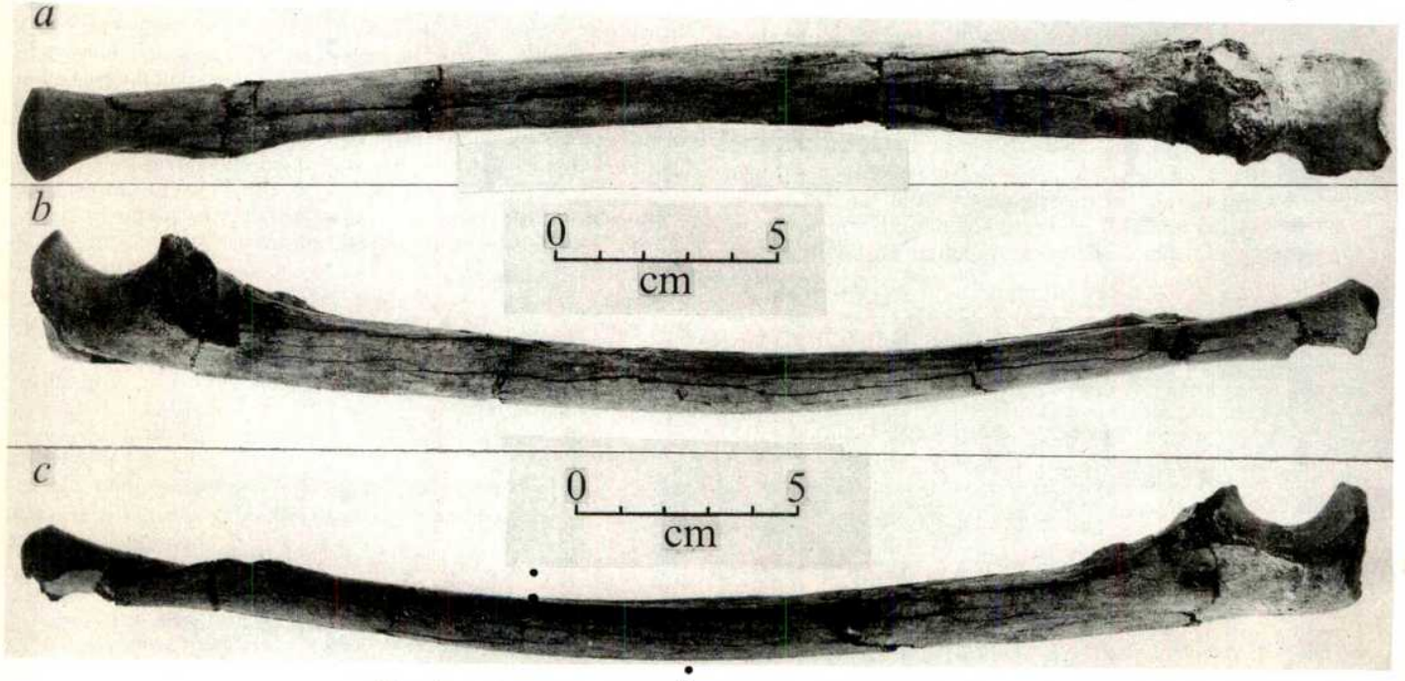


FIG. 1 *a*, Superior view of Omo ulna (Loc 40–19); *b*, lateral view; *c*, medial view.

TABLE 1 Length and curvature indices of Omo specimen and of ulnae of other higher primates

Specimens		*Homo* (=45)	*Pan* (=11)	*Gorilla* (=14)	*Pongo* (=10)	*Hyalobates* (=3)	Omo (Loc 40–19)
Maximum length	Mean	250	291	346	367	249	315
	s.d.	19.0	14.8	38.5	24.8	23.0	—
	s.e.	2.8	4.6	10.3	8.0	13.5	—
Relative length index	Mean	12.0	14.5	12.5	16.0	24.9	13.0
	s.d.	1.3	2.5	6.6	1.9	2.4	—
	s.e.	0.2	0.8	1.8	0.6	1.4	—
Curvature index	Mean	7.2	11.0	11.4	8.1	3.4	10.5
	s.d.	1.1	1.4	1.6	1.1	0.2	—
	s.e.	0.2	0.4	0.4	0.3	0.1	—

profundus. The shaft does not project proximal to the olecranon process as it does in baboon ulnae.

The coronoid process is extensively damaged along its margins. Its anterior surface, and the adjacent part of the shaft, are occupied by the ulnar tuberosity. The antero-medial side of the tuberosity is marked by a pronounced fossa for the insertion of brachialis. The lateral edge of the fossa is elevated and may be for a head of flexor pollicis longus.

The trochlear notch lies between the two processes, and is formed by two articular surfaces separated by a roughened area of bone. The proximal of the two articular surfaces is divided by a flattened central area into two nearly equal, slightly concave, areas for the flanges of the trochlea of the humerus. The distal articular surface on the coronoid process is incomplete and is divided by a ridge into a larger medial and smaller lateral surface. The lateral surface meets, at an angle, the laterally facing, concave, radial notch. Both the notch and the supinator crest, that leads distally from it, are extensively damaged.

Preliminary examination and comparisons indicate that this new fossil resembles in many respects modern human ulnae, but some features depart significantly from the modern human pattern. Morphological assessment has been supplemented by measuring some 20 parameters. The same measurements have been taken on samples of extant higher primates. These samples are small in some instances but prove adequate for this preliminary study. Features divergent from the modern human condition include:

(1) **Length of the shaft.** In the Hominoidea relative elongation of the forelimb is generally associated with suspensory postures, and this is demonstrated by the intermembral index. That this elongation is largely due to lengthening of the forearm can be demonstrated by the brachial index[5]. The maximum length of this fossil ulna and ulnae of other higher primates are given in Table 1. Using the *t* test, the probability that the fossil specimen could be included in the *Homo* sample is low ($P = <0.005$). Limb lengths are only useful when related to total body size, but from indirect evidence there is no reason to suppose that fossil hominids were taller than the modern *Homo sapiens* populations from which our sample was taken. The maximum length therefore suggests an elongated forearm for this particular fossil hominid. We have attempted to measure relative elongation of an isolated ulna by using the index

$$\frac{\text{Maximum shaft length (Martin}^{6}\text{ (1))}}{\text{Olecranon to coronoid distance (Martin (7))}}$$

The results (see Table 1) show that relative length can be related to locomotor habit—suspensory forms have high values. There is so much overlap, however, between the ranges of *Gorilla* and *Homo* that the significance of the fossil's value is difficult to assess.

(2) **Dorso-ventral curvature of the shaft.** The values for the curvature of the ulnar shaft, expressed as an index (Martin (4)), in which highly curved bones have high values, are also given in Table 1. In general, curvature is more marked in knuckle walkers than in suspensory forms. *Homo* occupies an intermediate position. The curvature seen in the African apes is presumably a response of the ulnar shaft to stresses peculiarly associated with knuckle walking, for *Papio*, with a digitigrade forelimb stance, has a straight ulna. The value of the index for this fossil specimen falls outside the human range and within that of the knuckle walkers; the probability levels are $P = <0.005$ and $P = 0.3$ respectively.

(3) **Cross-sectional profile of the ulna shaft.** Human ulnae generally have a prominent interosseus border which suggests that stresses on the interosseus membrane have dominated the final shape of the modern human ulnar shaft. A well marked bony shelf is uncommon in other hominoids and is absent in this fossil. Its absence, combined with the dorso-ventral flattening of the shaft, may indicate significantly different patterns of stress than those seen in modern human ulnae. It is possible that these differences are associated with changes in the relative bulk of forearm flexor and extensor muscles comparable with the changes which Tuttle[7] has demonstrated between knuckle walkers and humans.

(4) **Pattern of muscle origins and insertions.** The pattern of muscle markings in the fossil broadly parallels that found in modern human ulnae, though two differences may be significant. The first is the flatness and lact of buttressing of the ulnar tuberosity. The second is the weak development of the supinator crest compared with that of modern human ulnae. Further specimens may be required to determine whether these distinctions are merely the result of intra-group variability, which is substantial in man and other higher primates, or whether they reflect genuine differences in the development of the brachialis and supinator muscles in this hominid.

(5) **Shape of the ulna head.** Generally, the crescentic articular surface of the ulna head is more convex in modern human ulnae than it is in this fossil. Even if this difference is significant, its functional implications are not clear.

Overall, the functional implications of these differences from modern human ulnae point to a relatively elongated forearm and some morphological adaptations in the fossil not unlike those seen in modern knuckle walkers.

Unfortunately the ulna is still one of the least well represented parts of the skeleton among the early Hominidae. Three specimens are known for certain. Two are proximal fragments, one from Kromdraai (TM 1517) and one from East Rudolf (KNM-ER 1500); both are smaller than the Omo fossil. Their fragmentary state and the high intra-group and low between-group variability in ulnar morphology in higher primates strictly limits their usefulness.

OH, 36 ulna from Bed II Olduvai Gorge, Tanzania is complete apart from its distal end. As judged by the positions of structures that are common to both ulnae its relative length is shorter by approximately 5% than the Omo specimen and its maximum length is approximately the same as the Omo ulna. It is also more curved. The significance of any differences between the two fossils must await the detailed assessment of both specimens.

It is clear that the affinities of the Omo fossil are with the Hominidae. Mandibular and dental remains of Hominidae have been found at 11 localities within Member E of the

Shungura Formation. All, or nearly all, of this material seems to represent a robust australopithecine, but there are still no known associations of cranial and dental and postcranial parts of this hominid in most of the Omo succession. It is difficult therefore to determine the taxonomic affinities of this specimen. It seems to us that any such judgment must rely strongly on morphological features, and to a lesser extent on the functional capabilities inferred from them. These can then be related to existing hypotheses about the locomotor patterns of early hominids.

There is increasing evidence from other early hominid postcranial remains, from South and East Africa, that the morphology of robust australopithecines differed significantly from that of *Homo*[8–12]. This is now shown more clearly by material, recently announced, from East Rudolf, which is very like *Homo* and carries this dichotomy probably back beyond 2.6 million years[13]. Consequently, in view of the differences between the Omo fossil specimen and modern human ulnae, we feel that this ulna is probably that of a robust australopithecine and should be referred to *Australopithecus boisei*.

The relative elongation and the presence of certain features suggestive of a role in body support in this fossil do not necessarily mean that this creature used habitual suspensory postures or that it knuckle walked. Both these suggestions have been put forward, the first by Robinson[12], and the second by R. E. F. Leakey[14] in connection with a hominid humerus (KNM-ER 739) from East Rudolf. Tuttle, and others before him, have cogently argued that knuckle walking is a specialised means of fore-limb propulsion and support developed as a consequence of an arboreal form subsequently adapting to a terrestrial life. The morphological and functional evidence seen in this specimen is insufficient to infer such a specialised locomotor adaptation for this hominid.

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[1] Brown, F. H., in *Calibration of Hominoid Evolution* (edit. by Bishop, W. W. and Miller, J. A.) 273 (Scottish Acad. Press, Edinburgh, 1972).
[2] Howell, F. C., in *Calibration of Hominoid Evolution* (edit. by Bishop, W. W. and Miller, J. A.) 331 (Scottish Academic Press, Edinburgh, 1972).
[3] Howell, F. C., and Coppens, Y., *Am. J. phys. Anthrop.*, **39** (in the press).
[4] Bonnefille, R. *et al.*, *C. r. hebd. Seanc. Sci., Paris*, **276**, 2879 (1973).
[5] Knussman, R., *Bibliotheca Primatologia*, **5**, (Karger, Basle, 1967).
[6] Martin, R., Saller, K., *Lehrbuch der Anthropologie*, **1**, (Gustav-Fischer, Stuttgart, 1957).
[7] Tuttle, R. H., in *The Chimpanzee*, **2**, (edit. by Bourne, G. H.), 167 (Karger, Basle, 1970).
[8] Napier, J. R., *Archs. Biol., Liège* (Suppl.), **75**, 673 (1964).
[9] Patterson, B., and Howells, W. W., *Science*, **156**, 64 (1967).
[10] Day, M. H., *Nature*, **221**, 230 (1969).
[11] Day, M. H., in *Symp. Zool. Soc. Lond.*, No. 33 (edit. by Zuckerman, Lord) 29 (Academic, London, 1974).
[12] Robinson, J. T., *Early Hominid Posture and Locomotion* (University of Chicago, Chicago, 1972).
[13] Leakey, R. E. F., *Nature*, **242**, 447 (1973).
[14] Leakey, R. E. F., *Nature*, **231**, 241 (1971).

The effect of prostaglandin $F_{2\alpha}$ on ovarian blood flow and corpora lutea regression in the rabbit

In many species luteolysis is dependent upon the presence of the uterus and an intact utero-ovarian vasculature. The occurrence of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in uterine tissue, its specific venoconstrictor properties and its ability to induce luteolysis exogenously, led to the hypothesis that its release by the uterus might cause luteolysis by constricting the uterine and utero-ovarian veins and reducing ovarian bloood flow[1]. The observation that total ovarian venous outflow fell during infusions of $PGF_{2\alpha}$ in rats and rabbits (after laparotomy and ovarian vein cannulation) supported this hypothesis. A subsequent investigation using similar methods failed to confirm this in rats[2]. Experiments in sheep with ovaries and uterus transplanted *in toto* to the neck produced no unequivocal evidence for a drop in ovarian blood flow following doses of $PGF_{2\alpha}$ producing luteolysis, at least until oestrus ensued[3]. With increasing doses however, an immediate fall in ovarian blood flow occurred[4].

In rabbits from mid gestation to near term, the corpora lutea receives about 80% of the total ovarian arterial flow[5]. It is therefore possible that a substantial decrease in corpora luteal blood flow could be effected by regional redistribution of blood within the ovary without affecting the total flow. Novy[6] used radioactive microspheres to measure ovarian blood flow distribution in pseudopregnant rabbits and observed a 25% reduction in luteal flow on injection of $PGF_{2\alpha}$ intravenously. No significant alteration in total arterial ovarian blood flow occurred. In three sheep, which were not pregnant, corpora luteal blood flow, measured with radioactive microspheres, fell within 5.5 h from the start of a $PGF_{2\alpha}$ infusion

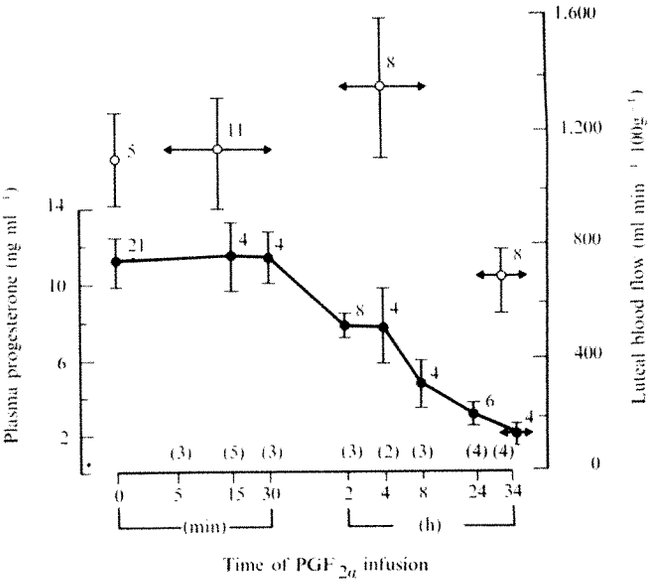


Fig. 1 Effect of $PGF_{2\alpha}$ infusion on peripheral plasma progesterone (●) and corpora luteal blood flow (○). Mean values with standard error and the number of observations are shown. The number of rabbits injected with microspheres at each time from the start of infusion is shown in brackets. Corpora luteal blood flows at time zero were determined by Abdul-Karim and Bruce[5].

TABLE 1 Ovarian blood flows (ml per min per 100 g (mean ± s.e.)) after 250 $\mu g\ h^{-1}$ $PGF_{2\alpha}$ given intra-arterially

Infusion time	*N*	Corpus luteum	Stroma	Total ovary
zero	5	1155 ± 212	127 ± 9	435 ± 59
5–30 min	11	1171 ± 196	674 ± 94*	809 ± 123
2–8 h	8	1360 ± 245	472 ± 62*	725 ± 127
24 h and delivery	8	689 ± 109	202 ± 42	327 ± 59

* significantly greater than zero time control ($P < 0.005$; *t* test)

into the uterine vein[7]. Luteolysis itself may affect the rate and distribution of ovarian blood flow. We therefore investigated the effect of $PGF_{2\alpha}$ on the chronological order of changes in ovarian blood flow and its distribution to test the hypothesis that alterations in these parameters may precede induced luteolysis.

Twenty-nine pregnant rabbits were used. The day of mating was denoted as day 0 of gestation. $PGF_{2\alpha}$ (5 ml h^{-1}) in saline solution was infused in 27 and saline alone in 2 rabbits through an aortic catheter introduced through a femoral artery, the tip being 4 cm above the origin of the ovarian arteries. The dose of $PGF_{2\alpha}$ was 250 $\mu g\ h^{-1}$ which induces labour in unanesthetised rabbits[8]. Anaesthesia was induced and maintained by an initial dose of 30 mg kg^{-1} sodium pentobarbitone and further small intravenous injections until the injection of microspheres as previously described[9]. When the infusion of prostaglandins lasted more than 8 h, sodium thiopentone anaesthesia was used for the initial placement of the infusion catheter; the rabbits were then allowed to recover and were anaesthetised with sodium pentobarbitone about 1 h before the microsphere injection. In preliminary trials, these procedures had little effect on ovarian blood flow. About 100,000 microspheres 25μm in diameter labelled with ^{46}Sc were injected into the left ventricle over 30 s, at various times after the start of the infusion (Fig. 1). Arterial plasma progesterone concentrations were measured before and during infusion with a radioimmunological method based on that of Thorneycroft and Stone[10] using petroleum ether (Mallinkrodt 30°–60°C Nanograde) instead of diethyl ether. The blood flow measurements were made on day 28 of gestation except in the two rabbits infused with saline (day 29 and 30). After the microspheres were injected, the rabbit was killed and the position of the infusion catheter was checked. The ovaries were separated into luteal and stromal components, weighed and measured for radioactivity to determine blood flows[9].

Arterial blood pressure did not alter significantly during the infusion of $PGF_{2\alpha}$, saline or during microsphere administration. There was a significant fall ($P < 0.01$; *t* test) in peripheral plasma progesterone within 2 h of the start of $PGF_{2\alpha}$ infusion (Fig. 1). This fall continued to delivery. The corpora luteal arterial blood flows were combined into three groups, those measured before the fall in plasma progesterone (5, 15 and 30 min after the start of $PGF_{2\alpha}$ infusion), those measured after a significant decline in plasma progesterone (2, 4 and 8 h) and those measured at 24 h or at delivery (Fig. 1). Blood flows in five control rabbits which were not infused[5] are also shown. There was no evidence of a decline in corpora luteal blood flow for at least 8 h after the start of $PGF_{2\alpha}$ infusion.

Even at 24 h, the mean flow was 825 ± 172 (s.e.) ml per min per 100 g ($n = 4$) or 71% of the control value. A substantial fall, to 552 ± 115 ml per min per 100 g was observed only in the four rabbits infused with $PGF_{2\alpha}$ to delivery. No difference was noted between flow rates in two rabbits receiving microspheres at delivery and one each at 0.5 and 4 h after delivery. Corpora luteal blood flow was 1,590 and 817 ml per min per 100 g in the rabbits infused with saline at 30 and 60 min following delivery, after infusions for 62 and 100 h respectively. At this time plasma progesterone was low (3.1 and 4.4 ng ml^{-1}). Table 1 shows corpora luteal, stromal and total ovarian arterial blood flows. Prostaglandin $F_{2\alpha}$ infusion had a rapid large effect on ovarian stromal flow. Five minutes after the start of infusion flow rose from a control value[5] of 127 ± 9 ($n = 5$) to 987 ± 209 (range 609–1330) ml per min per 100 g ($n = 3$). Stromal flow remained high for at least 8 h of infusion but returned to near normal at 24 h. When divided in the same way as corpora luteal blood flows, stromal flow was significantly greater between 5 min and 8 h infusion of $PGF_{2\alpha}$ but not at 24 h or delivery. Changes in total ovarian blood flows and corpora luteal blood flows were not statistically significant.

We conclude that in the rabbit luteolysis induced by $PGF_{2\alpha}$ is not preceded by a biologically significant reduction in the arterial blood flow to the corpora lutea. The mechanism of action lies elsewhere. The rise in ovarian stromal blood flow, also observed by Novy in pseudopregnant rabbits[6] is intriguing. In our experiments it was associated with a transitory decrease in placental blood flow and an increase in vaginal and cervical flow (N.W.B. and K.H., unpublished). Whether the circulatory changes in the stroma act indirectly to initiate luteolysis remains speculative, but a direct effect of $PGF_{2\alpha}$ on corpora luteal tissue must be considered.

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1 Pharriss, B. B., Cornette, J. C., and Gutknecht, G. D., *J. Reprod. Fert.* (Suppl. 10) 97–103 (1970).
2 Behrman, H., Yoshinaga, K., and Greep, R. O., *Ann. N.Y. Acad. Sci.*, **180**, 426–435 (1971).
3 McCracken, J. A., Barcikowski, B., Carlson, J. C., Green, K., and Samuelsson, B., *Adv. Biosci.*, **9**, 599–624 (1973).
4 McCracken, J., *Ann. N.Y. Acad. Sci.*, **180**, 456–472 (1971).
5 Abdul-Karim, R. W., and Bruce, N. W., *Fertil. Steril.*, **24**, 44–47 (1973).
6 Novy, M. J., *Biol. Reprod.*, **7**, 105–106 (1972).
7 Thorburn, G. D., and Hales, J. R. S., *Proc. Aust. physiol. pharmac. Soc.*, **3(2)** 145 (1972).
8 Nathanielsz, P. W., Abel, M., and Smith, G. W., *J. Endocr.*, **55**, 617–618 (1972).
9 Bruce, N. W., and Abdul-Karim, R. W., *J. Reprod. Fert.*, **32**, 15–24 (1973).
10 Thorneycroft, I. H., and Stone, S. C., *Contraception*, **5**, 129–146 (1972).

Placental transfer of asbestos

ASBESTOS fibres have been reported to penetrate the walls of the stomach and intestine of animals[1,2]. Earlier work at this institution showed that asbestos fibres can pass through the walls of the digestive tract and travel throughout the body[3,4]. Additional work with neutron-activated asbestos also indicated that asbestos could cross the placenta but definite confirmation of this was not obtained[4]. Here we show, by electron microscopy, that asbestos fibres can cross the

TABLE 1 Asbestos fibres in foetal liver and lungs from pregnant rats injected with asbestos

Rat No.	Total asbestos injected intravenously (mg)	Number of foetuses	Foetal liver fibres g^{-1} ($\times 10^6$)	Foetal lung fibres g^{-1} ($\times 10^6$)
		Experiment 1		
1	0	13	0	0
2	0	11	0	0
3	0	12	1.38	1.52
4	0	11	0	0.23
5	9*	11	2.83	1.00
6	9*	14	2.93	1.25
7	12	12	27.03	139.97
8	12	13	18.34	1.26
		Experiment 2		
9	0	13	0	0
10	0	16	0	0
11	0	12	0	0
12	0	16	0.45	0
13	4	12	7.59	0.14
14	4	10	0	4.32
15	4	7	3.95	0.38
16	4	8	1.38	1.38
17	10	9	2.67	1.33
18	10	14	100.12	2.90
19	10	8	39.32	0
20	10	8	5.65	3.91

* Young were born about 2 h after the third injection but were killed immediately.

placenta but that the extent to which this occurs is highly variable.

The same chrysotile asbestos preparation used in earlier work[3,4] containing approximately 10 × 10 fibres μg^{-1} was injected into the femoral vein of 300 g pregnant Wistar rats at levels of 1 to 3 mg (1 mg ml^{-1} water) at 2 d intervals beginning on the 10th to 14th day of gestation. The foetuses were removed by Caesarean section on the day before parturition to ensure that they did not inhale any asbestos fibres. To prevent cross contamination of the blood of the mother with the tissues of the foetuses the rats were killed by inhalation of ether and the foetuses within their intact amnionic sacs were removed from the uterus. Upon removal of the amnionic membranes the foetuses were opened and the lungs and livers saved for asbestos analysis. The livers from two foetuses (about 0.2 g) and the lungs from three (about 0.4 g) from each rat were analysed by previously described techniques[4].

In the first experiment four rats received 3 mg doses of asbestos. Two of these received 12 mg before they were sacrificed, but the other two bore young about 2 to 3 h after the third dose, apparently initiated by the stress of the injection. Preliminary experiments indicated that higher doses were usually fatal with the rats showing difficulty in breathing. This may be related to the large amount of intravenously injected asbestos which initially lodges in the lungs[4]. Extremely high values were recorded in the foetal liver and lungs from rat 7 and foetal liver from rat 8 (Table 1) but the level of asbestos fibres in the foetal tissues from rats 5 and 6 were close to the higher values in the control rats. For this reason a second experiment (Table 1, experiment 2) was conducted in which four rats again served as controls, four rats received four 1 mg injections of asbestos and four rats received five 2 mg injections.

The control values were quite low in this experiment with all but one falling below a detection level of one fibre on 10 electron microscope grid squares equivalent to 0.45 × 10^6 fibres g^{-1} in liver or about half this amount in lungs. Foetal liver and lung samples from rats receiving asbestos were again quite variable with extremely high values recorded in the livers of several rats. Such variable results are very difficult to analyse statistically but the high values are quite important in that they could result from a breakthrough of asbestos fibres in the placentas of some foetuses. A large inflow of asbestos into the blood of one foetus would probably raise the asbestos level in all its organs. This is not evident from the present data as the livers and lungs analysed were selected at random and could have come from different foetuses in the same uterus. It is possible that the extremely high values in livers of foetuses of rats 18 and 19 are the result of one or more particularly large asbestos fibres breaking through an opening between the maternal and foetal circulation in the placenta allowing many smaller fibres to follow in its wake. It is also possible that a large number of small fibres piercing the placental membrane could destroy one or more vital cells resulting in a disruption of the membrane. If this were the case and there was an intermingling of the two circulations this could suggest an additional hazard of asbestos fibres.

We conclude that the number of very high values recorded in the rats injected with asbestos compared with very low levels in the controls in the two experiments adds considerable evidence to the theory that asbestos fibres can cross the placenta. The way in which this occurs, however, by individual penetration, by a mass breakthrough or both has yet to be determined.

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1 Smith, W. E., Miller, L., Elsasser, R. E., and Hubert, D. D., *Ann. N.Y. Acad. Sci.*, **132**, 456 (1965).
2 Westlake, G. E., Spujt, H. J., and Smith, M. N., *Lab. Invest.*, **14**, 2029 (1965).
3 Cunningham, Hugh M., and Pontefract, R. D., *J. Ass. off. agric. Chem.*, **56**, 976 (1973).
4 Pontefract, R. D., and Cunningham, H. M., *Nature*, **243**, 352 (1973).

Magnetic field perception by electroreceptors in Black Sea skates

THE perception of the Earth's magnetic field by vertebrates for orientation purposes has been discussed for over a century[1–4]. New approaches to this, still unsolved, problem were made after the lateral line of some fish species was shown to contain electroreceptor structures[5,6]. The great sensitivity of these structures to electrical stimulation suggested that they may be an apparatus for detection of a

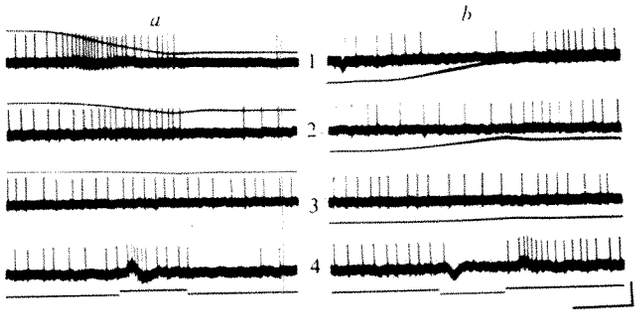


FIG. 1 Responses of the neurone in the area acoustico-lateralis of the medulla oblongata to magnetic stimuli of various intensities and opposite directions and to electrical stimuli of opposite polarities. Magnetic stimulus: 1, 80 gauss s^{-1}; 2, 36 gauss s^{-1}; 3, 9 gauss s^{-1}. *a*, South direction; *b*, north direction. 4, Responses to electrical stimulus of 3×10^{-9} A mm^{-2}. *a*, Cathodal stimulation; *b*, anodal stimulation. Time bar is 500 ms. Record of magnetic induction is 80 gs.

changing magnetic flux and thus have a role in fish orientation by means of the Earth's magnetic field[7-9]. The possibility of magnetic perception by animals having the electroreceptor apparatus, has been shown in behavioural experiments[6,10]. No direct neurophysiological data on this problem are available so far.

To investigate this problem we carried out experiments on the Black Sea skates (*Trigon pastinaca*), which have a well developed electroreceptor apparatus of ampullae of Lorenzini[10,11]. In our experiments, the single unit activity in the area acoustico-lateralis of the medulla oblongata was recorded with glass microelectrodes. Responses to electrical stimulation, as well as to the changing magnetic flux through the fish, were studied. Electrical stimuli were applied through the pair of silver electrodes placed on the skin of the fish. Magnetic stimuli were caused by a linearly increasing current of different characteristics, passed through the winding of electromagnet.

Our experiments showed that the changing magnetic field penetrating the fish evoked the response of neurones in the area acoustico-lateralis (Fig. 1), whereas the constant magnetic field failed to influence the electroreceptor system. The characteristic of the response to the changing magnetic field is dependent on the direction of the field; the intensity of the reaction is due to the rate of change of the magnetic field. The responses to magnetic stimulation could be recorded only in these neurones connected to the electroreceptor system which could easily be recognised by the responses of the neurones to electrical stimulation (current density thresholds were in the range of 10^{-9} to 10^{-10} A mm^{-2}).

The smallest change in the magnetic field evoking a neuronal response was found to be 2 gauss s^{-1}, obtained on a neurone showing no spontaneous activity. Apparently, this value could be reduced by improving the functional state of the animal.

The comparison between the magnetic flux change observed in experimental conditions and that which occurs during natural fish movements (both linear and turning) shows the possibility that the Earth's magnetic field may be perceived by electroreceptor apparatus of ampullae of Lorenzini.

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1 Middendorf, A. T., *Die Isepiptesen Russlands*, (St. Petersburg, 1855).
2 Yeagley, H. L., *J. appl. Physics*, **18**, 1035 (1947).
3 Thornton, W. H., *Proc. Univ. Durham phil. Soc.*, **8**, 301 (1931).
4 Jones, H., *Fish migrations*, Lond. (1968).
5 Lissman, H. W., *J. exp. Biol.*, **35**, 156 (1958).
6 Lissmann, H. W., Machin, K. E., *J. exp. Biol.*, **35**, 451 (1958).
7 Murray, R. W., *J. exp. Biol.*, **39**, 119 (1962).
8 Bennett, M. W. L., *Ann. N.Y. Acad. Sci.*, **188**, 242 (1971).
9 Kalnijn, A. J., *J. exp. Biol.*, **55**, 371 (1971).
10 Brown, H. R., Ilyinsky, O. B., Volkova, N. K., *Sechenov Physiol J. USSR*, **58**, 1499 (1972) (in Russian).
11 Akoev, G. N., Ilyinsky, O. B., *Experientia*, **29**, 293 (1973).

Is polymorphism in two-spot ladybird an example of non-industrial melanism?

THE polymorphism exhibited by the two-spot ladybird, *Adalia bipunctata* Linn., in which specimens have a red ground colour and black spots (the typical morphs) or a black ground colour with red spots (the melanic morphs) is a well known phenomenon. The reasons for the existence of this polymorphism, however, are not well understood. Analogy to the situation in some melanic Lepidoptera, such as *Biston betularia* Linn.[1,2], is unacceptable as it would seem that selective predation by birds upon the morphs which blend less well with their surroundings would not operate upon *A. bipunctata*. Ladybirds are distasteful, have a distinctive scent, are warningly coloured and therefore are avoided by vertebrate predators[3,4].

Studies of the frequencies of the morphs of *A. bipunctata* in Great Britain have been undertaken by Creed[5-7]. He postulates that the distribution of melanics is related to industrial pollution and speculates that some, as yet unidentified, component of the polluted atmosphere is toxic to the typical morph. This explanation is not altogether satisfactory, however, for example, melanics seem to be non-existent in central and eastern London, and were present at a very low frequency before smoke control regulations were introduced[8,9]. Furthermore, there are high frequencies of melanics in rural areas of north-west England. Specimens of the melanic morph were collected as early as 1696; 150 yr before the earliest records of industrial melanism in the Lepidoptera[10]. For these reasons, together with the absence of an identified selective toxin, we re-examined the alternative hypothesis of Lusis[11] that a climatic factor, in particular solar radiation, may determine the melanic frequency.

Differences have been recorded in the temperature of small brass objects painted black or white and exposed to sunlight[12], while melanism related to seasonal temperature variations has been demonstrated in an aphid[13]. Measurements of the metabolic rates of samples of *A. bipunctata* (containing both typical and melanic morphs) showed that in the dark oxygen consumption rose from 175 μl per g live weight per h at 10° C to 296 $\mu l\ g^{-1}$ at 15° C, 721 $\mu l\ g^{-1}$ at 20° C and 985 $\mu l\ g^{-1}$ at 25° C. Thus, even a small rise in temperature causes an increase in the metabolic rate of the insect. Pairs of melanic and typical insects were enclosed in tubes and chilled in ice for 30 min. Their movements were then recorded over a period of 12 min immediately following their introduction into an arena containing a 25 mm grid. Measurements of their activity were made at two temperatures, 5° C

TABLE 1 The activity of melanic and non-melanic forms of *A. bipunctata* measured as the number of 25 mm squares entered in 12 min

Temperature (°C)	Melanic	Non-melanic
5.0	33.4 ± 7.0	8.8 ± 2.04
7.5	67.0 ± 8.17	51.0 ± 9.23

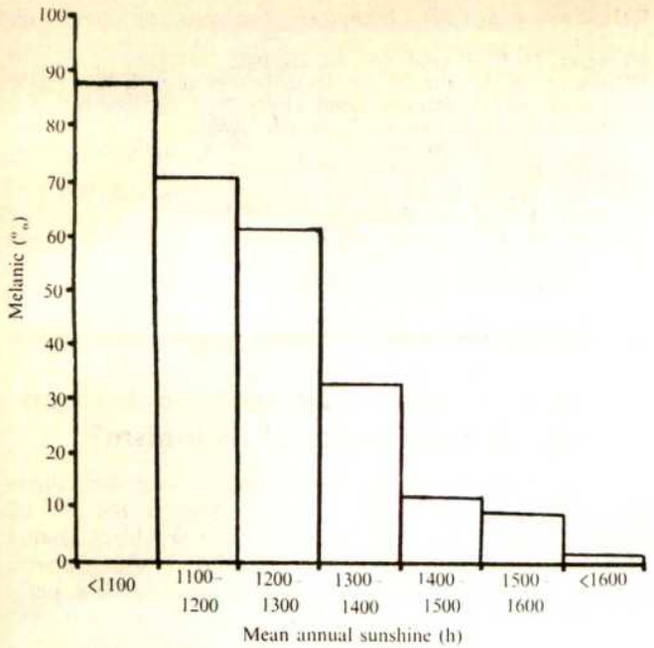


FIG. 1 Percentage of melanics in each 'sunshine class'.

and 7.5° C. Illumination was provided by a 100 W lamp suspended above the arena. The melanic insects showed greater activity at both temperatures (Table 1).

In view of the effect of illumination on activity, we believe that a lack of sunshine is the principle factor in the selection of the melanic morphs. The higher level of activity attainable by the melanic morphs will give them considerable competitive advantages in the search for food. This is especially true in areas where sunshine levels are low enough to reduce the period of activity to a point where it becomes a critical factor in the life of the insects. We have found that related species require warm, sunny conditions for mating and egg laying[14] and the same may be true for *A. bipunctata*. Thus, in localities with low sunshine levels, the mating and egg laying activity of the melanic morphs may greatly exceed that of the typical morphs. Smoke pollution may be expected to be a secondary factor in the determination of melanic frequencies as it will reduce the incident solar radiation in some areas to the critical level.

We have estimated the mean annual sunshine hours for the sites listed by Creed[5,7] from records for the years 1921-50 (ref. 15). The sites were then placed in 'sunshine classes' representing 100 h increments in the annual total. The % of melanics in each sunshine class was calculated from the numbers of the different morphs at each site. The results (Fig. 1) show a very close relationship between melanic frequency and annual sunshine hours. A correlation between melanic frequency and sunshine level at each site shows a highly significant negative correlation ($r = -0.59$, $P = <0.001$).

It seems that a re-examination of the theory of industrial melanism, as applied to *A. bipunctata*, is justified.

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1 Kettlewell, H. B. D., *Heredity, Lond.*, **9**, 323–42 (1955).
2 Kettlewell, H. B. D., *Heredity, Lond.*, **10**, 287–301 (1956).
3 Frazer, J. F. D., and Rothschild, M., *Proc. 11th int. Cong. Ent.* **3**, 249–56 (1960).
4 Rothschild, M., *Trans R. ent. Soc. Lond.*, **113**, 101–21 (1961).
5 Creed, E. R., *Heredity, Lond.*, **21**, 57–72 (1966).
6 *Ecological Genetics and Evolution*, (edit. by Creed, E. R.) (Blackwell, Oxford, 1971).
7 Creed, E. R., *Evolution, Lancaster, Pa.*, **25**, 290–93 (1971).
8 Hawkes, O. A. M., *Entomologist's mon. Mag.*, **63**, 262–266 (1927).
9 Hawkes, O. A. M., *Proc. zool. Soc. Lond.*, **1920**, 475–490 (1920).
10 Kettlewell, H. B. D., *The evolution of melanism* (Clarendon Press, Oxford, 1973).
11 Lusis, J. J., *Latv. Ent.*, **4**, 3–29 (1961).
12 Digby, P. S. B., *J. exp. Biol.*, **32**, 279–98 (1955).
13 Dixon, A. F. G., *J. anim. Ecol.*, **41**, 689–97 (1972).
14 Benham, B. R., and Muggleton, J., *Entomologist*, **103**, 153–70 (1970).
15 *The Atlas of Britain and Northern Ireland*, (edit. by Bickmore, D. P., and Shaw, M. A.), (Clarendon Press, Oxford, 1963).

Changes in the plankton community of the Western English Channel

MARINE biologists and oceanographers will probably be familiar with the story of the biological and chemical changes that occurred in the Channel waters off Plymouth during the 1930s: a summary of how the plankton community altered, the numbers of young fish declined, adult demersal fish became scarcer and herring were replaced by pilchard can be found in many textbooks. The most striking feature, and one of the first to be detected, was the changeover from a plankton community characterised by the chaetognath *Sagitta elegans* and associated 'plankton indicators' to a much poorer community characterised by *Sagitta setosa*[1–3].

Since 1965, signs of a reverse change in biological conditions have been evident, and reports on the striking improvement in the numbers of young fish since then have already been published[4,5]. By 1970 the numbers of the copepod *Calanus* during the spring peak of zooplankton (May and June) were equal to those found in 1930 (ref. 6). From 1933 to 1971, however, *Sagitta setosa* remained the dominant chaetognath in the weekly samples taken off Plymouth; *S. elegans* was either completely absent or occasionally present in very small numbers[3,6–15], although in

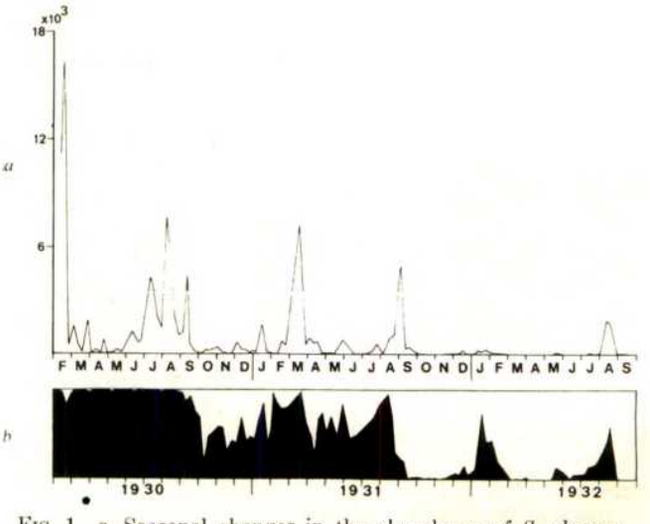


FIG. 1 *a*, Seasonal changes in the abundance of *S. elegans* (as numbers per standard haul of a 2 m net at station L5) in 1930-32, and, *b*, the proportion of *S.* elegans in the mixed population of *S. elegans* and *S. setosa* (redrawn from Russell).

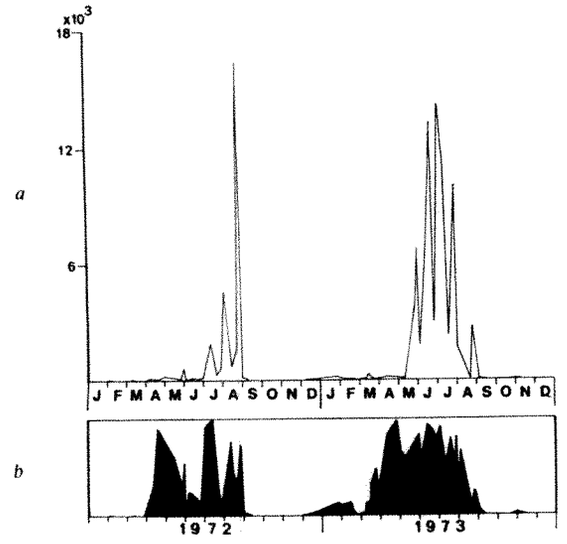


FIG. 2 *a*, Seasonal changes in the abundance of *S. elegans* (as numbers per standard haul of a 2 m net at station L5) in 1972-73; *b*, the proportion of *S. elegans* in the mixed population of *S. elegans* and *S. setosa*.

the later years after 1958 it was slightly more common in mid-channel and to the west[6,16].

It is therefore of great interest to report that in 1972, for the first time since 1932, *Sagitta elegans* was found in considerable numbers off Plymouth, even at inshore stations, and that in 1973 it was even more abundant and was the dominant species at the Eddystone station (L5) for 5 months. Over the period May to August inclusive, *S. elegans* was present in numbers equal to or greater than those recorded in the 1930s (Figs 1 and 2). The agreement between the results of weekly sampling in 1930–32 and more recent results is not complete, especially with regard to the proportions of the two species in certain of the months. The recent samples are collected with a net of very slightly narrower meshes, which retains more of the smaller sizes of chaetognaths: the total numbers of *S. elegans* (Fig. 1*a* and Fig. 2*a*) are somewhat greater, and their proportion in the combined chaetognath population (Fig. 1*b* and Fig. 2*b*) somewhat less than in the earlier period. Although the 1972 and 1973 samples did not show the spring peak of *S. elegans* found in 1930 and 1931, there were clear signs of a recurrence of this peak in 1974 and therefore the seasonal trends in abundance agree quite well.

That this return of *S. elegans* is not an isolated change is shown by the occurrence of the medusa *Aglantha* and the siphonophore *Nanomia* during the recent summers when *S. elegans* was abundant. Furthermore, in winter the euphausid crustaceans *Meganyctiphanes* and *Thyssanoessa* have been taken in night samples to the west and south-west of the Eddystone. A combination of all these north-western plankton indicators[7] with large numbers of *Sagitta elegans* has not been observed off Plymouth in any year since 1930 (ref. 17). Other indicators, such as the pteropod mollusc *Spiratella retroversa* and the larvae of the starfish *Luidia sarsi* have been abundant at certain times in the past few years, while warmer water species have become scarcer.

The recent evidence thus reinforces the view that the biological reversal reported in 1971 (ref. 6) was not a minor fluctuation but part of a much longer trend. It also seems that the order of the changes is being repeated inversely. Thus, in 1930–35 the replacement of *S. elegans* by *S. setosa* was followed by a decline in numbers of young fish and other macroplankton, and later by the appearance of large numbers of eggs of pilchard during the summer. In the more recent period a reduction in the summer pilchard eggs has been experienced since 1963, the young fish have returned in great numbers since 1966, and the numbers of the copepod *Calanus* came back to their former peak in 1970: only now, in 1973 is there a real abundance of *S. elegans*. An analysis of the corresponding hydrographic changes during the past ten years is being made by my colleague, E. I. Butler, but there seems no reason to doubt the suggested overall link with climatic fluctuation: some of the temperatures in the deeper waters below the thermocline in the past 2 yr have been the lowest recorded since the 1920s.

It could be suggested that what we have been observing off Plymouth since the 1920s is part of a cyclic fluctuation, with a peak-to-peak frequency of the order of 40 to 50 yr. *Sagitta elegans* seems to have been the first of the plankton species to show the change and the last to return, suggesting that in some way it could be more 'sensitive' to the causes of the fluctuation. Therefore a continued monitoring of this species and its associated 'indicators' may be helpful in detecting future changes in the ecosystem of the western Channel, whether naturally induced or otherwise.

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1 Russell, F. S., *J. mar. biol. Ass. U.K.*, **18**, 559 (1933).
2 Russell, F. S., *J. mar. biol. Ass. U.K.*, **20**, 147 (1935).
3 Russell, F. S., *J. mar. biol. Ass. U.K.*, **20**, 595 (1936).
4 Russell, F. S., *J. mar. biol. Ass. U.K.*, **49**, 305 (1969).
5 Russell, F. S., *J. mar. biol. Ass. U.K.*, **53**, 347 (1973).
6 Russell, F. S., Southward, A. J., Boalch, G. T., and Butler, E. I., *Nature*, **234**, 468 (1971).
7 Southward, A. J., *J. mar. biol. Ass. U.K.*, **42**, 275 (1962).
8 Russell, F. S., *J. mar. biol. Ass. U.K.*, **21**, 679 (1937).
9 Russell, F. S., *J. mar. biol. Ass. U.K.*, **22**, 493 (1938).
10 Russell, F. S., *J. mar. biol. Ass. U.K.*, **23**, 381 (1939).
11 Russell, F. S., *J. mar. biol. Ass. U.K.*, **24**, 265 (1940).
12 Russell, F. S., *J. mar. biol. Ass. U.K.*, **26**, 605 (1947).
13 Corbin, P. G., *J. mar. biol. Ass. U.K.*, **27**, 718 (1948).
14 Corbin, P. G., *J. mar. biol. Ass. U.K.*, **28**, 707 (1949).
15 Corbin, P. G., *J. mar. biol. Ass. U.K.*, **30**, 271 (1951).
16 Southward, A. J., *J. mar. biol. Ass. U.K.*, **41**, 17 (1961).
17 Russell, F. S., *J. mar. biol. Ass. U.K.*, **20**, 309 (1935).

Microorganisms participate in the construction of manganese nodules

THE geographical distribution and formation of manganese nodules have inspired investigation and speculation for more than a 100 years (for example, refs 1 and 2). Although photographs have revealed that some organisms attach themselves to Mn nodules on the deep seafloor (for example, ref. 3), these were apparently epiphytes, not contributing to the formation of the nodules. I now wish to suggest, on the basis of an ultrastructural examination of Mn nodules, that several groups of organisms are active in their construction.

I have found that: (*a*) numerous shelter-building organisms reside on the surface of deep-sea Mn nodules; (*b*) benthic foraminifera of the genus *Saccorhiza* attach Mn micronodules to the surfaces of macronodules as part of their tube-building activities; (*c*) some tubes of *Saccorhiza* and other benthic organisms originally constructed on nodule surfaces are buried and preserved (not replaced) within the interior of nodules; (*d*) other organisms on nodule surfaces build structures that contain large amounts of Fe and Mn compounds; (*e*) biologically derived structures cover large portions of surfaces of all the seventy-one nodules from the Pacific Ocean basin that I have examined so far.

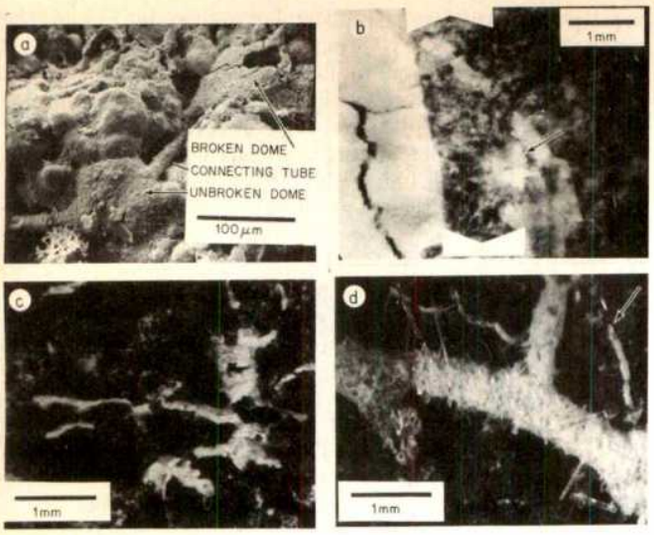


FIG. 1 Micrographs of nodule surface structures (oblique reflected light was used on all photographs except (*a*) which is from scanning electron microscopy). Small tube and dome structures (*a*) have very little contrast with the general nodule background. These structures occur in patches, or active regions, and are internally clean and empty. Structures near the borders of active regions are filled with a secondary matrix. One dome is ruptured and vacant while a second dome is intact. Massive, white, spined body is presumably a sponge (*b*) across which is a trail (marked) rich in Mn. The trail clearly proceeds from the nodule surface across the white mass and back onto the nodule surface. The arrow shows where a portion of the trail was removed to confirm that it is not the nodule surface protruding through the white mass. The organism responsible for this trail may be important in modifying the existing nodule surface by depositing or redistributing sheets of manganese oxides. Broken, partially infilled and partially covered *Saccorhiza* tubes (*c*) have some infilling material that appears to be pre-existing micro-nodules cemented in place by the tube builder while some appears to have been deposited in place as smaller tube and dome structures. This should be compared with the undamaged tube in (*d*). The smaller white tube (*d*) should be compared with Fig. 2.

Most organisms potentially important in the origin and development of nodules are microscopic (<0.5 mm across). They create fragile primary structures that are usually destroyed by any but the most careful collection process, partly explaining the lack of study of the organisms until modern sampling devices made possible recovery of undisturbed nodules.

The structures discussed here are mostly tubular or dome-shaped. The larger tubes are made of ferromanganese micronodules, mineral grains, radiolarian fragments and sponge spicules, and are identified tentatively as the remains of agglutinating *Saccorhiza*. Some of the smaller tubes and domes also seem to be agglutinated particulates. The smallest structures I have observed are hollow opaque tubes and domes, rich in Fe and Mn (Fig. 1*a*). Scanning electron microscopy suggests that these structures are precipitated *in situ*; they are dark reddish brown in reflected light, compared with dark brown or black for other spheroids on the surface of nodules. There is evidence of other microscopic life forms on nodules (Fig. 1*b*). In some regions of the nodules, particularly the bottom surfaces, thin, fragile, transparent membranes form large (1–100 mm²) canopies under which there is no sedimentary debris. (Other evidence of life forms will be detailed elsewhere.)

Some *Saccorhiza* tubes had formed on all the nodules I examined. The tubes are often broken open and the remaining fragments partially filled or covered by ferromanganese hydroxyoxides (Fig. 1*c*). Some of the infilling material seems to have formed *in situ*, and often has a distinguishable tube and dome structure (Fig. 1*a*). These observations suggest that the remains of the organisms inhabiting the nodules are incorporated into them as they grow, and that Mn micronodules are agglutinated to become part of the nodule.

If the structures produced by the organisms are preserved within the nodule, they would contribute to the overall growth of the nodule. The extreme of this hypothesis would be to consider nodules as a superstructure of surface-deposited tubes and fragments, within which would accumulate micronodules and matrix deposited organically or inorganically. To test this hypothesis, thin unsupported cross-sections of nodules were prepared. Opaque metal hydroxyoxides were leached away, leaving a fragile, nearly white framework of the original section (details to be presented elsewhere). Examination of this skeleton confirmed that preserved tubes are abundant inside nodules (Fig. 2). Preserved sections of complete tubes of *Saccorhiza* have been found, and assemblages if mineral grains of the appropriate size and arrangement are frequent. Fewks[4] and Sorem[5] have described features that are probably cross-sections of *Saccorhiza* tubes.

The preserved tubes seem to alternate with the concentric rings seen in Fig. 2, indicating that the onion-like structure of the nodules is a consequence of the particular type of remnant structure present. Alternatively, the zoning may be a result of diagenetic alteration of the nodule, perhaps under the influence of the microenvironment which is controlled by the presence of the tube material.

No leaching agent has been found that does not remove the Fe and Mn rich tubes and domes, and so direct observation of these structures has been impossible. It seems that they are either not preserved within the nodule (being altered into general matrix material) or become filled with a substance so chemically similar to themselves that leachants able to remove the filling also dissolve the structure.

The fragile remnant structure includes more than tubes and domes: there are globules of clay, thin stratiform sheets and many rather large (~0.1 mm) mineral grains. (Adjacent stratiform sheets can be confused with longitudinal cross-sections of tubular structures.)

The localisation of tubes and domes on parts of the

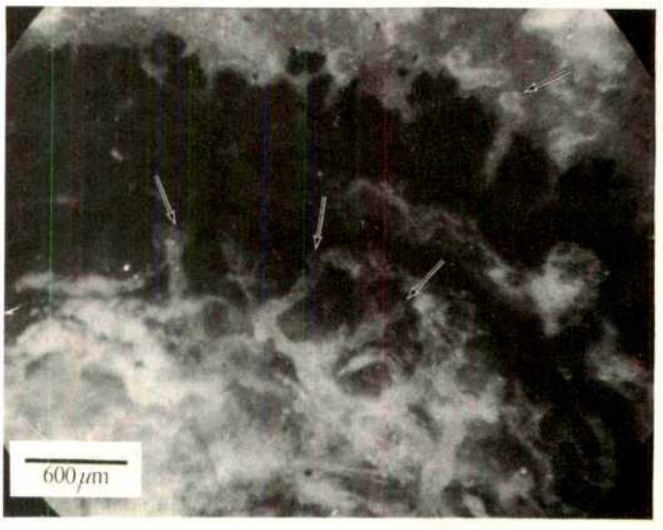


FIG. 2 Photomicrograph of tubes preserved in interior of a nodule and exposed by leaching. Leached material is nearly white, with no contrast so that dark-field photography was necessary, accounting for the poor contrast. The exposed tubes should be compared with those of Fig. 1*d* (arrow). Note the relationships of tubes and incompletely leached dark concentric rings shown in this cross-section.

nodule surfaces suggests that the organisms were 'active' at the time of collection. Most areas, however, yield no unfilled structures and are assumed to be 'dead' with respect to the relevant organisms. The greatest abundance (up to complete surface coverage) of 'active' structures occurs in regions of nodules believed to be near the sediment-water interface, suggesting that this type of growth could be responsible for the equatorial bulges in some nodules. Although all nodules investigated have had 'active' structures, 'dead' nodules with no discernible external biological produced surface structures, yet containing preserved tubes, might be expected.

It is unclear how the preserved structures were filled. Ehrlich *et al.* (ref. 6 and references therein) have adduced evidence that bacteria could play a role, but it is often suggested that slow, continuous inorganic precipitation of Mn and Fe is widespread on the ocean floor, leaving open the possibility that the infilling is entirely inorganic. The similarity of concentration ratios of several elements in adjacent sediments and nodules[7] indicates that the deposition of the metal-rich phases in both nodules and sediments is similar, whatever the process.

Graham[8] suggested that Mn nodules were of biological origin when he detected organic material in Mn crust from the Blake Plateau. He and Cooper[9] also suggested a biological origin for the 'manganese-rich' deposits of the seafloor, citing their observation of Mn coating on samples of arenaceous foraminifer skeletons from the Atlantic Ocean. The suggestions have remained unproven, principally because Graham and Cooper were unable to rule out contamination with organic matter or inorganic precipitation of Mn. Glasby and Hodgson[10] concluded from the low concentrations of organic pigments that organic influences were minimal in the formation of Mn deposits taken from the Indian Ocean. There is, however, no reason why structures such as I have found should contain significant concentrations of such pigments.

The abundance of biological structures throughout each nodule strongly suggests that the growth of deep-sea Mn nodules is aided by the activity of a diverse community of microorganisms. In an otherwise mushy environment, they build a solid superstructure within which ferromanganese oxides can accumulate.

This scheme fits well with reported observations. For example, if nodule development were dependent on biological activity, then, in the light of experience with other marine communities, nodules would be expected to be irregularly distributed geographically, which they are. They would further be expected to exhibit regularity in size, spacing and morphology within any one patch, which they do.

Radiometeric studies show that nodules are older than the sediment on which they lie, and this might be due to the activity of the microorganisms. The ability of small organisms to move large objects is well documented by Darwin's description of earthworms moving stones. The presence of microorganisms on nodule surfaces reinforces suggestions that Mn nodules could be moved by predators searching for food[2].

It is often suggested that the internal structure of nodules is the result of alternating periods of growth and decay ('alive' and 'dead'). This would explain results obtained with Be^{10} (ref. 11 and references therein), which show that nodules only grow 10% of the time. Even when 'alive', the nodules do not grow over all their surface simultaneously. Because most of the organisms involved are very small, it would require a long time to produce a new surface over the entire nodule. These observations conform with growth rates determined by several workers (1–100 mm per 10^6 yr).

Finally, if the communities of microorganisms vary from one locality to another, the nodules in the different areas might be expected to differ in shape, structure and composition. Again, such differences are common.

Of course I do not wish to imply that all constituents of Mn nodules are of biological origin. They may, in this and other respects, be analogous to coral reefs. But any model intended to explain the origin and development of deep-sea Mn nodules must take account of the extensive biological activity on their surfaces.

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1 Mero, J. L., *The Mineral Resources of the Sea*, 103–241 (Elsevier, Amsterdam, 1965).
2 Arrhenius, G., *Trans. Am. Geophys. Union*, **48**, 604 (1967).
3 Heezen, B. C., and Hollister, C. D., *The Face of the Deep*, 423–444 (Oxford University Press, New York, 1971).
4 Fewks, R. H., *Acta Mineral.-Petrog.*, Szeged, XX/2, 386 (1972).
5 Sorem, R. K., *Econ. Geol.*, **62** (1967).
6 Ehrlich, H. L., in *Ferromanganese Deposits on the Ocean Floor* (edit. by Horn, D. R.,) 63 (National Science Foundation, Washington, DC, 1972).
7 Elderfield, H., *Nature phys. Sci.*, **237**, 110 (1972).
8 Graham, J. W., *Science*, **129**, 1428 (1959).
9 Graham, J. W., and Cooper, S. C., *Nature*, **183**, 1050 (1959).
10 Glasby, G. P., and Hodgson, G. W., *Geochim. cosmochim. Acta*, **35**, 845 (1971).
11 Krishnaswami, S., Somayajulu, B. L. K., and Moore, W. S., in *Ferromanganese Deposits on the Ocean Floor* (edit. by Horn, D. R.) 117 (National Science Foundation, Washington, DC, 1972).

Photoperiodic responses in experimental hybrids of cockleburs

Hybrid intermediacy in night requirements for flowering has been reported in a study[1] of latitudinal variation of photoperiodic adaptation in cockleburs (*Xanthium strumarium* L.). The report stated that the data showed that different strains produced F1 hybrids photoperiodically intermediate between the parents and that segregation for critical night lengths was observed in the F2 generation. Ray (personal communication) feels, however, that the hybrid evaluation was possibly affected by facultative apomixis. I have also demonstrated [2,3] intermediacy of the photoperiodic response in F1 hybrids in *Xanthium* but have not found evidence of apomixis in emasculated plants.

To determine the potential role of hybridisation in their wide distribution, cockleburs from different parts of the world were crossed after they were induced to flower under experimentally controlled conditions. Plants with diverse night requirements between 7.5 h and 10.75–11 h (refs 1, 4, 5) were hybridised with day neutral plants[6,7] as well as with other photoperiodic types. The ease of producing F1 hybrids[3,8,9] within and between the members of the eight morphological complexes of Löve and Dansereau[10] extends to the production of F2 progenies; however, in crosses between the indigenous Eurasian complex, *strumarium*, and various American complexes, the F1 plants are only partially fertile[11,12].

TABLE 1 Photoperiodic adaptation of F1 hybrids involving the introduced *italicum* complex of Tahiti*

Selfed parental progenies (and morphological complex) and F1 hybrids†	Manifest night (h)‡	Apparent critical night (h)‡	Time of bud appearance outdoors in Texas §
Tahiti-A (*italicum*)	10.75–11.00	10.5–10.75	September 5–12
Tahiti-B (*italicum*)	11.00	10.75–11.00	August 25–September 8
F1 Tahiti × Pullman, Washington	9.25	9.00	June 28
Pullman (*oviforme*)	8.25	8.00	June 17–24
F1 Eureka, California × Tahiti	9.00	8.75	July 29–August 2
Eureka (*pennsylvanicum*)	8.25	8.00	July 4–8
F1 Tahiti × San Diego, California	10.00	9.75	July 29
San Diego (*italicum*)	10.00	9.50–9.75	July 22–29
F1 Tahiti × Wellington, Australia	10.25	10.00	August 8–19
Wellington (*italicum*)	10.5	10.0–10.25	August 19–26
F1 Tahiti × Bangalore, India	10.75	10.50	August 19–23
Bangalore (*chinense*)	11.00	10.50–10.75	August 26–29
F1 Tahiti × Bombay, India	10.75	10.50	August 23–26
Bombay (*chinense*)	11.00	10.75–11.00	September 5–12

* Tahiti burrs were originally collected near Maraa caves. The two selfed progenies were germinated from burrs produced in growth chambers[14]. The Tahiti plants show approximately 72% acetocarmine-stained pollen similar to that of some plants (65%) along the Tamaulipas coast of Mexico. All of the F1 hybrids involving a Tahiti parent had stained pollen within the range from 54 to 68%, most exceeding 60%. For the other parents, stained pollen exceeded 99% except for San Diego plants, 65%.

† In each cross except with Eureka, California the Tahiti plant is the pistillate parent.

‡ The manifest night under varying nights in the growth chambers is the dark period under which macroscopic floral buds become visible. The apparent critical night is a preceding dark period under which floral induction probably occurred. This terminology follows that of Ray and Alexander[1].

§ The time of bud appearance is for five plants of each selfed parental or hybrid progeny under outdoor garden conditions in Austin, Texas. The plants were germinated under continuous light and transplanted to natural daylengths in mid-June approximately six weeks after sowing of the burrs. The night length, which reaches a minimum near 9 h in mid-June, should have resulted in rapid floral induction of plants with night requirements less than 9 h. The Eureka, California plants and the Eureka—Tahiti hybrids were delayed in flowering possibly because of a response to high outdoor temperatures. Other California and Australia *pennsylvanicum* plants showed a delay in the time of expected bud production.

Selfed parental progenies were compared with hybrid progenies in growth chambers under fixed and/or varying dark periods with a day temperature of 30° C and a night temperature of 24° C. Although fixed photoperiods are necessary for establishing a critical night requirement, the increasing nights enable simultaneous testing of diverse responses. In most cocklebur, the longer night accelerates bud development and, therefore, the manifest night[1] under which macroscopic floral buds appear is preceded by a shorter critical night for floral induction.

Five plants in each progeny were compared under each of two varying nights: 7.5 to 9.0 h, 8.0 to 10.0 h, 9.0 to 10.5 h, or 10.25 to 11 h. By determining the number of nights required for bud production in dark periods near the inductive one, the apparent critical night[1] was estimated. In F1 studies, the dark period was increased 15 min at 5-10 d intervals, but for testing of F2 progenies the varying nights were increased 5 min every 5 d. The various F1 progenies were germinated and kept under continuous light for 6 to 9 weeks before the varying night comparisons. The Tahiti plants were 12 weeks from sowing of the burrs when initially exposed to 10.75 h nights. Selfed progenies of day neutral parents were germinated under fixed 16 h dark periods as well as under continuous light, and subsequently flowered and produced mature achenes in both conditions. Five to ten plants of each progeny were studied outdoors from mid-June in Texas.

F1 hybrids involving a day neutral parent and/or a parent

TABLE 2 Photoperiodic adaptation in hybrid progenies of *Xanthium* under varying nights*

Selfed parental progeny (and morphological complex) and hybrid progeny†	Number of plants with macroscopic floral buds in each varying night (h min)‡							Total number of plants
	935	940	945	950	955	1000	1100	
P1 Marfa, Texas (*italicum*)		1	3					4
P2 Lexington, Kentucky (*chinense*)				6	3			9
F1			1	4				5
F2(P1 × P2)(P1 × P2)	1	3	10	18	10	2		44
F2(P2 × P1)(P2 × P1)	1	3	7	24	11	1	1	48
ΣF2	2	6	17	42	21	3	1	92
P1 Wilberforce, NSW, Australia (*cavanillesii*)			6					6
P2 Manilla, NSW, Australia (*italicum*)							12	12
F2(P1 × P2)(P1 × P2)				13	9	3	1	26
F2(P2 × P1)(P2 × P1)				9	12	7	1	29
ΣF2				22	21	10	2	55

* Plants were 25 d from sowing of the burrs under continuous light when they were placed in a growth chamber nights of 9 h 20 min. The night length was increased by 5 min (±10s) each 5 d until it reached 10 h. The night length was subsequently fixed at 11 h to accelerate floral bud development. Test plants (25 d) produced macroscopic floral buds after 10 cycles of 16 h darkness. Experimental plants were 35 d from sowing at the first cycle of 9.5 h, the night expected to induce Texas *italicum* plants and Australia *cavanillesii* plants.

† F1 plants of the Australian cross were studied previously[3] and showed near midparent intermediacy.

‡ The critical night for floral induction probably preceded the manifest night by approximately 10 d, the Texas plants under nights of 9 h 30 min to 9 h 35 min and the Kentucky plants under 9 h 40 min to 9 h 45 min. In previous studies Marfa, Texas, plants[2] had indicated a critical night near 9.5 h and the Lexington, Kentucky plants[15] near 9.75 h. In other studies the Wilberforce plants[15] had shown induction under 9.5 h nights and the Manilla plants[15] under 10 h nights. My present study suggests a critical night for Wilberforce near 9 h 35 min and for Manilla near 9 h 55 min.

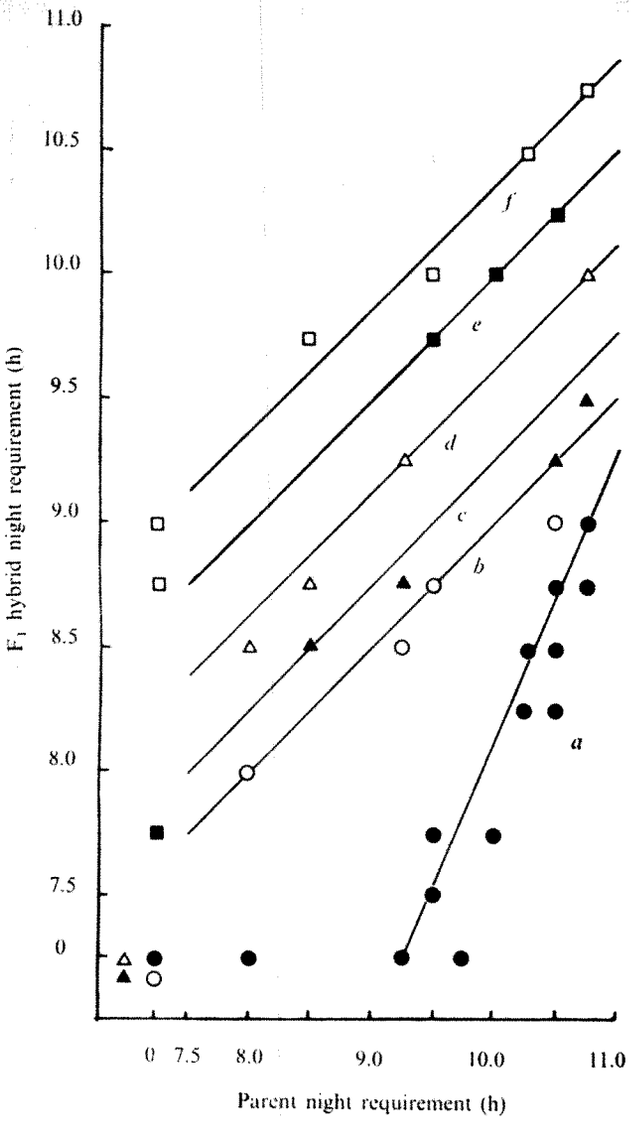


FIG. 1 Comparison of F1 hybrid night requirements for flowering. *a*, Hybrid responses with a day neutral parent (Varanasi, U. P.; Bangalore, Mysore, India) of the *strumarium* morphological complex (●). Other hybrid responses are with one of the following parents: Washington, *oviforme* (○); Italy, *italicum* (▲); Australia, *pennsylvanicum* (△); Australia, *italicum* (■); and Mexico, *italicum* or Bombay, India, *chinense* (□). The responses of hybrids between parents with night requirements are plotted along lines representing midparental intermediacy; *b*, × 8.0 h; *c*, × 8.5 h; *d*, × 9.25 h; *e*, × 10.0 h; *f*, × 10.75 h. Other parental plants used in the experimental crosses include: Eureka, California, *pennsylvanicum*, 8.0 h; Australia, *cavanillesii*, 9.5 h; Kentucky, *chinense*, 9.75 h; San Diego, California, *italicum*, 9.5–9.75 h; Brazil, *cavanillesii*, 10.25 h; Australia, *chinese*, 10.5 h; India, *chinense*, 10.25–10.5 h. Points on the graph represent the shorter night if two are indicated for a parental response.

with a critical night of 10.75–11 h show a wide range of photoperiodic adaptation (Fig. 1). The hybrids with a day neutral parent have an adaptive range from day neutrality to a 8.75–9 h night requirement[13]. Those involving a parent with a night requirement of 10.75–11 h show adaptation to nights of 8.75–9 h or longer. The crosses between day neutral plants of India and plants of Mexico[14] with the 10.75–11 h requirement produced the hybrid response to 8.75–9 h.

Approximate midparent intermediacy of the F1 hybrid response was demonstrated by most crosses involving two parents with a night requirement. Hybridisations included in Fig. 1 are those involving the *oviforme* complex from Washington, 8.0 h; the *italicum* complex from northern Italy, 8.5 h; the *pennsylvanicum* complex from Australia, 9.25–9.5 h; the *italicum* complex from Australia, 10.0 h; and either a Mexican plant in the *italicum* complex or an Indian plant in the introduced *chinense* complex, 10.75–11 h. Midparent intermediacy was also demonstrated by a series of crosses[8] involving plants from America and introductions to Australia with adaptation to various night lengths from 9.25 to 10.5 h.

F1 hybrids involving a Tahiti parent showed responses not reflecting midparent intermediacy (Table 1). Hybrid night requirements were slightly shorter than those of either parent in crosses with Bangalore and Bombay plants. The response of hybrids with San Diego and Wellington parents were similar. The hybrids with parents requiring 8 h nights showed near midparent intermediacy but were closer to the parent with the shorter night requirement. The hybrid responses may indicate a temperature interaction, differences in ripeness-to-flower (maturity) responses and/or partial dominance by one parent. Hybrids involving Hong Kong *strumarium* plants with a 9.25–9.5 h night requirement also failed to show midparent intermediacy[12,13]. With an American parent requiring a shorter night, the hybrid response was to a similarly short night. Hybrids involving an American parent with a longer requirement, 10.25–10.5 h, were intermediate in response but more similar to that parent.

F2 progenies examined under varying nights showed segregation of the photoperiodic response (Table 2). In the cross involving *italicum* from Marfa in western Texas[2] and *chinense* from Lexington, Kentucky[15] the parental requirements were separated by less than 15 min and the F2 generation, consisting of 92 plants, responded to nights differing by approximately 30 min. The cross between *italicum* and *cavanillesii* introductions[3,15] to Australia included an F2 progeny of 55 plants and a range of response to nights differing by approximately 20 min. Burr morphology and sesquiterpene lactone chemistry also show segregation in the F2 generation but the studies suggest no genetic linkage of either characteristic with photoperiodic response (my unpublished results). The F3 generation and other F2 progenies are being investigated for additional information about photoperiodic adaptation.

The F2 progenies of day neutral plants in the *strumarium* complex and *chinense* plants introduced to India[7,13] and to Australia[15,16] consisted of few plants because of partial fertility of the F1 hybrids[11,12]. Tested under natural day-lengths (and dark periods lengthening from 9 h in mid-June) in an outdoor garden following 6 weeks under continuous light in growth chambers, none of the F2 plants showed day neutrality. Each of the 13 plants that germinated from a total of 100 burrs produced on five F1 plants, showed a flowering reaction suggesting adaptation to nights shorter than 9.75 h. The selfed progeny of the day neutral parent flowered in growth chambers under continuous light; the selfed progeny of the *chinense* parents with a 10.5 h night requirement produced macroscopic floral buds in late August; the F1 and eight of the F2 plants in early July and the remaining five F2 plants before the end of July. The F1 had shown a requirement of nights of 8.25–8.75 h previously[13].

The intermediate photoperiodic response in F1 hybrids and the segregation for critical night length in the F2 generation suggest quantitative inheritance of the dark requirement for flowering. The hybridisation results also suggest that day neutrality in *Xanthium* is not dominant over a photoperiodic response as was indicated by the original studies of inheritance of photoperiodism in *Nicotiana*[17]. Day neutrality in *Xanthium* seems to result from greater negative physiological effects. Conversely, an imbalance towards increasing positive effects seems to produce the broad range of increasing night length requirements.

The diversity of photoperiodic adaptation in wild populations of *Xanthium* and the range of hybrid response may allow a broad base for future study of photoperiodic mecha-

nisms. Current physiological studies in *Xanthium* have concentrated almost exclusively on one or more strains from the vicinity of Chicago, Illinois[18] with a critical dark period usually given as 8.3–8.5 h. The actual mechanisms of photoperiodic control has been elusive but many physiological aspects of the dark induction have been reported for the Chicago strain(s)[19]. The present hybridisation studies were not intended to investigate the photoperiodic mechanism, but the results suggest that the photoperiodic response in *Xanthium* may be the total of 'negative' and 'positive' enzymatic effects and may also involve a timed reaction.

It seems unlikely that photoperiodic adaptation in *Xanthium* extends beyond the range of 7.5 to 11 h. Day neutrality is thinly separated from photoperiod controls at the short end of the range in indigenous European plants (my unpublished results) and tropical plants with a requirement near 11 h seem to have secondary controls involving temperature and maturity effects[14]. The results of hybridisation are providing insight into the adaptive capacity of *Xanthium* as a worldwide coloniser of habitats disturbed by man. In India the diversity of photoperiodic responses[6,7] can be explained as the result of hybridisation between day neutral indigenous plants and plants with photoperiodic responses introduced from America[11–13].

I thank Drs A. Lang, D. Löve, P. M. Ray, F. B. Salisbury, G. L. Stebbins, and J. A. D. Zeevaart for their advice. Dr Löve has examined burrs of most of the parental plants and many of the hybrids and has agreed with the treatment of the morphological complexes.

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1 Ray, P. M., and Alexander, W. E., *Am. J. Bot.*, **53**, 806 (1966).
2 McMillan, C., *Am. J. Bot.*, **60**, 397 (1973).
3 McMillan, C., *Can. J. Bot.*, (in the press).
4 McMillan, C., *Science*, **165**, 293 (1969).
5 McMillan, C., *Am. J. Bot.*, **57**, 881 (1970).
6 Kaul, V., *New Phytol.*, **70**, 799 (1971).
7 McMillan, C., *Nature*, **240**, 485 (1972).
8 Löve, D., and Nadeau, L., *Can. J. Genet. Cytol.*, **3**, 289 (1961).
9 Hicks, A., thesis, Univ. Illinois (1970).
10 Löve, D. and Dansereau, P., *Can. J. Bot.*, **37**, 173 (1959).
11 McMillan, C., *Nature new Biol.*, **246**, 151 (1973).
12 McMillan, C., *Am. J. Bot.*, (in the press).
13 McMillan, C., *Am. J. Bot.*, (in the press).
14 McMillan, C., *Am. J. Bot.*, **60**, 277 (1973).
15 McMillan, C., *Can. J. Bot.*, **51**, 221 (1973).
16 McMillan, C., *Science*, **171**, 1029 (1971).
17 Lang, A., *Z. indukt. Abstamm. U. Vererblehre*, **80**, 214 (1942).
18 Hamner, K. C. and Bonner, J., *Bot. Gaz.*, **100**, 388 (1938).
19 Salisbury, F. B., in *The Induction of Flowering* (edit. by Evans, L. T.) (Cornell Univ. Press, Ithaca, 1969).

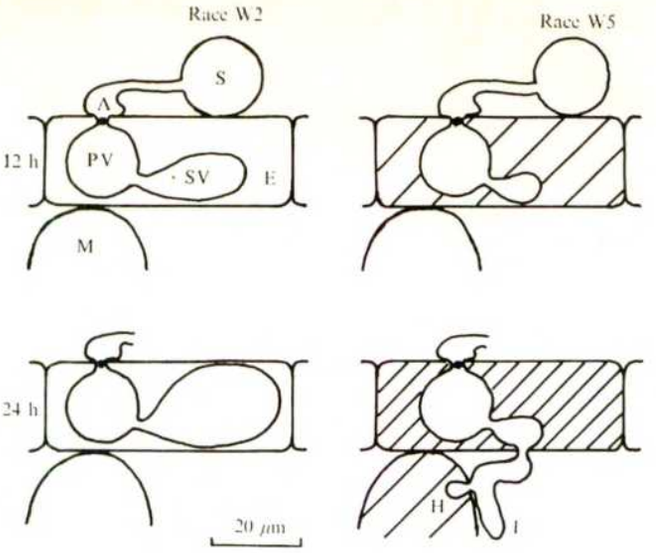


Fig. 1 Diagrammatic representation of the development of a compatible race (W2) and an incompatible race (W5) of *Bremia lactucae* in the lettuce cultivar Avondefiance. Primary and secondary vesicles are drawn to scale, measurements being derived from the means of ten replicates. Shading represents the extent of granulation and melanisation of the host cytoplasm. S, Spore; A, appressorium; PV, primary vesicle; SV, secondary vesicle; I, intercellular hypha; H, haustorium; E, epidermal cell; M, mesophyll cell.

Hypersensitivity as the primary event in resistance to fungal parasites

The hypersensitive reaction of plants to infection by incompatible strains of biotrophic fungi (organisms which, as parasites, derive their nutrients from living tissues[1]) involves the rapid necrosis of one or more cells at the penetration site, accompanied by restriction of the invading fungus. It is generally believed that this reaction by the host leads to a disruption of nutrient supplies to the fungus and the production of fungitoxic metabolites (for example, phytoalexins), resulting in the cessation of hyphal growth[2]. This traditional view has, however, been challenged by Brown *et al.*[3], who have suggested that the hypersensitive reaction of wheat to rust fungi is not the cause, but a consequence, of the earlier inhibition or death of the invading fungus. Király *et al.*[4], on the basis of indirect evidence, have extended this suggestion to include the resistance of bean to *Uromyces phaseoli* (rust) and potato to *Phytophthora infestans* (blight), and have questioned whether the term 'hypersensitive reaction' of hosts in connection with plant resistance has been correctly used in the past. Since this is a continuing debate among plant pathologists, we consider

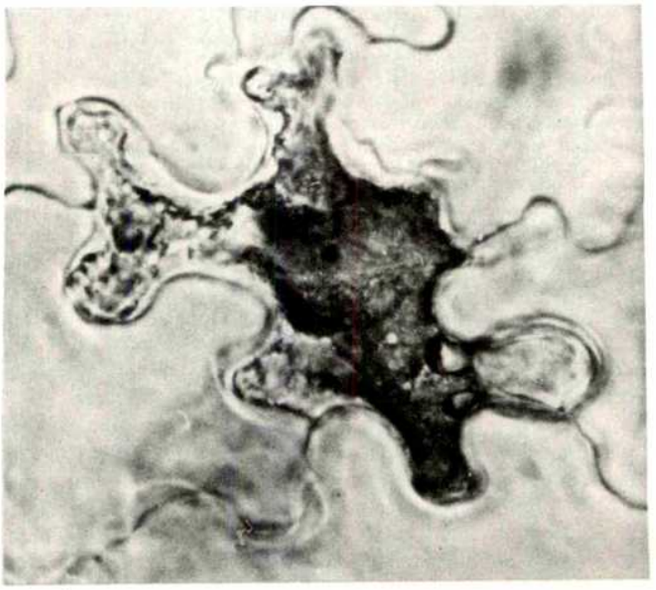


Fig. 2 Surface view of the hypersensitive reaction of an epidermal cell of the lettuce cultivar Avondefiance 60 h after inoculation with the incompatible race W5 of *Bremia lactucae*. Cells adjacent to the infected cell are apparently unaffected.

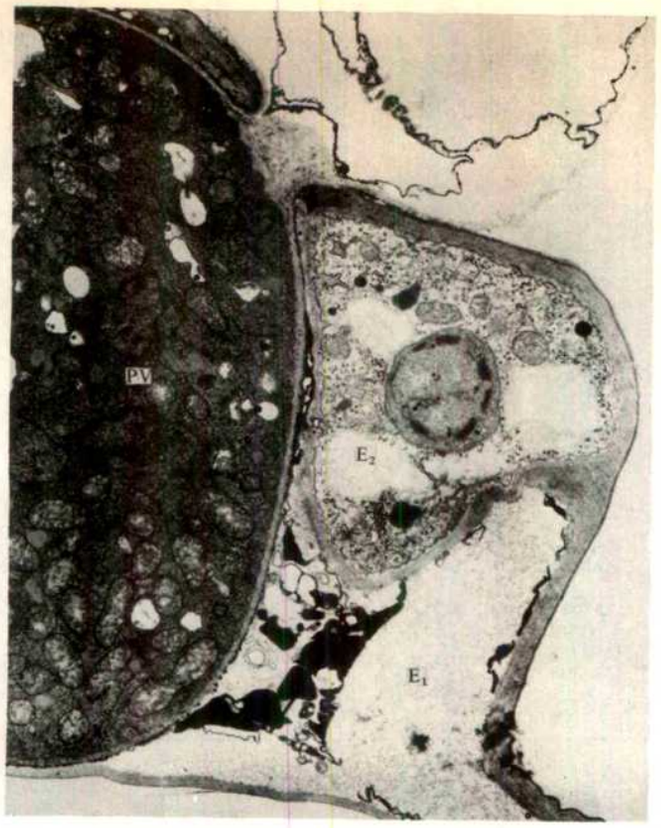


FIG. 3 Electron micrograph showing a primary vesicle (PV) of an incompatible race of *Bremia lactucae* (race W5) in an epidermal cell (E_1) of the lettuce cultivar Avondefiance. Note the complete disruption of the membranes and cytoplasm of this cell (arrowed) and the apparent normality of the cytoplasm of the fungus and the adjacent, non-penetrated cell (E_2).

it pertinent to report here information which accords with the traditional view of the significance of the hypersensitive reaction. We show that in the resistance of lettuce (*Lactuca sativa* L.) to incompatible races of the downy mildew fungus *Bremia lactucae* Regel, death of penetrated host cells is the primary event, preceding the cessation of fungal growth by several hours.

During a study of the interaction between four races of *B. lactucae* and five cultivars of lettuce we observed that incompatibility was normally accompanied by a hypersensitive reaction in the host. The time course of the infection process was followed in detail after inoculating the abaxial surfaces of cotyledons of the lettuce cultivar Avondefiance with aqueous suspensions of conidia of *B. lactucae* race W2 (compatible) or W5 (incompatible). Light microscopy showed that the germ tubes of both races penetrated epidermal cells and by 12 h after inoculation had produced a spherical primary vesicle and an elongated secondary vesicle[5]. The primary vesicles of both races developed at the same rate. Growth of the secondary vesicle of the incompatible race, however, was markedly retarded compared with that of the compatible race (Fig. 1); the former was fully expanded (5×9 μm) by 12 h and the latter continued to enlarge for 24 h, reaching a final size of 14×26 μm. Observations at intervals up to 60 h showed that the compatible race caused no necrosis of the epidermal cells it penetrated. In contrast, the epidermal cells entered by the incompatible race showed progressive granulation and melanisation, although adjacent non-penetrated cells remained apparently unaffected (Fig. 2). From 24 h onwards, the compatible race formed an intercellular hypha giving rise to an extensive mycelium bearing haustoria. The incompatible race, however, initiated an intercellular hypha as early as 12 h, although this stopped growing by 24 h, after producing approximately three haustoria which caused a hypersensitive reaction in the cells they entered.

Cytological examination of both strains of the fungus from spore germination onwards showed that for the first 12 h there was no significant increase in the number of nuclei per colony. Thereafter, the number of nuclei increased in colonies of the compatible but not of the incompatible race. Nuclear division in *B. lactucae* is probably dependent on the establishment of a biotrophic relationship between host and fungus and the failure of the nuclei to divide in the incompatible race is a reflection of its inability to establish such a relationship.

Electron microscopy of infected cotyledons showed that in compatible interactions the host cell plasmalemma was invaginated to accommodate both the primary and secondary vesicles of the fungus, and there was no evidence of cytoplasmic damage[5]. In contrast, within 4 h the membranes and cytoplasm of epidermal cells penetrated by the incompatible race were severely disrupted (Fig. 3), although adjacent cells were apparently normal. The fungal cytoplasm also appeared perfectly normal at this time, being dense with apparently functional organelles.

These observations make it clear that the primary event in the interaction between Avondefiance and the incompatible race W5 of *B. lactucae* is the rapid death of the penetrated cells. The fungus continues to grow for at least 12 h after this, although it too eventually dies. The most likely cause of death of penetrated cells is a rapidly-acting toxin produced by the healthy fungus during the early stages of the interaction. Since adjacent cells are not killed, this toxin could be a molecule which is closely associated with the fungal cell wall, or which cannot diffuse in sufficient quantity through host cell walls.

In the specific example reported here, light and electron microscopic studies during the very early stages of the host–parasite interaction have produced a clear sequence of events during the hypersensitive reaction to infection by an incompatible fungus. It is likely that more than one mechanism for hypersensitive resistance in plants exists, but it would be interesting to know whether the contradictory conclusions of earlier studies[3,4] would be supported by microscopic observations during the early stages of the host-parasite interactions.

Experimental details of this report will be published elsewhere. We thank Professor P. W. Brian, for discussions.

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1 Lewis, D. H., *Biol. Rev.*, **48**, 261–278 (1973).
2 Wood, R. K. S., *Physiological Plant Pathology*, (Blackwell's Scientific Publications, London and Oxford, 1967).
3 Brown, J. F., Shipton, W. A., and White, N. H., *Ann. appl. Biol.*, **58**, 279–290 (1966).
4 Király, Z., Barna, B., and Érsek, T., *Nature* **239**, 456–458 (1972).
5 Sargent, J. A., Tommerup, I. C., and Ingram, D. S., *Physiol. Plant Path.*, **3**, 231–239 (1973).

New species of *Dryopithecus* from Kenya

Two new species of Pongidae, assigned to a new subgenus of *Dryopithecus*, are here described for the first time. These assignations are based mainly on new specimens discovered recently in Kenya, but some other specimens[1,2] are reassigned to the hypodigms of the new species. A more complete revision of the African early Miocene dryopithecines is in preparation.

Three species of *Dryopithecus* from East Africa—*D. africanus*, *D. nyanzae*, and *D. major*—have been accepted as valid species, and are grouped in the subgenus *Proconsul* (Hopwood)[3]. The two new species are grouped in another subgenus to distinguish them from the other African and Eurasian species of *Dryopithecus*. Moreover, *Limnopithecus legetet*, which has pongid affinities, both dentally and postcranially, and which differs mainly in size from the species of *Dryopithecus*, is also included in the Dryopithecinae. As the subgeneric affinities of this species are not clear it seems best at this stage to retain it as a separate genus of the Dryopithecinae. The other species assigned to *Limnopithecus*, '*L. macinnesi*', is retained in the Hylobatidae[1], so that it will be necessary to change the generic classification of this species. Thus there are two genera and six species of the Pongidae, Dryopithecinae, and one genus and species of the Hylobatidae, now recognised in the early Miocene of East Africa. The dentitions of the six dryopithecine species range in size from slightly smaller than those of the gibbon to about the size of those of a female gorilla.

Le Gros Clark and Leakey[1], when describing a maxilla fragment assigned to *Proconsul africanus* (field no. 415'47, KNM-SO 401), noted its aberrant features and stated that further similar material might make a new species necessary. Such new material came in Leakey's 1966 collection from Songhor in the form of a complete palate which I described in 1970 (ref. 2). At that time, as my study of the total sample was incomplete, I referred it to *Proconsul* sp. with the intimation that it probably belonged to a new species; this views is now found to be justified. The entire dentition, based on a total of of 79 specimens, is known and a complete half mandible has just been found at Songhor (KNM-SO 1112) so that the lower dentition is now as well known as the upper. This new specimen is here described as a new species, *Dryopithecus gordoni*.

Another recent discovery, this time from Rusinga Island, was a partial maxilla and upper dentition (KNM-RU 2058) which, although almost identical morphologically to *D. gordoni*, is much smaller. Unfortunately the smaller form is known only from the upper dentition and from only seven specimens. One of these, a maxilla fragment (field no. R 593'47, KNM-RU 1778), had previously been allocated[1] to *L. macinnesi*. There are no morphological differences between these small specimens and *D. gordoni*, but examination of the ranges of variation and significance tests established that samples are sufficiently different in size to make it unlikely that they come from one population ($P < 0.001$). The smaller sample is therefore described here as a new species, *Dryopithecus vancouveringi*.

TABLE 1 Measurements of M^1 of *D. gordoni* and *D. vancouveringi*

		md	bl	bl/md
D. gordoni				
MW	52	8.4	8.0	95.3
SO	401	8.1	7.9	97.5
	487	8.4	7.9	94.1
	700	8.5	8.1	95.3
	930	8.1	8.5	104.9
	931	8.1	8.0	98.8
	932	7.8	8.2	102.6
Mean		8.2	8.1	98.0
Number		7.0	7.0	7.0
sd		0.24	0.21	4.05
D. vancouveringi				
RU	1778	7.0	6.8	97.2
	1801	6.4	6.6	103.0
	2058	6.8	6.5	95.6
SO	944	6.8	6.4	94.2
	1134	6.5	6.5	100.0
MB	125	7.1	6.7	94.4
Mean		6.8	6.6	97.0
Number		6.0	6.0	6.0
sd		0.27	0.15	2.48
Value of t		9.9	13.8	
Degrees of freedom		11.0	11.0	
Probability (P)		<0.001	<0.001	

The similarity of the two new species is illustrated in Fig. 1 where the upper tooth rows have been reduced to the same P4-M3 length. For comparison the equivalent tooth rows of *D africanus* and '*L. macinnesi*', which correspond most closely in size to *D. gordoni* and *D. vancouveringi* respectively, are

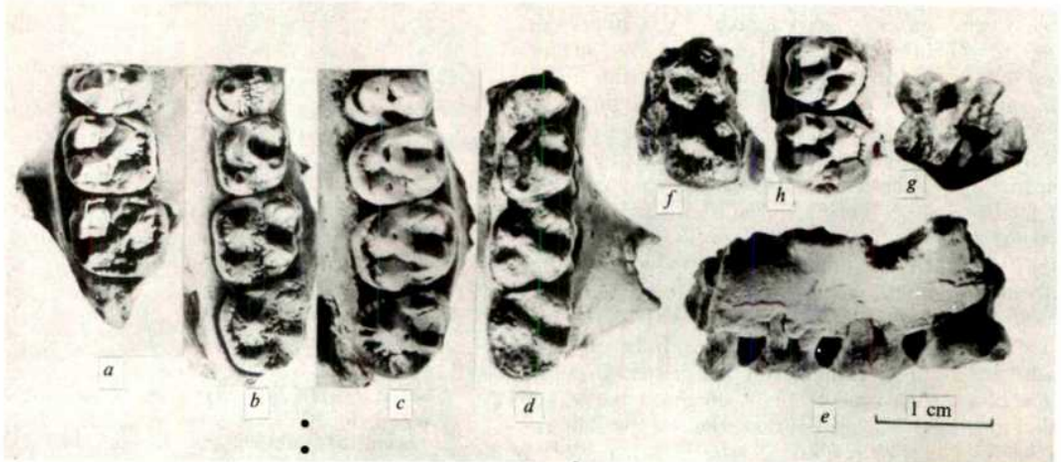


FIG. 1 Occlusal views of upper dentitions. *a*, *D. africanus*, P4-M2, KNM-RU 2036; *b*, *D. gordoni*, P4-M3, KNM-SO 700; *c*, '*L. macinnesi*,' P4-M3, KNM-RU 1849; *d*, *D. vancouveringi*, P4-M3, KNM-RU, 2058; *e*, *D. vancourveringi*, P4-M3 (lateral view), KNM-RU 2058; *f*, *D. vancouveringi*, dp4-M1, KNM-RU 1778; *g*, *vancouvering*, dp4-M1 (lateral view), KNM-RU 1778; *h*, *D. major*, dp4-M1, KNM-SO 542. *d*, *e*, *f*, and *g* are all to the same scale as indicated, but the other specimens are reduced or enlarged to the same P4-M3 length as in *d*, or to the same dp4-M1 length as in *f*.

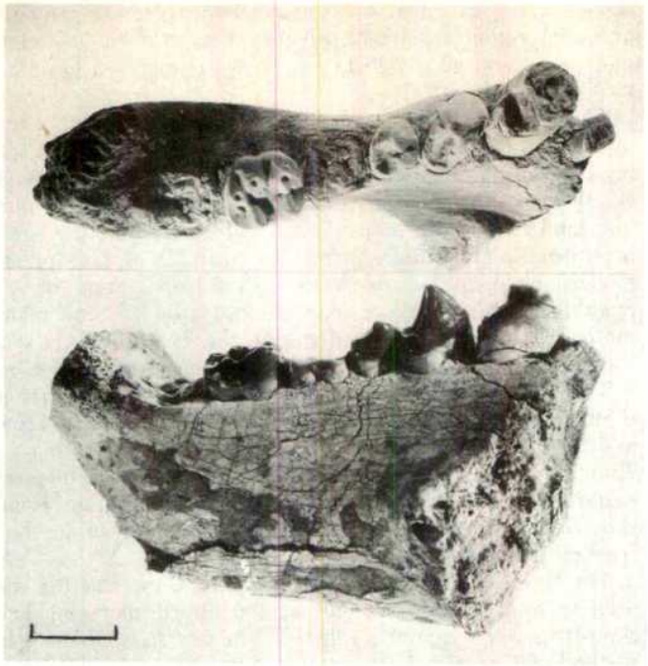


FIG. 2 Occlusal and lingual views of the new mandible of *D. gordoni* (KNM-SO 1112).

also included. The figure illustrates the distinctive morphology of the two new species, particularly the molariform premolars, with large lingual and distal cingula, the elongated upper molars (also shown in the tooth dimensions given in Table 1), the large size of the protocone on the upper molars, and the increase in molar size from M1 to M3. Other features not immediately evident from the figure are the lowness of the molar and premolar cusps, the degree of wrinkling of the occlusal surfaces, and the relatively marked wear gradient on the molars. Other features which are present in the larger sample of *D. gordoni* but which are not known for *D. vancouveringi* include the remarkably high-crowned structure of the incisors and to a lesser extent the canines, the elongated and wrinkled lower molars, and the large size and depth of the mandible (Fig. 2). In all these shared features, the two species differ from all other species of *Dryopithecus*, so they are here combined into a single higher taxonomic category. Since recent usage in pongid taxonomy has been to recognise subgeneric rather than superspecific groups[3], this category was selected, and the name *Rangwapithecus* was chosen. When more is known about the Eurasian dryopithecines, and in particular when better upper dentitions and skulls are found, it may well be necessary to resurrect some of the genera sunk by Simons and Pilbeam[3], and this may include establishing *Rangwapithecus* as a valid genus.

Diagnosis of the two new species are as follows:

Subgenus: *Rangwapithecus* subgen. nov.

Diagnosis: species approximating in size to the gibbon and siamang. Incisors high crowned and relatively very narrow compared with (*Proconsul*) and (*Dryopithecus*). Upper molars and premolars elongated, the molars usually longer (mesiodistally) than broad, low cusped and the occlusal surface often has more secondary wrinkling than in (*Proconsul*). Upper molars increase in size from M1–M3 and premolars from P3–P4 unlike both (*Proconsul*) and (*Dryopithecus*). No reduction of M3. Lower molars and premolars also elongated. The molars have a marked wear gradient, such that M1 may have dentine exposed on the occlusal surface when M3 is only just coming into wear, unlike the condition in (*Proconsul*) and (*Dryopithecus*). Strong lingual cingula are developed on all the upper molars and premolars, and the premolars also have a prominent distal cingulum. Zygomatic process is set very low over M1/2. The floor of the maxillary sinus is greatly extended.

Contained species: (1) *D. gordoni* sp. nov. (2) *D. vancouveringi* sp. nov.

(1) *Dryopithecus* (*Rangwapithecus*) *gordoni* sp. nov.

Holotype: KNM–SO 700 maxilla with complete upper dentition except for the incisors. Housed at the Kenya National Museum, Nairobi.

Locality: Songhor, Kenya.

Reference: Andrews[2], not named but referred to *Proconsul* sp. (Individual specimens attributed to this taxon have been assigned in the literature to *Limnopithecus macinnesi*, *Proconsul africanus* and *Proconsul nyanzae* (ref. 1).)

Distribution: Early Miocene. Most common at the Tinderet site of Songhor but a few specimens are known from the Rangwa sites of Rusinga and Mfwangano Islands.

Hypodigm: seventy-nine specimens which cover the entire dentition, the mandible except for the ascending ramus, the maxilla and palate. A few postcranial fragments may belong to this species. BM(NH) M 16637; KNM–RU 1686, 1692, 1722, 1768, 1833, 1894; KNM–MW 52; KNM–SO 89, 373–5, 377, 383, 401, 419, 420, 428, 434, 445, 449, 450, 463, 464, 467, 473, 475, 486–8, 507, 521–3, 531, 540, 550–3, 555–63, 575, 577, 578, 583, 588, 590–3, 700, 904–9, 921, 923–5, 930–2, 938, 943, 945, 948, 1081, 1112, 1225.

Diagnosis: a species of *Dryopithecus* intermediate in dental size between the siamang and pygmy chimpanzee; similar in size to *D. africanus*. Upper teeth similar to those of *D. vancouveringi* as described in the subgeneric diagnosis, differing only in larger size. In the lower dentition the incisors are very high crowned and narrow; the canine is high crowned and bilaterally compressed; the P3 is very bilaterally compressed with nearly parallel buccal and lingual sides; the P4 is elongated; the M1–M3 are also elongated, the cusps are low, the buccal cusps are divided by deep buccal sulci like the condition in the gorilla, the occlusal ridges and buccal cingulum are poorly defined, and secondary wrinkling is often present. Mandibular body and symphysis relatively deep and robust, prominent superior transverse torus, no inferior torus. P3–M3 lengths are, upper 40 mm and lower 44 mm for single specimens. Upper M1–M3 length is 29 mm. Named in honour of H. L. Gordon, formerly Government Medical Officer at Koru, who collected many of the early specimens at Koru.

(2) *Dryopithecus* (*Rangwapithecus*) *vancouveringi* sp. nov.

Holotype: KNM–RU 2058 left maxilla with crowns of P4–M3. Housed at the Kenya National Museum, Nairobi.

Locality: Rusinga Island, Kenya.

Distribution: Early to ?middle Miocene at the sites centred on Rangwa: Rusinga and Mfwangano Islands; and the Tinderet site of Songhor. It also may occur at Maboko Island.

Hypodigm: seven specimens which cover only the upper postcanine dentition and parts of the maxilla. Also referred provisionally to this species are three specimens from Maboko Island, KNM–MB 53, 125, 148. KNM–RU 1778, 1801, 2058; KNM–MW 48; KNM–SO 942, 944, 1134.

Diagnosis: a small species of *Dryopithecus* approximately the dental size of the siamang. It is like *D. gordoni* in morphology, as defined in the subgeneric diagnosis, but differs from it in size. The M1 is significantly different from the M1 of *D. gordoni*. The upper tooth row lengths are M1–M3, 22 mm and P3–M3, estimated 31 mm for a single specimen. Named in honour of Judith and John Van Couvering who found the type specimen in 1968 at the Kaswanga Point deposits of Rusinga Island.

The six species of dryopithecines apparently represent a Miocene radiation of the Pongidae in Africa. There are several degrees of differentiation between the species. The species pairs *D. nyanzae*/*D. major* and *D. gordoni*/*D. vancouveringi* probably represent near contemporary speciation, inasmuch as the members of each pair are largely allopatric in distribution and differ from each other principally in size. *D. africanus* is much more distinct, both in size and morphology, from the *D. nyanzae*/*D. major* pair, and it is sympatric with *D. nyanzae*: it is probable that it represents an older stage in speciation. Finally, the distinctive morphological difference between the species assigned to the two subgenera and genera must represent a still more ancient divergence.

The Miocene proliferation of pongid species in Africa seems strange in the light of the distribution of present day African pongids. Rather, closer evolutionary parallel might be with the later Cainozoic radiation of African cercopithecine monkeys, which are even more diverse than were the Miocene pongids. The fossil pongids are known only from one corner of their range in Africa; it seems unlikely from geographical

and palaeogeographical evidence that East Africa represented the centre of the Miocene pongid radiation, and the main centres of distribution were more probably in West and Central Africa (as are those of modern monkeys). No Miocene pongids have been recovered in these regions, but it is quite possible that if they were, the pongid radiation would be seen to be at least as rich and diverse as is the cercopithecine radiation of today.

The relationship of these fossil hominoid primates with the living ones remains uncertain. In my opinion it is no longer feasible to suggest direct ancestral-descendent relationships between fossil and living species[4]. Presumably one or more of the Miocene species was ancestral to the later pongids, but which this was, and whether one or more species was also ancestral to the Eurasian dryopithecines, can not be known from the available evidence.

I thank Drs D. R. Pilbeam and A. C. Walker for help with the preparation of this note. I also thank the Wenner-Gren Foundation for Anthropological Research for financial support, the Boise Fund for support of field work and the Museum Trustees of Kenya for permission to work on this material.

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1 Clark, W. E., Le Gros, and Leakey, L. S. B., *Fossil Mammals of Africa*, No. 1 (British Museum (Natural History), 1951).
2 Andrews, P., *Nature*, **228**, 537–40 (1970).
3 Simons, E. L. and Pilbeam, D. R., *Folia Primatol.*, **3**, 81–152 (1965).
4 Pilbeam, D. R., *Bull. Peabody Mus. nat. Hist.*, **31**, 1–185 (1969).

Hair of the mammoth

THERE have been few detailed studies of the frozen remains of woolly mammoths (*Elephus primigenius*)[1]. Here I describe a study of evolutionary change based on the grouping of the hair follicles and the distribution of hair diameters. This approach has hitherto been possible only with domestic animals[2].

The ancestral elephant *E. meridionalis*, a warm latitude forest dweller of the Early Pleistocene, gave rise to two lines. One remained in the same environment and underwent only moderate evolutionary change, giving rise to the straight-tusked *E. antiquus* of the Middle and Late Pleistocene. The other line evolved more rapidly, passing through two species stages in the same time; *E. trogontherii*, a cool steppe dweller of the Middle Pleistocene, and *E. Primigenius*, the woolly mammoth of the Arctic tundra in the Late Pleistocene[1].

A notable adaptation of the mammoth to a cold climate was the development of a long, dense coat. The ancestor of the elephants must have had hair, but it may already have been as much reduced in *E. meridionalis* as it is in modern elephants. The coat of the mammoth is therefore likely to represent a redevelopment of hair, and not the retention of the ancestral condition. What was the structure of the mammoth coat, and what follicle grouping was involved in its development?

The coat of the mammoth has been described as having an underwool, a longer, coarser yet fluffy outer hair, and an even longer, scarcer, bristly but flexible hair[3] (Table 1). Another account described the underwool as soft and reddish, one kind of hair as coarse, wiry and black, and the other as brownish and much finer[4]. The scarcity of hair in the modern elephant is associated with a corrugation of the epidermis, and a lack of skin glands. Although the mammoth, too, apparently lacked glands, the increase of the hair was associated with a loss of the epidermal corrugation[5].

The ancient material available comprised some hair from a Siberian mammoth about 10,000 yr old sent from Moscow, and three samples of skin from the Liakhov mammoth (No. 1912-23) in the Palaeontology museum, Paris. The modern material for comparison was some elephant leather, some skin from an African elephant, and two specimens of hair from an Indian elephant.

The International Standards Organisation method 181 was used to measure the diameter of 100 fibres from each hair sample in whole mount[6], and the skin was sectioned by the method of Ryder and Stephenson[6], after treatment in Sandison's softening fluid[7,8].

It has been concluded[5] that no fading has occurred in the colour of mammoth hair. By electron spin resonance[9] the Moscow sample had been shown to have a concentration of free radicals equal to that in white wool, and so it was concluded that it had been white, and that the yellow-brown colour was merely discoloration. It seems unlikely that the mammoth was in fact white, since stomach contents indicate that it was vegetarian like the musk ox[1], and only predators or prey in the Arctic turn white in winter.

Although I was unable to detect pigment granules, the coarser mammoth hairs were darker. A feature common in double-coated mammals, this suggests that the hairs were originally pigmented, the pigment having become diffuse with time. Chemical degradation has been demonstrated in mammoth hair[10].

The hair diameter distribution of the ancient and modern samples is shown in Table 2. The Moscow sample had a double coat but owing to its irregular nature, the length of the underwool could not be measured. The outer hair was shorter than the shorter (20 cm) of the two lengths quoted[4] although not much less than the longest (12 cm) in Neuville's unknown specimen[5] (Tables 1 and 2). The lack of the longest hairs (45 cm) cannot be due to breakage, because no hairs of commensurate coarseness were present in either the Moscow or Liakhov samples (Table 2). Their absence could be due to shedding some time before death, which could imply death at the end of winter, and not at the end of summer as previously surmised[1].

The three samples of flank skin were of similar thickness: fresh, 23 mm; leather, 22 mm; Liakhov, 22 mm (leg only 12 mm). It was impossible to section the leather. The fresh

TABLE 1 Previous measurements

Source	Specimen	Underwool length	Outer hair length: Short	Outer hair length: Long
Lang[3]		5 cm	45 cm	longer (diameter ≤ 1 mm)
Quakenbush[4]		3.8 cm (red)	15–20 cm (brown)	45 cm (black and bristly)
Neuville[5]	unknown	2.5–3 cm (40–75 μm diameter) blond	12 cm (90–325 μm diameter) red or black	
	Liakhov (leg)	light yellow	25* cm light red (blond) at base	

* Hair length confirmed in present study (see Table 2).

TABLE 2 Coat measurements

Specimen	Length (cm)	Diameter (μm) Range	mode	Mean ± s.d.	CV (%)	Distribution
Moscow	10	24–132	40	56.8 ± 24.7	43.5	skewed-to-fine
Liakhov (leg)—straight	25	26–110	40	50.0 ± 15.8	31.5	skewed-to-fine
Liakhov (flank)—curly	stubble only	40–114	60	66.5 ± 15.0	22.6	slightly skewed
Indian elephant (body)	13	114–586	224	293.8 ± 104.4	35.5	continuous*
Indian elephant (head)	15		~500			

* Although to naked eye the few coarser ones stand out among the remainder

skin confirmed the corrugation[5] of the epidermis, which had closely-packed pillars about 1 mm high resembling the villi of the intestine but hexagonal in section. The pillars were grouped into clusters separated by channels, and the hairs emerged at the junction of several channels. The hair density was 2 to 3 cm^{-2}, and were of two sizes, suggesting origination from primary central (larger) and primary lateral (smaller) follicles. The presumed centrals were deeper than the presumed laterals, extending 4 to 5 mm into the skin.

Sections cut parallel to the skin surface revealed sparse, round, non-medullated hairs with no glands or erector muscles. The follicle density was 6 cm^{-2} (that is, two groups). The trios had a V configuration (90° angle) with the larger central follicle at the base and the two laterals at the tips of the arms, which were 2 to 5 mm long. At least one central follicle had a surrounding sinus which suggests that it was a tylotrich (special sensory) follicle[11].

The best sections of the Liakhov skin came from the leg. The collagen fibres were very coarse, and stained green with picro-indigo carmine, as found previously with undecayed remains[8]. Follicle debris stained maroon with basic fuchsin. The hairs exhibited slight basophilia, staining orange rather than yellow, with picric acid and fuchsin.

The hairs in the leg skin were noticeably circular in section and of two widely differing sizes (outer coat and underwool). One fine group of 15 ranged from 48 to 76 μm in diameter (mean 62 μm), and a coarser group of five ranged from 88 to 180 μm (mean 129 μm). Both groups were thus coarser than the similar hairs measured immediately above the skin surface (Table 2).

There were a few follicle groups with trios of larger 'primary' follicles in which the laterals were 0.2 to 0.3 mm from the central. Between the central and the lateral were smaller 'secondaries' (Fig. 1). Distributed at random were a few even larger empty follicles, one of which had a collapsed lumen measuring 140 × 400 μm. These could be the source of the much larger hairs recorded by previous workers.

The central hair of the 'primary' trio in Fig. 1 is 68 μm, whereas the lateral is 128 μm, in diameter. The 'secondaries' between are 50, 56 and 62 μm in diameter. The hairs within the skin seemed to have narrowed preparatory to shedding, in spite of the observation that those above the surface were finer. For example, the hairs within the skin had only a tiny medulla, although there was little medullation in either the hair above the surface, or in that of the modern elephant. These hairs are remarkably coarse to lack a medulla. Neuville[5] mentioned elliptical as well as circular hairs, and variable medullation, although his measurements show that any medulla occupied no more than one-third of the width of the hair.

The Liakhov flank had more open connective tissue, and this seemed to be the cause of a loss of hairs during sectioning. No groups were evident but nine hairs ranged from 60 to 80 μm in diameter (mean 70 μm).

In attempting to draw conclusions from this evidence, the tendency is to think of the modern elephant as having lost the hair seen in the mammoth, but the modern elephant must be regarded as the ancestral form. Thus the ancestral elephant probably lacked skin glands and hair erector muscles, and it seems that these were not developed by the mammoth. Erector muscles, but not the skin glands, would have been advantageous in a cold climate. Why the elephant lost these glands when they are retained in the similarly 'hairless' rhinoceros is not clear.

From the earlier descriptions it seemed that the longest and coarsest hairs of the mammoth could have grown in the central follicles of primary trios, and so be 'bristles', while the shorter and less coarse hairs might have grown in the two laterals, being therefore 'awns'[6]. Although the modern elephant has central and lateral primaries, the hairs differ little in size, and both compare with the longest and coarsest 'bristles' of the mammoth. Evidence of even larger follicles was found in the mammoth, but these did not seem to belong to trio groups. Since at least some of the centrals in the elephant may be tylotrich follicles, the largest hairs of the mammoth could also be tylotrichs. The development of these sensory hairs in the elephant could be associated with the thick and apparently insensitive epidermis, and they could have been retained in the mammoth. The mammoth acquired a greater 'primary' follicle density not only by reducing the distance between the central and lateral follicles within trios, but by reducing the distance between the groups. The resulting greater hair density was associated with a reduction in the hair diameter of the 'primaries' (Table 2), which is a common phenomenon in sheep[8].

The modern elephant lacks an undercoat and so has none of the smaller secondary follicles that are developed later than the primaries. Such an adaptation to a hot environment is seen in the domestic 'hair' sheep (for example, the southern Desert type of India) which has less underwool as a result of a reduction in the number of secondary follicles[2]. The mammoth redeveloped an undercoat by the acquisition of smaller follicles lacking in the elephant (Fig. 1).

It is tempting to regard these smaller follicles as true 'secondaries', having developed later in the foetus, but unfortunately the lack of glands removes one distinguishing

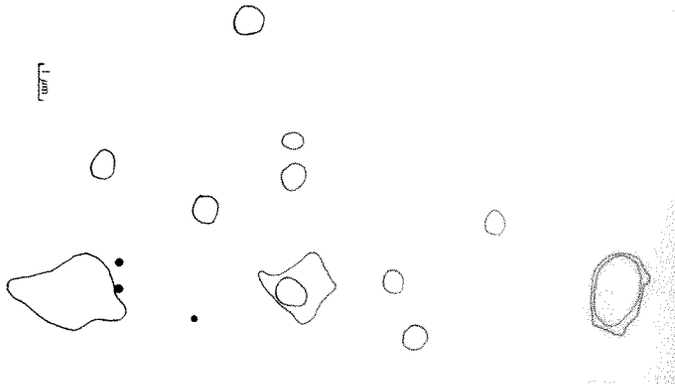


FIG. 1 Tracing of hair group in skin from leg of Liakhov mammoth.

feature[6]. The undercoat usually forms a distinct group of hairs in the diameter distribution which are separated by a wide gap from the much coarser outer coat hairs, as, for example, in the wild sheep[2]. In the mammoth (Table 2) it is difficult to detect any gap, so that the diameters seem to have a continuous distribution.

It may be therefore that once secondaries have disappeared in evolution, they cannot be redeveloped, and so any new undercoat must be formed by increasing the number of follicles, and extending the diameter range, as seems to have happened in the mammoth. An objection to this interpretation is that the size difference between the primaries and secondaries is thought to be due to their development at different times and to follicle competition[6]. If the smaller follicles of the mammoth were developed later, they could therefore be regarded as secondaries.

I thank Dr M. R. Brambell of London Zoo and Mr R. E. Chaplin of Edinburgh Zoo for supplying samples of modern elephant hair, Dr S. K. Eltringham of the Uganda Institute of Ecology for some elephant skin, Dr L. Ginsburg of the Palaeontological Museum, Paris for mammoth skin, H. B. Scott and F. M. Hay for technical assistance, and Dr G. C. Priestley for comments on the manuscript.

Note added in proof: After this letter had gone to press two more small samples were received from the USSR. Seven hairs from the Levar mammoth ranged from 14 to 39 cm in length, and from 98 to 260 μm in diameter with a mean of 179 μm. Three hairs from the Bereleh specimen ranged from 35 to 50 cm in length, and from 240 to 380 μm in diameter. The underwool of this sample ranged from 20 to 46 μm with one hair 70 μm in diameter, and an overall mean of outer hairs and underwool of 57 μm. These are evidence of the longer and coarser hairs mentioned in previous reports, but not found in the other samples of the present study.

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[1] Farrand, W. R., *Science*, **133**, 729–735 (1961).
[2] Ryder, M. L., in *The Domestication and Exploitation of Plants and Animals*, (edit. by Ucko, J. J. and Dimbleby, G. W.) 495–521 (Duckworth, London, 1969).
[3] Lang, H., *Zoologica*, **4**, 25–53 (1925).
[4] Quakenbush, L. J., *Am. Mus. Nat. Hist. Bull.*, **26**, 87–130 (1909).
[5] Neuville, H., *Bull. Mus. Nat. Hist.*, **23**, 374–387 (1917).
[6] Ryder, M. L., and Stephenson, S. K., *Wool Growth*, (Academic Press, London and New York, 1968).
[7] Sandison, A. T., *Stain Technol.*, **30**, 277–283 (1955).
[8] Ryder, M. L., in *Science in Archaeology*, Second ed. (edit. by Brothwell, D. R., and Higgs, F. S.) 529–544, (Thames and Hudson, London, 1970).
[9] Saccki, S., Lanzi, C., and Zanoth, L., in *Advances in the Biology of Skin* **IX** (edit. by Montagna, W., and Dobson, R. L.) 169–175. (Pergamon, London, 1969).
[10] Gillespie, J. M., *Science*, **170**, 1100–1102 (1970).
[11] Straile, W. E., *Am. J. Anat.*, **106**, 133–148 (1960).

Kinetic Cyclopean effect

While watching noise displayed on a television screen recently I noticed a visual effect which I report here. As normally viewed, displays of video noise ('snow') have the appearance of fields of small speckles which seem to dart about at random. When, however, the noise is viewed monocularly, the texture of the field seems finer and the speckles more nearly static. The display seen monocularly has the appearance of a field of smaller speckles which scintillate *in situ*. I have shown the effect to about ten friends and colleagues, without telling them what to expect. All noticed some difference between binocular and monocular viewing and several promptly described exactly what I had seen. In general, the size change was more consistently reported than the motion change.

I have made a rudimentary effort to explore the effect using a noise display generated by passing the green (514 nm) beam of an argon-ion laser through a pair of ground glass plates, one of which could be translated at a controlled speed. The speckle pattern so generated was projected on a screen. The resulting field of wideband, randomly changing visual noise bore a strong resemblance to television 'snow', though its statistics were not identical[1]. Under the conditions of the arrangement the speckle pattern produced by interference at the retina was so fine that it was nearly invisible, and so could not be confused with the intended display.

The following observations were made by three people. (1) The difference between monocular and binocular viewing was clearly evident, and resembled the effect obtained with the television screen; excessively rapid translation of the ground glass, however, did diminish the effect. (2) There was no difference between monocular and binocular viewing appearances of the stationary noise field obtained when both ground glass plates were still. (3) The effect could be obtained when the images in the left and right eyes were uncorrelated. Such images were obtained by presenting a different portion of the display to each eye using cardboard tubes to facilitate binocular fusion. (4) The effect could also be obtained when half the visual field of one eye was blocked by a card. In this case, one half of the field consists of the noise display as seen monocularly, and the other half consists of the same display as seen binocularly.

The last observation indicates that the effect is not induced by any process occurring in the eye itself, such as a transient misfocussing caused by the change from one mode of viewing to the other. The effect is therefore Cyclopean[2] in nature. Since it can only be seen in a non-static visual noise field, the classification 'kinetic Cyclopean effect' seems appropriate.

That the effect can be obtained whether or not the noise is binocularly correlated might suggest that even when the binocular images of the noise fields discussed here are correlated, they in some way exceed the ability of the visual processor to perceive the correlation. In any case it is not clear why a planar field of randomly changing visual noise should not appear the same to two eyes as to one. An adequate explanation of this effect might yield new information about the functioning of the visual system.

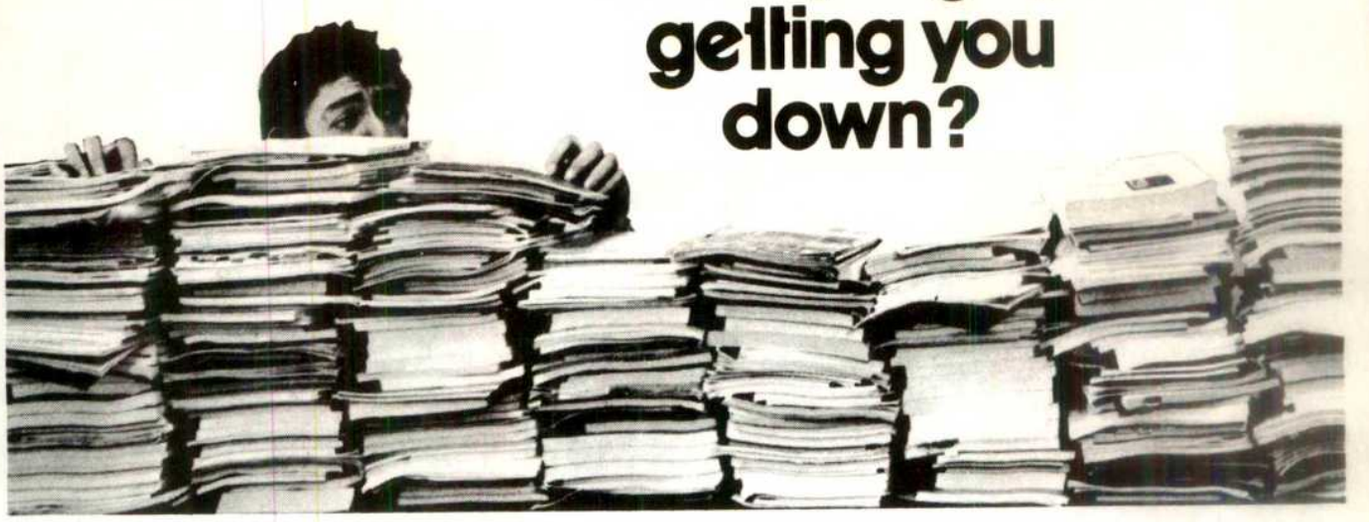
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[1] Nagata, K., and Umehara, T., *Japan J. appl. Phys.*, **12**, 694 (1973).
[2] Julesz, B., *Foundations of Cyclopean Perception*, (University of Chicago Press, Chicago and London, 1971).

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Electrons in Fluids

The Nature of Metal-Ammonia Solutions

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Express emotion

Darwin and Facial Expression: A Century of Research in Review. Edited by Paul Ekman. Pp. xi+273. (Academic: New York and London, December 1973.) $17; £8.

In recent years there has been a new spate of popular books dealing with man's animal origins. These, together with claims about racial differences in intelligence and the biological basis of sex differences, have reinstated the old 'nature-nurture' controversy within psychology, and have led to heated disputes which are unlikely to be scientifically fruitful so long as the question is seen in terms of absolutes rather than matters of degree. Ekman's collection of papers is therefore doubly timely. Not only does it assess the century of research which has followed Darwin's valuable and delightful book, *The Expression of the Emotions in Man and Animals*, but it also provides an object lesson in how the confrontation of genetic and environmental viewpoints can lead to advances beyond the usual, and vague, "interactionist' compromise.

Taken together, the chapters give a balanced picture of Darwin's general approach to the explanation of behaviour, his specific theories of expression, his research methods, and the data he collected. They show that his position was in fact a sophisticated one, although he emphasised the genetic links between animal and human expressions, and the apparently innate universality of many of our emotional responses. In fact he was sensitive to many methodological pitfalls in research on emotion that later investigators have often overlooked. The present volume presents what is, by and large, a vindication of Darwin's insights. It also shows that we are only just starting to make significant advances on what he discovered a century ago.

Ekman's collection concentrates on the face, mentioning other aspects of expressive behaviour and the structure of emotion itself only in passing. Of the four main chapters only that by Petrinovich takes a broader view, giving an interesting presentation of how Darwin's theoretical and methodological paradigms have influenced psychology and ethology, and emphasising the importance of a functional view of human activities which takes full account of their biological and ecological context.

Chevalier-Skolnikoff reviews research on primate expressions, discussing their determinants and functions and relating them to the evolution of facial musculature and of the nervous system. Charlesworth and Kreuzer review the development of expressions and their recognition in infancy and childhood, remaining firmly at the descriptive level because they believe there is too little data to support elaborate theorising. It must be said, however, that on the topics of infant social smiling and fear of strangers there are other data which do go some way beyond Darwin's work and surely permit some theorising about underlying processes.

The other major chapter is Ekman's thorough review of cross-cultural studies, although some other aspects of adult expression are scarcely represented in the book at all. Recent studies, especially his own, contradict the long-standing view of anthropologists that Darwin was wrong in claiming an innate basis for emotional responses. In both Western-influenced and isolated cultures 'basic' emotions like happiness, disgust, surprise, anger, sadness and fear are displayed and recognised in relatively universal ways. Ekman's major contribution is a theory which accepts an innate basis to the linkage between eliciting stimuli, felt emotions, and behavioural expressions, and yet can deal with individual and cultural variations by reference to learned 'display rules' and other complicating factors. Although his theory gives a plausible role to both nature and nurture, and is to be welcomed, it is unfortunate that he does not entirely do justice to the environmentalists.

In fact the book as a whole takes too little account of two important facts about expressive behaviour. One is that facial expressions occur in association with other bodily movements, vocalisations, and even speech. Such multi-channel activities are simultaneously organised at several levels of instantaneous and temporal structuring, as the linguistic anthropologists have correctly emphasised. The second, more crucial point, is that these behavioural performances normally occur within, and are perceived within, a context of continuing social interaction. In fact much non-verbal behaviour serves several functions other than the purely expressive one. Darwin was certainly aware of the first of these points, and would surely have been glad to accept the second, so it is a pity that Ekman's book lacks a further chapter on the empirical and theoretical evidence relating to them. In fact it is on these topics that the most interesting nature-nurture questions now rest.

Despite these strictures, and other minor defects of the volume, Ekman has produced a valuable book which should be widely read. The contributors' careful avoidance of undefined technical terms, coupled with its very general lessons, should extend its appeal beyond the confines of the specialist disciplines represented. It is therefore inexcusable that its absurdly inflated price should threaten to deter many readers who would learn much from it.

Ian Vine

Critical approach

Exercises in Chemical Physics. By J. R. Riter, jun. Pp xiv+313. (Gordon and Breach: New York and London, 1972.) n.p.

Significantly new ideas in science teaching are rare and by no means necessarily translatable into book form. Professor Riter's method consists in a kind of inspired exegesis of selected papers in his field, the broad and delightfully ill-defined area of chemical physics. The papers he treats, or perhaps one should say draws upon, all appeared in about the last ten years of the *Journal of Chemical Physics*, a convenience which by no means takes away from the author's breadth of vision. In all some 166 articles are discussed under headings which include: thermodynamics, quantum mechanics, statistical mechanics, rate processes, mass spectroscopy, infrared, optical, rotational and NMR spectroscopy and the states of matter.

I used the term 'inspired' above quite advisedly. Professor Riter's studies are far more than a simple run-down of the background theory followed by routine, examination-type questions. The content of each article and the student's awareness of it are continuously probed as the author puts questions, makes suggestions for further reading and points to cross-connections. The main results of the article are usually presented in facsimile and a short precis of its aims and achievements given; thereafter the student is asked to reflect, criticise, calculate and cross-refer—sometimes even invited to find mistakes in the original. The result, as will have been deduced from the number of papers given, is

extremely condensed; with almost every other sentence in some passages putting a question or making some pertinent demand on the student, the quantity of food for thought provided is enormous and quite out of proportion to what one would expect from a book of such modest size. That the result is not indigestible owes much to Professor Riter's sense of style and feeling for the subject matter. His questions have a provocative and searching flavour difficult to convey here, which would be altogether more cramped in a conventional textbook. There is a droll humour, an unwavering sense of relevance, an infectious but entirely mature enthusiasm for chemical physics as a field and a sympathy with the authors whose work is dissected in such fascinating detail.

This adds up to one of the most successfully achieved ideas in advanced teaching that I have seen in a long time, and one which could no doubt be copied, though perhaps not so suitably, in many other branches of science.

M. R. Hoare

The nature of sleep

The Functions of Sleep. By Ernest L. Hartmann. Pp. ix+198. (Yale University: New Haven and London, January 1974.) £3.50 cloth; £1.25 paper.

This is a short readable book which should often succeed in interesting and informing the non-expert as well as the specialist. The author has been a prominent worker and prolific writer in the field of sleep research for over a decade. The emphasis in much of the book is on his own sleep research findings and he concludes by advancing yet one more theory concerning the nature of sleep. This latter is contained within the now generally accepted proposition that sleep has a restorative and reprogramming function. In contrast to some others, Hartmann's view is that the former is associated with slow wave sleep wherein synthesis of protein, RNA and other macromolecules crucial to cerebral function occurs, while the latter process is related to paradoxical or D sleep which follows on slow wave sleep and within which reconstruction of memory at the synaptic level can then occur.

Hartmann builds up his case systematically in the early chapters. Other theories are given brief and initial attention before a series of chapters within the rump of the book is given over to the various experimental approaches that can be made. Sleep deprivation studies are presented as having proved to be of limited heuristic value. Consideration is given to his own investigations of personality differences between long and short sleepers, and also to the clinical correlates of variable sleep which include behavioural, affective, nutritional and occupational items. Some physiological and chemical accompaniments of the various sleep stages are described as are also the ways in which their manipulation can affect sleep in animal studies and the human. A brief but interesting chapter on the different kinds of tiredness and a final one in this section concerning the nature of dreaming serve to remind the reader of the author's firm clinical roots.

The final chapter of the book concerns the integration of his theory of sleep into a more extensive one concerning the nature of the mind and he outlines the possible chemical bases of a variety of psychological mechanisms. Although speculative, it also reflects an important attempt to mobilise psychoanalytic concepts in physiochemical terms in a way which opens up the prospect of experimentation.

For all its chauvinism this is an attractive monograph in which the author, from a position of wide experience in the field of sleep research, has come clean to stoutly defend his holistic and multi-faceted approach and the need for it at this stage in our attempt to understand the chemistry of mind.

The style in which the references are set out (placed at the bottom of each page as well as in an alphabetical list at the end of the book) also makes for easy reading.

A. H. Crisp

Text on bacteria

Fundamental Principles of Bacteriology. By A. J. Salle. Pp. x+1094. (McGraw Hill: New York and London, May 1973.) £8.85.

This book is intended for "introductory majors courses in microbiology or bacteriology". But although there are chapters on yeasts, moulds, bacterial viruses and viral diseases of man, it cannot be regarded as a satisfactory microbiology text because protozoa and algae are scarcely mentioned in their own right and because, in the chapters on various aspects of the environment, microorganisms other than bacteria are more or less completely ignored. The short chapter on associations of bacteria does not compensate for these shortcomings and the book must therefore be judged as a bacteriology text.

In the preface the author states that he "attempts to include only sound fundamental material" and that he places emphasis "on the use of chemistry for a clearer understanding of the composition of bacteria and the reactions they produce". The extent to which these aims are achieved varies, of course, but in some chapters there are striking differences in the treatment of comparable topics. For example, the composition and chemistry of the bacterial cell wall are inadequately treated. There is no mention of teichoic acids although the existence of these polymers is acknowledged in the previous edition. Does the author now doubt the soundness of the work on their composition and structure? In contrast, immediately following the section on the cell wall there is a comparatively full and satisfactory treatment of flagella.

Although the book has many illustrations of chemical structures and equations for reactions, often these do not add very much to the student's understanding of the processes in which they take part. Perhaps the most striking instance is in the chapter on enzymes, where there is a 30-page incomplete list of bacterial enzymes "to show how enzymes are named and classified". This is somewhat extravagant if space when many other topics could, with advantage, have been dealt with more fully.

In many instances archaic nomenclature is used. For example, although it is true that some bacteriologists are extremely conservative in the names they use for carbohydrates, surely nobody now uses 'levulose' (*sic*) in preference to 'fructose' these days, and even if they do it must be confusing for any student to find such inconsistent usage as ". . . sucrase hydrolyzes sucrose to glucose and levulose," (page 354). Another criticism is that some at least of the reading lists are not very well chosen.

The index is inadequate and the layout of the print on the page is irritating in that although there are large outer margins the print approaches the bottom of the page so closely as to appear to be in danger of falling off!

It is remarkable that although the first edition appeared in 1939 most of the one thousand or so pages of this the, seventh edition, are still by the original author. Collaborators are responsible for only four of the thirty-one chapters—some eighty-nine pages. It would perhaps have been better had more authors been involved, for clearly it is beyond the capabilities of any one person to be sufficiently up to date in the many different fields that are covered by this book to be able to extract and present the essential information from each area within the time scale required in the preparation of a new edition.

Of course there is a lot of sound, thoroughly well presented material but deficiencies and distortions of relative importance prevent it being a serious contender for selection as a text for an undergraduate course. Perhaps the nicest way to summarise is to adapt the comment made by one British ex-politician about a recently deceased ex-colleague, and say that it is not fundamentally a bad book.

R. C. W. Berkeley

Red algae

Biology of the Rhodophyta. By P. S. Dixon. Pp. xiii+285. (University reviews in Botany.) (Oliver and Boyd: Edinburgh, October 1973.) £5.

A COMPREHENSIVE review of the Rhodophyta has been wanted for some years now. Professor Dixon has presented an analysis of current knowledge of certain aspects of the biology of these intriguing algae, drawing on information published up to 1972. The clear purpose is to make the reader aware of the deficiencies in knowledge, as well as indicating the advances made.

Within the scope of the seven chapters there are four in particular which present concise and stimulating accounts of key areas in present day research on red algae: morphology, reproduction, life histories and systematics and phylogeny. The chapter on morphology is of especial merit as it illustrates the numerous modifications of the filamentous habit and the potential of further work on morphogenesis both experimentally and with field observations. These approaches enliven a topic all too often avoided by students. The chapters on reproduction and life histories set out clearly the areas of conjecture, fact and theory. Through all of these the author steers a steady course which leaves the reader with little doubt as to what is reliable in the mass of conflicting information. The lines of future research are also clearly shown. Although the deficiencies in some aspects of the classification system are recognised, the lack of credible alternatives at present is also underlined.

Here then is a book of fundamental importance to students (of whatever age) of red algae. Minor blemishes there are. There is some repetition in later chapters of points covered in the introduction. Many of the diagrams are 'old friends' and some new interpretations would have been valuable. Many readers will regret the lack of a chapter on ecology. But the basic value of the book to continuing studies on red algae cannot be overemphasised.

A. D. BONEY

Man and society

The Riddle of Man: An Introduction to Psychology. By Richard S. Lazarus. Pp. xii+628. (Prentice-Hall: Englewood Cliffs, NJ, January 1974.) $10.95.

THIS is an outstanding, balanced and humane multidisciplinary book which is much more than an introduction to psychology as its modest subtitle might suggest. The 'riddle of man' the book takes as its subject matter concerns man's biological nature and its interaction and interdependence with his social and physical environments.

Man's biological make-up is seen as influencing the social order he creates and in turn social patterns are shown to modify the way biological processes operate. But the author does not conceptualise man as a passive responder to environmental stimuli and he explicitly rejects the view that in order to understand human behaviour one has to examine it solely in terms of rewards and punishments which follow actions (as does Skinner). The author defines psychology as the science of behaviour and mental activity—a deliberate choice of words since he considers it as one of man's remarkable qualities that he not only acts but is an observer and critic of his own actions. Psychology is presented as one of many interrelated disciplines—biology, anthropology, sociology and economics, all of which attempt to contribute to an understanding of man and society. The author draws on all these sciences and borrows their insights but his focus is a psychological analysis of the individual's biological and social development, his internal motivation and overt responses, his mental and emotional states and his reactions to stress and change.

The book is unusual for a textbook in psychology in that it tells a coherent and compelling story; it addresses itself to some of the unsolved issues of current—and perennial—human concern and to this end has been organised around four key issues: the physical and social environments of man; the psychology of aggression; prejudice and intergroup hostility; and adaptation versus alienation. This book, then, is problem-centred and continuously forges imaginative links with the student's own world and experience. But, within this framework, it also introduces the reader to a great many basic psychological and social psychological processes, concepts and theories. It presents competing paradigms and theories and makes explicit their assumptions and consequences and hence challenges the student to come to his own conclusions and to forego a premature and too exclusive theoretical commitment of his own—be this a commitment to behaviourism or to Freud or to anything else. Thus, at all times the student is presented with scientific controversies which are patiently and skilfully explored to give the student a sophisticated grasp of the issues. Thus, for instance, in discussing the failure of individuals to cope successfully with the stress of living and to find inner harmony, both the 'illness model' and the 'social learning model' are examined as a basis for understanding adaptive failure.

This book is beautifully produced, superbly illustrated with imaginatively chosen photographs, cartoons and diagrams. A further feature are quotations in the margins from important primary sources and detailed notes on theoretical, methodological and empirical issues which provide a useful underpinning of the text.

HEDY BROWN

Semiconductors

Semiconductor Physics. By Karlheinz Seeger. Pp. xv+514. (Springer Study Edition.) (Springer-Verlag: Vienna and New York, 1973.) 430 schillings; 60 DM.

"THIS book has been designed primarily as a text-book for a three-semester, three-hour per week senior or graduate course in semiconductor physics for students in electrical engineering or physics"; to quote its preface. The course is no easy option, for the book covers with encyclopaedic thoroughness nearly every aspect of semiconductor physics, in breadth if not always in depth. After introductory chapters on elementary properties, band structure and semiconductor statistics, the following topics are discussed: charge and energy transport in a non-degenerate electron gas; carrier diffusion processes, with a brief treatment of transistors; scattering processes in a spherical one-valley model; charge transport and scattering in the many-valley model; carrier transport in the warped-sphere model; quantum effects in transport phenomena; impact ionisation and avalanche breakdown; optical absorption and reflection; photoconductivity; semiconductor light generation; surface properties; and miscellaneous semiconductors. The text is well illustrated with 372 figures, many taken from the literature. There are five useful appendices and author, subject and substance indices.

There are two features which are unusual in a book of this nature. Complete derivations, including the intermediate equations, are given in the text of the numerous mathematical relations. This feature, which is designed to assist the less numerate student, unfortunately increases the algebraic content of the book at the expense of its physics content. The second feature is the provision of over 650 references in the text, many of them to books of similar size. In most cases these references provide a useful supplement to information already given in the text. In some cases, however, they are used as a substitute for such information. The important semiconductor technique of zone refining is mentioned on pages 1 and 153. In the first case it is accompanied by a reference to three books and several other volumes, and in the second by a reference to a book in German. In neither case in any explanation given of the nature of zone refining, an omission ac-

ceptable in a research paper but not in a textbook.

Despite these blemishes, the book provides a well written, comprehensive account of the transport and optical properties of semiconductors, which should prove useful not only to 'senior or graduate' students, but also to research scientists in the area of semiconductor physics.

J. B. BIRKS

Leukocytes collecting

Chemotaxis and Inflammation. By Peter C. Wilkinson. Pp. viii+214. (Churchill Livingstone: Edinburgh and London, 1974.) £4.

THERE is an analogy between the way in which research on leukocyte chemotaxis has been conducted and the movement of leukocytes themselves. For years there was more or less random observation by pathologists, bacteriologists and immunologists, who reported chemotactic activity for neutrophils or mononuclear phagocytes in a variety of products of bacterial growth, tissue injury and immune reactions. A distinction was made between cytotaxins, which themselves stimulate the directional movement of cells, and cytotaxigens, which bring about a chemical change in non-chemotactic molecules, usually serum constituents, as a result of which cytotaxins are generated. From all this work a long list of chemotactic factors has accumulated, some of which are controversial.

Two technical innovations improved the quality of research in this field. One was the introduction of the Boyden chamber and developments of it which made observations on chemotaxis more quantitative. The second was the availability of new methods for purifying chemotactic factors such as complement components. At present research on chemotaxis is gaining fresh impetus. Random activity and accumulation of miscellaneous observations is now being replaced by studies directed towards the purification and characterisation of cytotaxins and ascertaining how they act on leukocytes. Although the progress so far made is limited, the direction is obviously right.

Some cytotaxins, such as α_s-casein and the cleavage products of complement components, C3a and C5a, have been identified and quite a lot is known about the chemical composition of casein and C3a. The first steps have been taken towards defining the physicochemical properties of a molecule that make it cytotaxic. Wilkinson's own research on the generation of chemotactic activity when proteins are denatured or coupled with acid anhydrides or other groups is a contribution in this direction.

It seems as though basic peptides with specific conformations, such as the arginine-rich C3a, or proteins with rather high concentrations of surface non-polar groups, such as α_s-casein and denatured or substituted proteins, are cytotaxic. Possibly the former interact with and cluster certain acidic glycoproteins in the surface of the leukocyte whereas the latter penetrate into the membrane of the leukocyte. Both could increase the permeability of ions, such as calcium, through the leukocyte membrane, thereby triggering chemotactic responses.

Books on chemotaxis are out of date and out of print. This year will see the publication of two books on chemotaxis, a multi-author volume edited by Sorkin and the shorter monograph by Wilkinson under review. The latter is a well written introduction to the subject which will provide enough information for all but specialists. Scotland is neutral ground in the skirmishes that have sometimes developed between Switzerland and the eastern seaboard of the United States, and Wilkinson provides a balanced appraisal of the situation. Where available information is confusing, such as in the role of cyclic nucleotides in the responses of leukocytes to chemotactic factors, this is plainly stated. Perhaps observations on activation of guanylate cyclase, too recent to be included by Wilkinson, will help to resolve the problem.

In any case, there is current interest in the motility of cells, which depends on an actomyosin system similar to that in smooth muscle, on mechanisms by which attachment of various substances to plasma membranes can trigger cellular responses and on the behaviour of embryonic and other cells in concentration gradients. These developments in cell biology should soon be applicable to chemotaxis. Wilkinson's book provides a useful summary of the first fifty years of research on chemotaxis and can also serve as an introduction to the next phase of development. Perhaps it will stimulate cell biologists to use leukocyte chemotaxis as a model system which is of interest in itself and also relevant to medicine.

A. C. ALLISON

Climatic change

Éléments de Paléoclimatologie. By Raymond Furon. Pp. 216. (Sciences de la Terre.) (Librairie Vuibert: Paris, 1972.) n.p.

THERE is a clear need for a new objective, systematic review of palaeoclimatology, but Furon's contribution, instead of improving on the three main books is more likely to retard this interdisciplinary science. It commences with a chapter of statements about the characteristics of present climatic zonation, but without emphasising the underlying causes, and is followed by an inadequate outline of uncomprehended theories that could cause climatic change. Forty-nine pages are given, according to the title, to methods of palaeoclimatology. They turn out to be seriously incomplete yet space is wasted returning to arguments about continental drift instead of making necessary qualifications to bald statements such as striated and polished stones proving a glacial origin; illite, mica and feldspars in shales associated with Carboniferous coal being incompatible with a humid, tropical climate; and so forth. The objective evaluation of climatic indicators should be possible irrespective of the status of theories of the Earth's surface evolution.

The second section comprises five chapters dealing with different time spans which contain patchy lists of stratigraphic features, only some of which are of palaeoclimatic usefulness—for example, the thickness of Mesozoic volcanics in Mozambique is surely less climatically significant than the coral reefs of the North American and European Permian that are neither mentioned nor shown on a map purporting to show their distribution. Despite his own warnings, the author interprets all occurrences of *Gangamopteris* and *Glossopteris* as indicating warm, humid conditions, and the interbedded glacial debris as always from mountain glaciers associated with an unexplained 300 million year climatic cycle of ice ages. He admits, however, to being "un peu troublé" by the absence of glacial evidence from continents north of the Tethys and sprinkles the section dealing with the Carbo-Permian of Europe with "climat chaude", "chaude et humide", and so on, sometimes based on the presence of evaporites, but often without specifying the evidence. Luckily, the data are plotted on maps using the present geography and so avoid arguments about reconstructions, which Furon disbelieves, and which even those on the plate-tectonic bandwagon must still regard as subject to review.

The 16 palaeoclimatic maps are regrettably small, less than 12 × 8 cm, so that symbols (often only one or two per map) often overlap areas far distant from the actual outcrop and are all the same size, irrespective of such factors as the thickness of the evaporites represented. There is no index, but quite long bibliographies follow each chapter. This arrangement leads to repetition, so that Furon's *La Paléogéographie* is listed five times but Termier and Termier's *Atlas de Paléogéographie* only appears once and Harland's work on the late Precambrian is not mentioned anywhere. Nonetheless, the bibliographies are the most useful part of this unsatisfactory book.

D. H. TARLING

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Ciba Foundation Symposium 23

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matters arising

Solar activity and climatic change

SIR,—Following John Gribbin's article (*Nature*, **246**, 453; 1973), we wish to draw your attention to an unpublished thesis by Dr J. Laszewski (University of Warsaw) which supports Gribbin's suggestions (see also ref. 1). A translation of this thesis is available in the library of The Institution of Civil Engineers.

Laszewski (now professor at the University del Zulia in Maracaibo, Venezuela) has collected long term records of the mean annual discharges of many rivers in Europe, North and South America, Africa and Australia. He demonstrated that the mean annual discharge varies in a cyclic manner which is connected in a complex way with the cycles of sunspots. This is a result of the changes in the atmospheric circulation influenced by the sunspot cycle. The long cycles are divided into shorter cycles, so the story is very complex.

Although none of the records of river discharges examined cover more than about 150 yr and most cover less than 100 yr, one may suspect that the cycle of 200 yr suggested in Gribbin's article is detectable.

Yours faithfully,

T. M. PRUS-CHACINSKI
C. H. Dobbie and Partners,
Francis House,
Francis Street,
London SW1

J. R. D. FRANCIS
Imperial College,
Prince Consort Rd,
London SW7

1 Stachy, J., *Long term variation of the runoff of Polish rivers* (Institute of Meteorology and Hydrology, Warsaw, 1970) (in Polish).

Refining geoid heights

SIR,—In their recent paper refining the longitude-averaged geoid height with improved values for the zonal harmonics in the geopotential, King-Hele and Cook[1] show their latest version of the height of the meridional geoid section relative to a spheroid of flattening 1/298.25. In this definition an axially symmetric Earth is assumed.

If the distribution of Precambrian exposed and overlaid rocks[2] is expressed as the ratio of their occurrence along a particular line of latitude to the total length of that line (that is longitude 0° to 360°) and plotted against latitude, there seems to be reasonable correlation with geoid height. The actual proportions are plotted in Fig. 1 together with a smoothed curve on twice the scale.

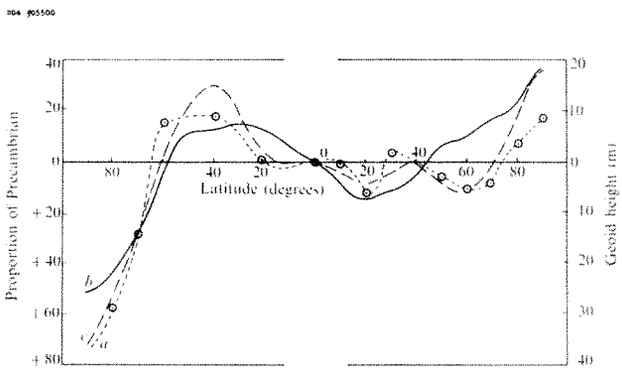


FIG. 1 Comparison of geoid heights given by King-Hele and Cook[1] with proportion of Precambrian stable areas. *a*, Proportion of Precamprian rock; *b*, geoid height, after King-Hele and Cook[1]; *c*, precamprian proportion smoothed on double scale.

A similar correlation exists with longitude for the gravimetric geoid published by Marsh[3] which agrees well with the Smithsonian Standard Earth 1969 (ref. 4). The geoid height at latitude 35°N is plotted against longitude in Fig. 2 and the distribution of Precambrian rocks is shown together with the extent of regions of Tertiary and Hercynian folding, which appear to have some significance, particularly in regions of orogeny such as 35°N, 65°E.

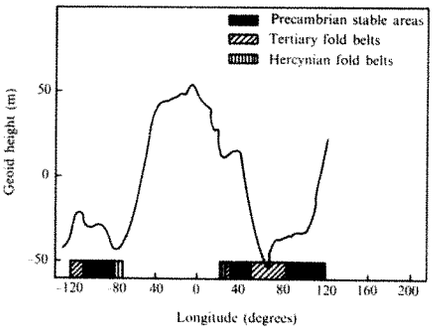


FIG. 2 Detailed gravimetric geoid at latitude 35° N showing correlation with geological formation.

The surprising feature of these effects is that the geoid height seems to be lower than the 1/298.25 spheroid in the stable land areas and higher than it in the oceanic areas. This is the reverse of what would have been expected intuitively.

Yours faithfully,

B. P. DAY
Chedworth,
Dippenhall Street,
Crondall, Farnham, Surrey

1 King-Hele, D. G., and Cook, G. E., *Nature*, **246**, 86–8 (1973).
2 *The Times Atlas of the World* (Times Newpapers Ltd., London 1968).
3 Marsh, J. G., *Significant Accomplishments in Sciences NASA Goddard SFC*, SP-331, 212 (1972).
4 Gaposchkin, E. M., and Lambeck, K., *Smithson. astrophys. Obs. Spec. Rep.*, **315**, (1969).

Left and right in Goya's portraits

MCMANUS and Humphrey[1,2] have described biases in the frequency with which more of the right or left sides of faces are shown in paintings. Examination of 1776 portraits by different artists revealed that, overall, 57% showed more of the left side of the sitter's face. Whilst self-portraits tended to show more of the right side of the face (61% of those examined), male portraits tended to show more of the left side of the face (56%) and female portraits showed an even stronger bias towards the left (68%).

In an analysis of 335 portraits by Rembrandt, the same authors found that the frequency with which more of the left face is shown increases progressively across the following categories of sitter: self, male kin, male non-kin, female kin, female non-kin. To explain this it was suggested that the categories represent positions on a dimension 'socially like myself—socially unlike myself' along which Rembrandt placed his sitters. The choice of mainly left or right face was an indication by Rembrandt as to how he perceived his social relationship with the subjects of his portraits.

Table 1 summarises an analysis of 295 portraits by Goya taken from a comprehensive illustrated account of his works[3]. Each portrait was classified simply as

TABLE 1 Relative frequencies with which more of the left face is shown in Goya's portraits.

	Self	Male kin	Female kin	Male non-kin	Female non-kin
No. of portraits examined	14	14	12	185	70
No. showing more of the left face	7	9	4	95	50
%	50.0	64.3	33.3	51.4	71.4

showing either full face, or more of the left or right side. The classification of each sitter's relationship to Goya follows that adopted by McManus and Humphrey. The male kin and female kin categories include members of the Bayeu y Subias and Goicoechea families to whom Goya became related through his marriage and that of his son Javier.

Analysis of the data in Table 1 shows, firstly, that Goya's portraits fit the general pattern discovered by McManus and Humphrey in that a majority (56% overall) show more of the sitter's left face. This result is statistically significant (Binomial Test, $Z = 1.98$, $P = 0.024$, one-tailed). There is also a significant sex difference in that, overall, female portraits are more likely to show the left side of the face ($\chi^2 = 4.4$, $P < 0.05$); this result agrees with the original finding by McManus and Humphrey. Analysis of the data in terms of kin compared with non-kin, however, does not reveal any significant relationship ($\chi^2 < 1.0$). More detailed examination of Goya's portraits makes it seem even more unlikely that perceived social relationships influenced Goya's choice of which side of faces to paint: thus of 15 of Goya's self portraits, one is full face, seven show more left face and seven more right; of the four portraits of Goya's son, two show more left face, two more right; all three portraits of Goya's beloved grandson, Mariano, show more left face.

Whilst Goya's works provide evidence which agrees with McManus and Humphrey's interesting general findings about left and right in portraits, their hypothesis relating choice of side and the relationship between artist and sitter is not supported. This could mean either that the effect occurs only with certain artists, or that certain relationships discovered in Rembrandt's portraits are due to other causes, some of which have already been suggested[4].

Yours faithfully,

I. E. GORDON

Department of Psychology, The University, Exeter

[1] McManus, C., and Humphrey, N., *Nature*, **243**, 271–273 (1973).
[2] Humphrey, N., and McManus, C., *New Scient.*, **59**, 437–439 (1973).
[3] Gassier, P., and Wilson, J., *Goya, His Life and His Work* (Thames and Hudson, London, 1971).
[4] Corlett, T., McGlone, B., and Wheale, N. H., *New Scient.*, **59**, 581–582 (1973).

Announcements

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

Natural Resources, the Engineer and the Environment. By Sir Kingsley Dunham, FRS. (19th Graham Clark Lecture). Pp. 24. (London: Council of Engineering Institutions, 2 Little Smith Street, SW1, 1973.) [3112

Department of the Environment. Report of the Committee on the Use of Valuers in the Public Service. (Cmnd. 5518) Pp. v + 33. (London: HMSO, 1973.) 24p net. [3112

Department of Education and Science. Statistics of Education 1972. Vol. 5: Finance and Awards. Pp. xvii + 59. (London: HMSO, 1973.) £1.70 net. [3112

Proceedings of the Royal Society of London. B: Biological Sciences. Vol. 184, No. 1076: A Discussion on the Biology of an Equatorial Lake—Lake George, Uganda. Organized by P. H. Greenwood and J. W. G. Lund. Pp. 227–346. Vol. 184, No. 1077: A Discussion on the Value of Automation in the Health Service. Organized by D. A. J. Tyrrell. Pp. 347–481. (London: The Royal Society, 1973.) [31

The Commonwealth Foundation. Report IV, July 1971–June 1973. Pp. 81. (London: The Commonwealth Foundation, 1974.) [141

Progress Without Pain. (Text of Speeches given at the Seminar on the Potential of Humane Non-Animal Research Techniques. London, March 1973.) Pp. xiii+76. (London: The National Anti-Vivisection Society, 51 Harley Street, W1, 1974.) 75p. [141

Proceedings of the Royal Irish Academy. Vol. 73, Section A. No. 21: Tensor Products, Multiplication Operators and the Spectral Mapping Theorem. By R. E. Harte. Pp. 285–302. 42p. No. 22: Strictly Singular and Strictly Cosingular Operators on Topological Vector Spaces. By G. P. Shannon. Pp. 303–308. No. 23: Operators Generating Weakley Compact Groups II. By T. A. Gillespie and T. T. West. Pp. 309–326. 42p. Vol. 73, Section B. No. 23: Drumlins of County Clare. By T. F. Finch and M. Walsh. Pp. 405–414+ plates 18–20. 27p. No. 24: Population Dynamics of Barnacle Geese, *Branta leucopsis*, in Ireland. By D. Cabot and B. West. Pp. 415–444. 45 p. No. 25: Littoral and Benthic Investigations on the West Coast of Ireland—II. (Section B: Shellfish Investigations, the Occurrance of *Palinurus mauritanicus* Gruvel, 1911 on the West Coast of Ireland, Decapoda Palinuridae). By J. P. Mercer. Pp. 445–450+ plate 21. 16p. No. 26: Littoral and Benthic Investigations on the West Coast of Ireland—I. (Section A: Faunistic and Ecological Studies). The Sponge Fauna of Kilkieran Bay and Adjacent Areas. By G. Konnecker. Pp. 451–472. 35p. (Dublin: Royal Irish Academy, 1973.) [141

Other countries

Smithsonian Contributions to Zoology, No. 127: A Revision of the Family Lichomolgidae Kossmann, 1877, Cyclopoid Copepods Mainly Associated with Marine Invertebrates. By Arthur G. Humes and Jan H. Stock. Pp. v+368. (Washington, DC: Smithsonian Institution Press, 1973. For sale by US Government Printing Office.) $4.30. [2012

Carnegie Institution of Washington. Report of the President, 1972/1973. Pp. 38. (Washington, DC: Carnegie Institution of Washington, 1973.) [2812

Australian Atomic Energy Commission. Twenty-first Annual Report, 1972/1973. Pp. 128. (Coogee, NSW: Australian Atomic Energy Commission, 1973.) [3112

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Chemical Physics, 1972/1973. Pp. 64. (Clayton, Victoria: CSIRO, 1973.)

Australian Academy of Science. Report of the Committee on Solar Energy Research in Australia. (Report No. 27). Pp. 63. (Canberra, ACT: Australian Academy of Science, 1973.) [31

Annals of the Transvaal Museum, Vol. 28, No. 13: Human Skeletal Remains from Border Cave, Ingwavuma District, Kwazulu, South Africa. By Hertha de Villiers. Pp. 229–256 + 9 plates. Vol. 28, No. 14: Behaviour and Biology of *Leptoglossis membranaceus* (Fabricius) in the Transvaal, with Descriptions of the Genitalia (Heteroptera: Coreidae). By J. A. Van Reenen. Pp. 257–286. Vol. 28, No. 15: Two Species of *Antidorcas* Sundevall at Swartkrans (Mammalia: Bovidae). By E. S. Vrba. Pp. 287–352+9 plates. Vol. 28, No. 16: Notes on Stomach Contents Analysis of Various Carnivores in Southern Africa (Mammalia: Carnivora). By S. Viljoen and D. H. S. Davis. Pp. 353–364. (Pretpria: Transvaal Museum, 1973.) [71

The Walter and Eliza Hall Institute of Medical Research 1972/1973. Pp. 136. (Melbourne: Walter and Eliza Hall Institute of Medical Research, Post Office, Royal Melbourne Hospital, 1973.) [71

New Zealand Meteorological Service. Rainfall Percentiles: Tables of Percentiles, Means, Standard Deviations, and Extremes, of Monthly, Seasonal, and Annual Rainfall for New Zealand and Pacific Island Stations. Pp. 98. (Wellington: Government Printer, 1973.) [71

Australia: Commonwealth Scientific and Industrial Research Organisation. Report of the Division of Horticultural Research 1971/1973. Pp. 148. (Adelaide: CSIRO, 1973.) [71

Fiji. Report of the Department of Agriculture for the year 1972. Pp. 40. (Suva: Department of Agriculture, 1973.) [71

La Vita di Copernico di Bernardino Baldi dell' Anno 1588, alla luce dei Ritrovati Manoscritti delle Vite dei Matematici. By Bronislaw Bilinski. (Wroclaw and Warszawa: Zaklad Narodowy Imienia Ossolinskich, Wydawnictwo Polskiej Akademii Nauk, 1973.) [81

Svenska Linnésällskapets Arsskrift, Argang 1970–1971. (Yearbook of the Swedish Linnaeus Society. (Pp. 135. (Uppsala: Swedish Linnaeus Society, 1973.) [81

OECD Nuclear Energy Agency. First Activity Report. Pp. 85. (Paris: OECD Nuclear Energy Agency, 1973.) [111

Australia: Commonwealth Scientific and Industrial Research Organization. Division of Soils Technical Paper No. 19: Techniques of Oblique Aerial Photography of Agricultural Field Trials. By J. R. Harris and T. G. Haney. Pp. 40. (Melbourne: CSIRO, 1973.) [111

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Paper 73-8: Granulate Facies Rocks on Northeastern Devon Island, Arctic Archipelago. By J. Krupicka. Pp. 41 (6 plates). $1.50. Paper 73-24: Medial Lower Cambrian Trilobites from the Mackenzie Mountains, Northwestern Canada. By W. H. Fritz. Pp. 43 (8 plates). $2. Pamphlet on Meteorites. (Ottawa: Information Canada, 1973.) [111

United States Department of the Interior: Geological Survey. Bulletin 1305: A Geochemical Reconnaissance for Gold in the Sedimentary Rocks of the Great Lakes Region, Minnesota to New York. By David A. Seeland. Pp. 16. (Washington, DC: Government Printing Office, 1973.) 35 cents. [111

Environmental Education at University Level: Trends and Data. Pp. 320. (Paris Cedex: Centre for Educational Research and Innovation, 1973.) 30 francs; £2.66; $7.50, DM 18.80. [111

South African Sugar Association. Annual Report of the Experiment Station for 1972/1973. Pp. 52. (Mount Edgecombe, Natal: South African Sugar Association, 1973.) [111

The Major Groups of Palms and Their Distribution. By Harold E. Moore, Jr. Pp. 115. (Reprinted from *Gentes Herbarium* 11(2), 1973.) Pp. 115. (Ithaca, New York: L. H. Bailey Hortorium of the New York State College of Agriculture and Life Sciences, Cornell University, 1973.) [111

World Health Organization—International Agency for Research on Cancer. Annual Report, 1972/1973. Pp. 133. (Lyon, France: WHI, International Agency for Research on Cancer, 1973.) [111

Scientist and Engineers for the Nuclear Age—Final Report on the Atomic Energy Commission Fellowship Programs, 1948–1973. Pp. 60. (Oak Ridge: Oak Ridge Associated Universities, 1974.) [141

European Organization for Nuclear Research—CERN. CERN 73-16: Transport: Computer Program for Designing Charged Particle Beam Transport Systems. By K. L. Brown, D. C. Carey, Ch. Iselin and F. Rothacker. Pp. vi+113. (Geneva: CERN, 1973.) [141

Health Effects of Environmental Pollution. Pp. 20. (Washington, DC: US Environmental Protection Agency, 1973.) [141

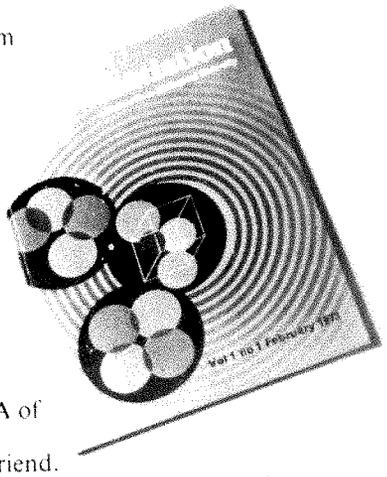
The Rising Tide: The ILO Looks at the Population Problem. Pp. 40. (Geneva: International Labour Office, 1973.) [141

United States Department of the Interior: Geological Survey. Water-Supply Paper 2029-C: Use of Finite-Difference Arrays of Observation Wells to Estimate Evapotranspiration From Ground Water in the Arkansas River Valley, Colorado. By E. P. Weeks and M. L. Sorey. Pp. iii+27. (Washington, DC: Government Printing Office, 1973.) 50 cents. [141

University of Alaska. Annual Report of the Geophysical Institute, 1972/73. Pp. 182. (Fairbanks: Geophysical Institute, University of Alaska, 1973.) [161

East African Community. Annual Report of the Freshwater Fisheries Research Organization for 1971. Pp. 56. (Jinja, Uganda: East African Freshwater Fisheries Research Organization, 1973.) 6s. [161

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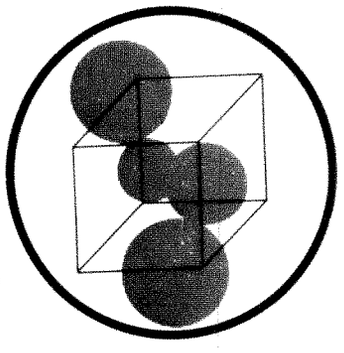
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The Personnel Officer, Australian Scientific Liaison Office, 64-78, Kingsway, London, WC2B 6BD by the 7th June, 1974.

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CHEMISTS

Two Chemists are required in the Chemistry Section to work on the chemistry of raw materials used in the brewing process.

One appointment will be made at Research Fellow (post doctoral) level for which experience in the chemistry of natural products would be a distinct advantage.

The other appointment will be made at Experimental Officer level and would be suitable for a first degree graduate whose main interest is organic chemistry. Students expecting to graduate this summer are invited to apply.

Present salary range for both appointments £1,849-£2,848 p.a.

Applications, together with the names of two referees and curriculum vitae should be sent to the Administrative Manager, Brewing Industry Research Foundation, Nutfield, Redhill to arrive not later than June 14, 1974. (1718)

THE NORTH OF SCOTLAND COLLEGE OF AGRICULTURE
ABERDEEN
POULTRY ENVIRONMENT SPECIALIST

Applications are invited for a new post in the Poultry Division of the College. Candidates should have an Honours Degree in Biology or a Biological Science together with post-graduate research experience preferably in whole animal biology. Excellent modern facilities for research and development.

Entry according to age and qualifications on scales:

Grade III—£2,328 x 16 increments to £4,061.

Grade IV—£1,584 x 7 increments to £2,148.

Forms of application and further particulars of the post (Reference Number 29/74), obtainable from the Secretary, The North of Scotland College of Agriculture, 581 King Street, Aberdeen AB9 1UD with whom applications should be lodged **not later than May 27, 1974.** (1719)

THE OPEN UNIVERSITY
BIOLOGY TECHNICIAN
(Temporary Post)

An immediate vacancy exists for a BIOLOGY TECHNICIAN (Grade III) with experience of Microbial Genetics, especially sterile techniques. He/she will assist in the preparation of experimental materials, including television and other display work, associated with a Nuffield/Open University Genetics Course. The appointment will be for three years.

Salary, based on qualifications and experience, will be within the Grade III scale: £1,650 to £1,920 per annum.

For details and application forms, please contact The Personnel Manager (BT2), The Open University, P.O. Box 75, Walton Hall, Milton Keynes MK7 6AL. (Tel: Milton Keynes 74066, extension 3068). Closing date for applications: Friday, May 24, 1974. (1788)

Glaxo

Glaxo Laboratories Limited
Technical Officer (Chromatography)
ULVERSTON

Applications are invited for a post as Technical Officer (Chromatography) in the Chemical Process Development Department at Glaxo Laboratories' bulk antibiotics plant, Ulverston, Cumbria. Experience or interest in the field of Chromatography is required to work primarily on the application of High Pressure Liquid Chromatography to problems connected with the manufacture of antibiotics. The successful candidate will form part of a well-established small group already well versed in paper, thin layer and gas chromatographic techniques. He or she will preferably have an HNC or HND in chemistry or be engaged upon such courses.

Every encouragement will be given to those wishing to continue their studies.

The Company offers good conditions of service including bonus, pension and sick-pay schemes and environmental aspects are second to none. The town of Ulverston is situated on the shores of Morecambe Bay and on the southern fringe of the Lake District National Park.

Apply in writing, quoting reference U.526, to The Personnel Officer (JE), Glaxo Laboratories Limited, Ulverston, Cumbria LA12 9DR. (1771)

BEDFORD COLLEGE
(University of London)
RESEARCH ASSISTANT
in
ZOOLOGY

Research Assistant required to work under a three year N.E.R.C. grant on respiratory physiology of annelids subjected to low oxygen tensions. Applicants should have a good Honours degree in Zoology or Biochemistry. The appointment will date from October 1, 1974. Salary £1,500 by 2/£87 to £1,674.

Application forms may be obtained from the Assistant Secretary (Personnel) (N), Bedford College, Regent's Park, London NW1 4NS (Tel. 486 4400 Ext. 312). (1793)

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY
Department of Physics
OPPORTUNITIES FOR RESEARCH IN OPTICS AND LASERS

Applications are invited for vacancies at Post-Doctoral Research Assistant and Postgraduate (M.Phil. and Ph.D. by thesis, M.Sc. by course) levels. The optics section undertakes research in laser and optical physics (including the interaction of high power laser with matter), coherent optics, optical design and visual optics. The Post Doctoral Research Assistant salary scale is from £1,929 to £3,048 plus £162 London allowance (under review).

The SRC is prepared to offer a limited number of advance course and research studentships. Support for research students and post-doctoral appointments is also available on contracts from government and industrial laboratories. Experienced researchers: students expecting to be awarded a good Honours degree in physics, electrical engineering or electronics: and graduates with relevent industrial experience can obtain further details and application forms from Professor D. J. Bradley, Optics Section, Department of Physics, Imperial College, Prince Consort Road, London SW7 2BZ. (1721)

THE EDINBURGH SCHOOL OF AGRICULTURE
RESEARCH ASSISTANT

Applications are invited for a research assistantship for work on animal behaviour under the direction of Dr. D. G. M. Wood-Gush. The work will be exclusively with pigs.

Salary: £1,500.

Further particulars and application form from the Secretary, The Edinburgh School of Agriculture, West Mains Road, Edinburgh EH9 3JG. (1786)

Avian Research

Pedigree Petfoods seeks a man or woman to take over and develop the Company's avian research programme in the broad areas of nutrition, reproductive endocrinology and toxicology. The person appointed will report direct to the Head of our Animal Studies Centre which is recognised as one of the foremost Small Animal Nutrition Centres in Europe, and will join a group of other scientists engaged in behavioural and nutritional research, establishing him/herself as the Company authority on avian matters.

Applicants should have a good primary degree in a biological subject with at least 2 years' relevant post graduate research, not necessarily in the avian field. Some practical experience in avian management would be ideal.

Starting salary will be at least £3,700 on a scale rising to £4,700. In addition we offer non-contributory pension and life assurance and generous re-location allowances. Housing prices are reasonable here in comparison with the South East and the Animal Centre is located in pleasant countryside 5 miles from our plant at Melton Mowbray yet only 35 minutes' drive from Leicester and Nottingham.



If you would like more information about this position and an application form, please telephone or write to: Elizabeth Hague, Management Recruitment Officer, Pedigree Petfoods Limited, Melton Mowbray, Leicestershire. Tel: Melton Mowbray 4141.

(1774)

National Institute for Medical Research
Mill Hill – London

GRADUATE Biologist or experienced technician required by the Division of Cytopathology to assist in work on the electron microscopy of viruses and cell membranes with particular emphasis on cryo-techniques using freeze etching equipment. Salary on scale £1533—£2199 p.a. Non-graduates with suitable experience will also be considered for this post on salary scales related to their qualifications. Please quote ref: JTO/Cyto.

GRADUATE Biochemist required to assist in research on membrane biochemistry. Biochemical experience will be an advantage. Salary on scale £1533—£2199 p.a. Please quote ref: JTO/LBTCS.

TECHNICIAN/JUNIOR TECHNICIAN, preferably with microbiological or biochemical experience, required to assist in work on mechanisms of resistance to Bacterial infections in the Laboratory of Bacterial Infectivity. Candidates with HNC or equivalent will be considered for appointment to the Technician grade, £1566 p.a.—£2418 p.a. Those who are currently following an HNC course or who have ONC will be appointed as Junior Technician, £946 (at 17)—£1521 p.a. Please quote ref: Tech/LBI.

Applicants for the above should contact P. A. Collingridge, N.I.M.R. The Ridgeway, Mill Hill, NW7 1AA, Tel: 959-3666. (1752)

The University of Leeds
DEPARTMENT OF PHYSIOLOGY
CARDIOVASCULAR UNIT

Applications are invited for a post of RESEARCH ASSISTANT. The post is financed by the Medical Research Council and previous training in neurophysiology is necessary. The appointment will be made for one year in the first instance and the salary will be on a scale rising to £2,500.

The successful candidate would work in the basic laboratories of the Unit which are fully equipped to study central nervous and reflex control of the cardiovascular system. The Unit operates its own computer facility. The problems on which the sucecssful applicant will be expected to work are within the field of central projections of cardiovascular afferent fibres to the brainstem. Informal enquiries may be made to the Cardiovascular Unit.

Forms of application and further particulars from the Registrar, The University, Leeds LS2 9JT (please quote 43/13/10). Closing date May 31, 1974. (1763)

ST. THOMAS'S HOSPITAL
MEDICAL SCHOOL
DEPARTMENT OF GYNAECOLOGY

Applications are invited for a Research Assistantship in Biochemistry in this Department. The successful candidate will join a group working on problems of oestrogen and gonadotrophin interaction and metabolism. Previous endocrine or radioimmunoassay experience is not essential but would be helpful. Starting salary up to £1,900 according to qualifications. Applications with full details and names of two referees and enquiries to: Dr. M. G. Brush, St. Thomas's Hospital Medical School, London SE1 7EH. (1736)

• UNIVERSITY OF BATH

Applications are invited for a

LECTURESHIP IN GENETICS

in the School of Biological Sciences. Persons interested in any aspect of genetics will be considered. Lecturer salary scale £1,929 to £4,548. Further particulars and application forms obtainable from Registrar (S), The University, Bath BA2 7AY, quoting ref. 74/65. Closing date for receipt of applications May 31, 1974. (1737)

INSTITUTE OF TERRESTRIAL ECOLOGY, GRANGE-OVER-SANDS, LANCASHIRE
ANALYTICAL CHEMIST

An analytical chemist (Scientific Officer) is required by the Chemical Section at Merlewood Research Station, Grange-over-Sands, Lancashire.

The work is concerned with the analysis of plant materials, soils and waters for a wide range of inorganic and organic constituents in connection with ecological and pollution studies.

Qualifications: Normally under age 27 with a pass degree, HNC, HND or equivalent in an appropriate subject. Previous laboratory experience with instrumental techniques would be an advantage.

Salary: £1,435 to £2,329. Starting salary may be above the minimum.

Superannuation arrangements.

Application forms and further particulars available from Institute of Terrestrial Ecology, Alhambra House, 27/33 Charing Cross Road, London WC2H 0AX.

Closing date: May 31, 1974. (1733)

UNIVERSITY OF ABERDEEN
RESEARCH ASSISTANTSHIPS IN BIOCHEMISTRY

Applications are invited for TWO POSTS—(i) to work with Dr. C. K. Pearson on the initiation and control of DNA synthesis in mammalian cells in tissue culture and (ii) to investigate the organisation of reiterated base sequences in eucaryotic DNA, under the supervsion of Dr. N. Hardman. Applicants should possess, or be in process of obtaining, an honours degree in Biochemistry or an allied subject. Both posts are for three years and suitably qualified applicants may register for a higher degree. Salary in range £1,479 to £1,659 (under review).

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by May 25, 1974. (1734)

UNIVERSITY OF QUEENSLAND
AUSTRALIA
LECTURER IN MATHEMATICAL STATISTICS
DEPARTMENT OF MATHEMATICS

An applicant should possess a higher degree and have research interests in any branch of statistics including probability and stochastic processes.

Salary: Within the range of $A8,698 to $A11,982 per annum.

Other Benefits: Lecturers Superannuation similar to F.S.S.U., housing assistance, study leave, and travelling and removal expenses.

Additional information and application forms are obtainable from the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF.

Applications close in London and in Brisbane on **May 27, 1974.** (1725)

AUSTRALIA

PUBLIC SERVICE OF VICTORIA
MINISTRY FOR CONSERVATION
FISHERIES AND WILDLIFE DIVISION
RESEARCH OFFICER
(THREE POSITIONS)

Yearly Salary: (a) $A6,229 minimum; $A8,981 maximum.
(b) $A9,151 minimum; $A10,674 maximum.

Note: Generally appointment will be within the salary range (a) according to qualifications and experience but applicants possessing special qualifications or extensive experience may be considered for appointment to salary range (b).

Position No. 1. Ref. No. (I/01)

Duties: Under the direction of the Officer in Charge, Freshwater Fisheries, to undertake a research programme based on the Seven Creeks system and the fish population therein. To carry out nutrition trials on both native and introduced fish species at the Snob's Creek Freshwater Fisheries Research Station and Hatchery. Other duties as directed.

Qualifications: An approved degree in Science or Agricultural Science or an equivalent qualification; research experience in an appropriate field is desirable. Experience in fisheries research and/or animal nutrition an advantage.

Note 1. The successful applicant will be required to occupy a residence within the Hatchery grounds. A rental of approximately 9% of salary a year will be charged.

Note 2. To reside at Snob's Creek Fish Hatchery. The successful applicant will be stationed at the Arthur Rylah Environmental Research Institute, Heidelberg, for approximately six months as from the commencement of duty.

Position No. 2. Ref. No. (I/02)

Duties: Under the direction of the Officer in Charge, Freshwater Fisheries, to undertake research into the freshwater eel stocks and to develop a policy for the commercial exploitation of these fish. To undertake other research programmes into the biology of selected freshwater fish inhabiting coastal streams. Other duties as directed.

Qualifications: An approved degree in Science or Agricultural Science or an equivalent qualification; research experience in an appropriate field is desirable. Experience in eel fisheries including intensive culture, and/or population dynamics of fish stocks an advantage.

Position No. 3. Ref. No. (I/03)

Duties: Under the direction of the Officer in Charge, Freshwater Fisheries to undertake research studies into the basic biology of selected freshwater fish species. Other duties as directed.

Qualifications: An Approved Degree in Science or Agricultural Science or an equivalent qualification; research experience in an appropriate field is desirable; specialist training or experience in physiology an advantage.

Note 1. The successful applicants for position Nos. 2 and 3 will be stationed at 123 Brown Street, Heidelberg.

Note 2. Separate applications must be submitted for these positions.

Applications quoting appropriate reference number should be addressed to the Secretary, Public Service Board of Victoria, State Public Offices, No. 1 Treasury Place, Melbourne, 3002, Australia, by not later than 9.30 a.m. on Wednesday, 5th June, 1974, together with statements of experience and qualifications and date and place of birth. (1743)

THE OPEN UNIVERSITY
RESEARCH ASSISTANT IN BIOLOGY

Applications are invited for a **Research Assistantship in Biology**, tenable from June 1, 1974 or as soon as possible thereafter, to work with Dr. Gary Dutton of the Brain Reesarch Group on **glycoprotein metabolism in the brain.** Applicants should possess a 1st or good 2nd class honours degree in biochemistry or have equivalent neurochemical experience. The appointment will be for one year initially with the possibility of further renewal.

Salary scale: £1,323 to £2,058 per annum with F.S.S.U. benefits.

Further details are available from The Personnel Manager (RB2), The Open University, P.O. Box 75, Walton Hall, Milton Keynes, MK7 6AL. (Tel: Milton Keynes 74066 extension 3068). Closing date for applications: **Friday, May 24, 1974.** (1758)

CENTRAL PUBLIC HEALTH LABORATORY

TECHNICIAN

required by the Salmonella and Shigella Reference Laboratory. Suitable qualifications would be a relevant scientific degree; A.I.M.L.T. or H.N.C. in Medical Laboratory Sciences. This is the National Salmonella and Shigella Centre for Great Britain and the Commonwealth and offers training in the most advanced methods of Salmonella and Shigella identification.

Written applications with full details of age, experience and qualifications to the Personnel Officer, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT. (1730)

A YOUNG SCIENCE GRADUATE

with a good honours degree is needed for work on Hospital infection. Laboratory and field studies are nivolved, including the development of tracer techniques using fluorescent and other particles. The problems involved are largely physical so that a basic training in the physical sciences may be the most appropriate, but a wide scientific outlook coupled with some mechanical aptitude and ability is more important than the precise course. F.S.S.U. provision and salary according to age, qualifications and experience on M.R.C. scale rising to £3,378 plus London Allowance.

Applications to Dr. O. M. Lidwell, Cross Infection Reference Laboratory, Public Health Laboratory, Colindale Avenue, London NW9 5HT. (1764)

UNIVERSITY OF EDINBURGH
DEPARTMENT OF FORESTRY AND NATURAL RESOURCES

A.R.C. RESEARCH ASSOCIATE

Applications are invited from candidates with three or more years research experience for the position of Research Associate in the Department of Forestry and Natural Resources, University of Edinburgh, to work on the effects of wind on the performance of forage grass species. The project is supported by a three-year grant from the Agricultural Research Council. The appointee will work both in the field and with a controlled environment wind tunnel, being concerned with plant water relations and photosynthesis. Suitably relevant research experience and ability to work in a team within a broadly based Department are essential.

Salary will be according to age and qualifications on the following scale: £1,929 by £129 to £2,058 by £165 to £2,223 per annum (under review), with F.S.S.U. benefits.

Further particulars on request to the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh EH8 9YL, to whom applications should be sent not later than May 30, 1974, giing full details of qualifications and experience together with the names of two academic referees. Please quote reference 5016. (1760)

Physiological Measurement Technician

£1332-£1803

To assist consultant anaesthetists and senior registrars at St George's Hospital, Tooting, London S.W.17 with research projects in General and Dental Anaesthesia, including pulmonary function tests, blood gas and gas analysis, circulatory and respiratory monitoring. Duties include preparation, setting up, calibration and checking equipment; preliminary calculations and checking results; preparation of charts and diagrams. Experience of blood gas measurement desirable, but not essential.

Facilities include Canteen, active Sports and Social Club, and Staff Health Service. The appointment will be for the duration of the research projects, i.e. until the end of January 1975. Further details and application forms from Personnel Office, The Royal Dental Hospital, 32 Leicester Square, London WC2H 7LJ. Tele: 01-930 8831 extension 11. (1720)

FOOD SCIENTIST

DEPARTMENT OF AGRICULTURE AND FISHERIES

The person appointed will advise the Department on all aspects of compositional food standards. The appointment will be made in the grade of Agricultural Inspector.

Essential: (i) 1st or 2nd class Honours University Degree in **Food Science, Dairy Science, Chemistry, Biochemistry** or **Microbiology** or an equivalent qualification.

(ii) At least five years' post-graduate experience including adequate experience of processing techniques and quality control in the food industry or in food analysis or research.

Salary: **£3,352–£4,742** (man)
£2,844–£4,221 (woman)
Entry up to maximum possible.

Age Limit: 45 years.

CLOSING DATE: 23rd MAY 1974

Application forms, etc. from Secretary, Civil Service Commission, 45 Upper O'Connell Street, Dublin 1. (1738)

ROYAL HOLLOWAY COLLEGE
(University of London)
Egham Hill, Egham, Surrey
CHEMISTRY DEPARTMENT

RESEARCH ASSISTANTSHIPS IN COMPUTER SIMULATION OF LIQUIDS

Applications are invited for two Postdoctoral Assistantships for work on the computer simulation of liquids at molecular level in a group led by Professor K. Singer. A background in statistical mechanics is desirable.

The appointments are for two years at a salary in the range £1,929 to £2,223 plus F.S.S.U.

Further details may be obtained from the Personnel Officer (N), to whom applications together with curriculum vitae and the names and addresses of 2 referees should be sent as soon as possible. (1795)

WESTFIELD COLLEGE
(University of London)
DEPARTMENT OF BOTANY AND BIOCHEMISTRY

DEMONSTRATOR

Applications are invited for this newly created post in the department, tenable from September 1, 1974. Candidates should have a good degree in Plant Sciences or Microbiology preferably with an interest in experimental, physiological or biochemical education. The main duties of the post are the organisation and supervision of undergraduate laboratory classes. The appointment will be for a period of three years, subject to annual renewal. The salary is within the range £1,404 to £2,388 plus London Allowance. Further particulars from the Personnel Officer (N), Westfield College, Kidderpore Avenue, Hampstead, London, NW3 7ST with whom applications close on June 1, 1974. (1797)

UNIVERSITY OF BIRMINGHAM
CHARLES HAYWARD CHAIR OF GERIATRIC MEDICINE

Applications are invited for the newly established Charles Hayward Chair of Geriatric Medicine in the Department of Medicine. Appointment from a date to be arranged.

Salary in the clinical professorial range, maximum £7,599.

Further particulars obtainable from the Registrar, University of Birmingham P.O. Box 363, Birmingham B15 2TT, to whom applications (12 copies: one from overseas applicants) naming 3 referees, should be sent by June 17, 1974. (1741)

UNIVERSITY OF AUCKLAND
NEW ZEALAND

PROFESSOR AND HEAD OF THE DEPARTMENT OF ELECTRICAL ENGINEERING

Applications are invited for the above-mentioned Chair; Professor A. G. Bogle assumes a Personal Chair on his election as Dean of the School of Engineering for a further term. The main fields of research being pursued at present are: antennas, surface wave propagation, radio astronomy, semiconductor device modelling, system simulation and control, active networks and medical intrumentation.

Professorial salaries are at present established within the range NZ$13,863 to $17,645 per annum having regard to the qualifications of the candidate concerned.

Superannuation is on FSSU basis. Fares and other removal expenses are allowed within specified limits.

Further particulars and application procedure available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close on **May 31, 1974.** (1757)

ICI in Australia

Research Leader

Animal Health

We are seeking a highly qualified and experienced scientist to head an Animal Health Research Team in our Associate Company in Australia. The laboratory is attractively situated near Melbourne.

ICI Australia has made a number of major contributions to veterinary science and is planning a substantial expansion in its animal health research effort.

The position: The Research Leader will be responsible to the Research Manager of ICI Australia Ltd. for the planning and direction of the Company's research in the area of Animal Health, particularly the development of agents for the control of animal parasites and for the regulation of animal metabolism, on a world-wide basis. The staff include chemists, biologists and biochemists and the final team will number about 40 research workers.

The Research Leader's first duty will be to build up this team with the assistance of colleagues and Personnel Department staff and he should be willing to undertake the difficult task of selecting the right people. Liaison with the parent company's extensive research organisation in the UK will be an important function and some overseas travel will be required.

Qualifications: The applicant should be a graduate in veterinary or biological science. Extensive research experience is essential and he should have proven ability in research planning and organisation. Industrial research experience would be desirable but not essential.

Age: Age is not critical. Qualifications, experience and suitability for the position are the major criteria.

Salary and Conditions: This is a senior staff position and an attractive salary will be offered. For an outstanding candidate a salary at Reader to Professorial level will be negotiated. Benefits include a contributory Staff Pension Fund, liberal recreational leave and a non-contributory medical benefits scheme. Removal and travel expenses will be met by the Company and housing assistance will be provided.

Applications in writing to:
J. Leak, Assistant Personnel Manager, ICI Ltd., Pharmaceuticals Division, Alderley House, Alderley Park, Macclesfield, Cheshire.

(1749)

THE MOTOR INDUSTRY RESEARCH ASSOCIATION

A research engineer is required in the Vehicle Safety Section for interesting and varied work connected with the role of vehicles in road accidents, including research on impact test methods, statistical aspects of road accidents and ergonomics. The successful applicant will probably have a good honours degree in mechanical engineering (or related subjects) and up to seven years' experience since leaving university. A knowledge of statistical methods is desirable.

Applications, in writing, to the Personnel Manager, The Motor Industry Research Association, Nuneaton, Warwicks, giving details of experience, qualifications and current salary and quoting MAM/JAS. (1775)

UNIVERSITY OF OTAGO

DUNEDIN, NEW ZEALAND

LECTURER OR SENIOR LECTURER IN CLINICAL BIOCHEMISTRY

Applications are invited from medical or science graduates for the position of Lecturer or Senior Lecturer in Clinical Biochemistry.

Salary, according to qualifications and experience, within the following scales:

Science Graduate: Lecturer NZ$6,753 to $8,568 per annum; Senior Lecturer NZ$8,718 to $11,139 with a bar at $10,232 per annum.

Medical Graduate: Lecturer NZ$9,172 to $13,107 with a bar at $10,836 per annum; Senior Lecturer NZ$13,485 to $15,528 per annum.

Salary scales are subject to both triennial review and cost-of-living adjustments.

Further particulars are available from the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University.

Applications close in New Zealand and London on **June 30, 1974.** (1756)

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF GENETICS

Applications are invited for a **RESEARCH ASSOCIATESHIP** to work on biometrical genetics and plant breeding.

Salary scale (under review: £1,569 to £2,223 (exceptionally £2,718) plus F.S.S.U.

Further particulars from the Assistant Registrar (S), P.O. Box 363, Birmingham B15 2TT to whom applications (3 copies) naming 3 referees by June 3, 1974. Please quote reference NN4. (1772)

AGRICULTURAL RESEARCH COUNCIL POULTRY RESEARCH CENTRE

Applications are invited for a post within the **SCIENTIFIC/HIGHER SCIENTIFIC OFFICER GRADES** in the Ethology Section of the Poultry Research Centre, for work on reproductive behaviour. The project involves a certain amount of surgical work and the person appointed should be prepared to supervise technical staff.

Candidates should have a pass degree or H.N.C. in an appropriate biological subject, but specialisation in Ethology is not a necessity.

Salary within the scales £1,435 to £2,329 or £2,221 to £2,854 per annum, with placement according to qualifications and experience. A minimum of five years' post-qualifying experience is required for appointment in the higher grade. The post is superannuable with a non-pensionable allowance to offset contributions.

Application forms, which may be obtained from the Secretary, Poultry Research Centre, King's Buildings, West Mains Road, Edinburgh EH9 3JS, should be completed and returned to him not later than May 21, 1974. (1680)

Brewing Industry Research Foundation

NUTFIELD, SURREY

BIOCHEMISTS

Two Biochemists are required for:

(a) Work on the purification and investigation of enzymes active in barley germination; and

(b) for biochemical studies of β-glucans and pentosans.

Appointments will be made as Research Fellows (post doctoral) on the salary range £1,849 to £2,848 p.a. or, for candidates with suitable post doctoral experience, as Scientists on the salary range £2,149 to £3,247 p.a.

Research Fellow appointments are for up to 3 years. Superannuation under F.S.S.U.

Detailed applications should be sent to the Administrative Manager, Brewing Industry Research Foundation, Nutfield, Redhill, Surrey, to arrive not later than May 31, 1974. (1770)

UNIVERSITY OF NEW BRUNSWICK, FREDERICTON, NEW BRUNSWICK, CANADA

Applications are invited for the following positions:

1. Assistant Professor with Ph.D. in Microbiology to teach undergraduate courses in Microbial Infection and Immunity (two-term course) and Applied Microbiology (one-term course). This position is renewable.
2. Assistant Professor with Ph.D. in Zoology to teach an undergraduate course in Experimental and Laboratory Techniques and assist in some other area of the candidate's own speciality. This position is renewable.
3. Assistant Professor with Ph.D. in Genetics to teach a one-term undergraduate course in Introductory Genetics and a one-term course in Cytogenetics. This position is for one year only, replacing a faculty member on sabbatical leave.
4. Assistant Professor with Ph.D. in Animal Physiology to teach a two-term undergraduate course in Comparative Animal Physiology and a one-term undergraduate course in Endocrinology. This position is for one year only, replacing a faculty member on sabbatical leave.
5. Assistant Professor with Ph.D. in Plant Pathology to assist in courses dealing with plant pathogens and to assist in General Biology (Botany). This position is for two years only, replacing a faculty member on leave of absence.
6. Three Instructors to assume major responsibility for laboratory instruction in one of the following areas: (i) General Biology; (ii) General Microbiology; (iii) Experimental and Laboratory Techniques.

Salaries for the above positions will be commensurate with qualifications and experience but appointments will probably be made at, or near, the floor of the ranks advertised: Assistant Professor —$12,500 per annum; Instructors—$8,000 per annum. Applications should be received by June 30, 1974; Appointments effective September 1, 1974 or earlier if possible.

Applications, including curriculum vitae and names of three referees should be sent to: Dr. M. D. B. Burt, Chairman, Department of Biology, University of New Brunswick, Fredericton, New Brunswick, Canada. (1768)

UNIVERSITY OF BIRMINGHAM BRITISH HEART FOUNDATION CHAIR OF CARDIOLOGY

Applications are invited for the British Heart Foundation Chair of Cardiology in the Department of Medicine. Appointment from a date to be arranged.

Salary in the clinical professorial range, maximum £7,599.

Further particulars obtainable from the Registrar, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, to whom applications (12 copies: one from overseas applicants) naming 3 referees, should be sent by June 17, 1974. (1742)

UNIVERSITY OF SOUTHAMPTON

FACULTY OF MEDICINE

LECTURESHIP IN HUMAN MORPHOLOGY

Applications are invited from biological graduates, preferably with a Ph.D., or from medical graduates for a teaching and research appointment in Human Morphology. Previous teaching experience is not essential; good research facilities are available in a variety of fields and experience in electron microscopy would be an advantage. Salary scale £1,929 to £4,548 per annum (by 16 increments) subject to review. Initial salary will depend upon qualifications and experience. Enquiries should be directed to Professor D. Bulmer or Professor D. Mayor, Medical and Biological Sciences Building, Bassett Crescent East, Southampton, from whom further particulars may also be obtained.

Applications should be addressed to the Deputy Secretary (Ext. 2804), The University, Southampton SO9 4NH (7 copies from applicants resident in U.K. and one from others) not later than May 31, 1974. Ref. Na. (1637)

GUY'S HOSPITAL MEDICAL SCHOOL

Paediatric Research Unit

Applications are invited for first or second year postgraduate research assistant to assist with biochemical research on human biochemical genetic disorders.

Applications should be sent to the Administrative Secretary, Paediatric Research Unit, Guy's Hospital Medical School, London Bridge, SE1 9RT. (1744)

SOUTH AFRICAN SUGAR ASSOCIATION EXPERIMENT STATION
Mount Edgecombe, Natal
Republic of South Africa

AGRICULTURAL ADVISORY CHEMIST

Applications are invited for the permanent post of Agricultural Advisory Chemist at the South African Sugar Association Experiment Station at Mount Edgecombe, Natal, Republic of South Africa. Applicants should have at least a B.Sc. degree with Chemistry as a major subject and some agricultural training. Post-graduate experience in soil and plant analysis will be a particular recommendation. The successful candidate will be required to supervise a large chemical services laboratory, and to prepare fertilizer recommendations based on soil and leaf analyses for sugarcane growers. In addition the Advisory Chemist will be expected to study methods of assessing crop nutrient requirements, and to participate in various co-ordinated research projects.

The commencing salary will be dependent upon qualifications and experience, and would be classified in one of the following grades:—

Grade 4: R422—626/month.

Grade 5: R496—755/month.

Application forms, a list of benefits (which include a 10% annual bonus) and conditions of service are obtainable from:

South African Sugar Association,
Fountain House,
125-135 Fenchurch Street,
London EC3M 5EH,
England.

Completed forms, together with curriculum vitae, should be air-mailed directly to:

The Director,
S.A.S.A. Experiment Station,
P.O. Mount Edgecombe, 4300,
Natal,
Republic of South Africa. (1717)

THE WELSH NATIONAL SCHOOL OF MEDICINE
(University of Wales)
DEPARTMENT OF PATHOLOGY

Applications are invited for a

RESEARCH ASSISTANT

for a period of two years, to join a team supported by the M.R.C. Salary £1,278 to £1,980. The appointment entails research on lymphocyte function in a variety of diseases, notably Sarcoidosis. Excellent facilities available in this new teaching centre.

Application forms available from the Registrar, The Welsh National School of Medicine, Heath Park, Cardiff, CF4 4XN to whom they should be returned within two weeks of the appearance of this advertisement. (1745)

THE EDINBURGH SCHOOL OF AGRICULTURE
POST-DOCTORAL RESEARCH ASSISTANTSHIP

Applications are invited for a research assistantship in the Department of Agricultural Biochemistry for work on embryo development in higher plants. Candidates should be qualified in biochemistry or a related discipline.

The appointment, financed by the A.R.C. (salary scale £2,221 to £2,413, with F.S.S.U. benefits), is tenable for three years from October 1, 1974, and will be associated with a small group working on seed development and maturation.

Further details and application form from the Secretary, Edinburgh School of Agriculture, West Mains Road, Edinburgh EH9 3JG (1683).

Glaxo

Clinical Trials Officer

An opportunity has arisen for a male or female pharmacist, or graduate in biological science, to join the clinical trial section of the Medical Department. Candidates, aged 24-30 years, must have a pleasant personality and be interested in clinical pharmacology and therapeutics. They will be expected to show initiative and enthusiasm and to aid this, the post offers a stimulating and varied environment as a member of a team involved in the evaluation of new and existing drugs in human disease. It is essential that they can communicate confidently with members of the medical profession, because to initiate and co-ordinate clinical trials, discussions are held with physicians and academic research workers. The successful applicant will, therefore, be playing a role in the battle against morbidity if not mortality. Some travel within the UK will be involved.

The Company operates excellent conditions of employment, including pension and bonus schemes. Please write giving brief details and quoting reference U.776 to the Personnel Officer (JSP), Glaxo Laboratories Limited, Greenford, Middlesex. Telephone: 01-422 3434. (1771)

Pathologist

Shell Research Limited, Sittingbourne, Kent, have a vacancy in the Pathology Division of their Tunstall Toxicological Laboratory for a medical or veterinary graduate. You will be a member of a multidisciplinary team of scientists involved with the investigations of the toxicology of chemicals. You should have a strong interest or experience in experimental pathology. Should you have post graduate experience in pathology this would be an advantage but is not considered to be an essential requirement. The level of appointment will depend on background and experience and the salary offered will be competitive. Please write or telephone for an application form to Shell Research Limited, Recruitment Division (N), PNEL/34, Shell Centre, London SE1 7NA 01-934 2948.

Shell

(1801)

Department of the Environment
Princes Risborough Laboratory

Information Scientist

■ **Take charge of library** ■ **Provide documentary and information services for laboratory staff working in field of timber and timber products for building and construction.**
☐ Degree/HNC or equivalent in science or engineering ☐ 5 years' post-qualifying experience ☐ Information science qualification and linguistic ability an advantage ☐ Age normally under 30 ☐ Appointment as Higher Scientific Officer (£2221-£2854 under review) ☐ Ref: SA/57/HA.
☐ Application forms (for return by 3 June 1974) from Establishment Office, Building Research Establishment, Princes Risborough Laboratory, Princes Risborough, Aylesbury, Bucks, HP17 9PX.

Centre for Overseas Pest Research
London

Entomologist

■ **Study insect migration** ■ **Population dynamics of migrant insects** ■ **Long overseas assignments.**
☐ 1st/2nd hons degree in scientific subject ☐ Interest in Ecology and experience of tropical pests desirable ☐ Age under 32 ☐ Appointment as Senior Scientific Officer (£2973-£4070), Higher Scientific Officer (£2396-£3029) or Scientific Officer (£1610-£2504) according to age, qualifications and experience ☐ Salaries under review ☐ Ref: SA/15/JA.
☐ Application forms (for return by 31 May 1974) from Administrative Secretary, Centre for Overseas Pest Research, College House, Wrights Lane, London W8 5SJ.

Ministry of Agriculture, Fisheries and Food
Food Science Division, Norwich

Chemist

■ **Join team investigating reactions of sulphite with food components** ■ **Study effects of conditions during processing, distribution and culinary preparation of foods** ■ **Development of analytical methods for measurement of sulphite products.**
☐ 1st/2nd hons degree, or equivalent, in chemical subject ☐ At least 2 years' postgraduate experience, preferably in identification and measurement methods ☐ Age under 30 ☐ Appointment as Higher Scientific Officer (£2221-£2854 under review) ☐ Ref: SB/7/AE.
☐ Application forms (for return by 31 May 1974) from Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, telephone Basingstoke 29222 ext. 500 or London 01-839 1992 (24 hour answering service).

Science group
CIVIL SERVICE

(1811)

THE UNIVERSITY OF ASTON IN BIRMINGHAM
DEPARTMENT OF PHARMACY

Applications are invited from good honours graduates with a special interest in Physiology and/or Pharmacology for the post of RESEARCH ASSISTANT to work with the Professor of Pharmacology. The post which will be for a period of three years is supported by a Medical Research Council grant to Professor C. B. Ferry and will be subject to M.R.C. regulations.

The project is concerned with the pharmacology of normal and disordered mamalian neuromuscular transmission.

Salary will be within the range £1,206 to £1,869 per annum. Preliminary enquiries may be made to Professor C. B. Ferry. Requests for application forms (which should be returned within 14 days of the appearance of this advertisement) should be sent, preferably on a postcard, quoting Ref. No. 971/ to: Staff Officer, University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET. (1784)

UNIVERSITY OF BRISTOL
SCHOOL OF CHEMISTRY
POSTDOCTORAL RESEARCH ASSISTANTSHIP

Applications are invited for a S.R.C. Postdoctoral Research Assistantship to work on the calculation of potential energy surfaces of electronically excited molecules in collaboration with Professor R. N. Dixon and Dr. G. G. Balint-Kurti. A background in the theory of electronic molecular structure or computing experience will be an advantage.

The appointment which is for one year in the first instance, with a possible extension to two years, is available from October, 1974. Salary range up to £2,247 p.a.

Applications with curiculum vitae and names of two referees to Professor R. N. Dixon, School of Chemistry, Bristol University, Bristol BS8 1TS by June 15, 1974. (1782)

McGILL UNIVERSITY
MONTREAL, QUEBEC, CANADA

Applications are invited for a two year appointment at the Assistant or Associate Professor level for a biologist-physiologist with strong BIO-MATHEMATICAL interests in the Department of PHYSIOLOGY starting September 1, 1974. The department hopes to be able to extend the appointment. Responsibilities include teaching undergraduate and graduate level Science courses. Send applications with curriculum vitae and names of 3 references to:

Ms. Maria Olejar
Dept. of Physiology
3655 Drummond Street
McGill University
Montreal, Quebec, H3G 1Y6, Canada (1779)

LABORATORY TECHNICIAN

required, lady preferred, for Merchants in W.1 area. Adequately equipped laboratory. Duties include analyses of routine samples of commodities, and future research after familiarisation. Ability to write reports and work on own initiative essential. Salary £1,500 neg. Phone 01-636 7525 ex 64. (1802)

UNIVERSITY OF LONDON
CHAIR OF DERMATOLOGY AT INSTITUTE OF DERMATOLOGY

The Senate invite applications for the above Chair. Salary within the range £6,480 to £7,599 a year. Applications (10 copies) should be received not later than June 7, 1974, by the Academic Registrar (N), University of London, Senate House, London WC1E 7HU, from whom further particulars may be obtained. (1776)

UNIVERSITY OF SOUTHAMPTON
FACULTY OF MEDICINE
PHARMACOLOGY—TEMPORARY LECTURESHIPS

Applicatioins are requested from registered medical practitioners or from science graduates with appropriate qualifications for two Temporary Lectureships in Pharmacology on a full- or part-time basis for the academic year 1974/75. Experience in teaching medical students an advantage. Salary within range £3,588 to £5,091 (clinical) or £1,929 to £4,548 (non-clinical). Superannuation.

Further particulars may be obtained from the Deputy Secretary, The University, Southampton SO9 5NH, to whom applications (seven copies from U.K. applicants and one from others) should be sent not later than May 24, 1974. (1657)

UNIVERSITY OF SOUTHAMPTON
FACULTY OF MEDICINE

Applications are invited from registered medical practitioners or from science graduates with appropriate qualifications for two TEMPORARY LECTURESHIPS IN PHARMACOLOGY on a full- or part-time basis for the Academic Year 1974/75. Experience in teaching medical students an advantage. Salary within range of £3,588 to £5,091 (clinical) or £1,929 to £4,548 (non-clinical). Superannuation. Further particulars may be obtained from the Deputy Secretary, The University, Southampton SO9 5NH, to whom applications (7 copies from U.K. applicants and one from others) should be sent not later than May 24, 1974. Ref. Na. (1713)

WELSH NATIONAL SCHOOL OF MEDICINE
(UNIVERSITY OF WALES)
TENOVUS SCHOLARSHIP

Applications are invited from candidates who are about to qualify with a degree of a recognised University for a Tenovus Scholarship tenable at Velindre Hospital. Value: £750, £800, £850 for three years.

Applications to the Registrar, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN. (1783)

UNIVERSITY OF DUNDEE
DEPARTMENT OF THARMACOLOGY AND THERAPEUTICS
RESEARCH ASSISTANT

Applications are invited for the post of Research Assistant available from October 1, 1974 and tenable for three years. The successful applicant may register for a Ph.D. degree and will work under the supervision of Dr. D. S. Hewick on a project relating to the role of nitroreduction as a route of drug metabolism. The net salary will be equivalent to the stipend awarded for an M.R.C. Scholarship for training in research methods.

Candidates should have, or expect to obtain this year, a first or upper second class honours degree or equivalent in pharmacy, pharmacology, biochemistry or related subject.

Applications, quoting Ref. Est/30/74 and naming two referees, should be sent to The Secretary, The University, Dundee DD1 4HN by May 31, 1974. (1781)

BIO-RESEARCH LABORATORIES LTD.
CANADA

Applications are invited from qualified Veterinary Pathologists interested in joining the Department of Toxicology in the capacity of Staff Pathologist. Responsibilities will include technical supervision of Autopsy Room and Histology Laboratory personnel and activities. Principally, however, the successful candidate will be responsible for the evaluation of histological specimen material arising out of toxicological research in a variety of laboratory animal species and for the preparation of reports appropriate to the research in question. Salary will be commensurate with qualifications and experience.

Bio-Research Laboratories Ltd. is a Canadian research company operating in the life sciences field. Applications, curricula vitarum and requests for further details may be directed to Dr. C. I. Chappel, Director, Bio-Research Laboratories Ltd., 265 Hymus Blvd, Pointe Claire, Quebec, Canada H9R 1G6. (1799)

UNIVERSITY OF LIVERPOOL
APPLIED ECOLOGIST

Applications are invited for the post of Environmental Advisory Officer in the Environmental rehabilitation Unit of the Department of Botany, providing ecological advice to local authorities and industry. Applicants should possess a good honours degree and postgraduate experience in plant ecology, soil science or agronomy. They should have interest, and preferably experience, in the application of ecological principles to solving practical problems of the environment. Salary £1,959 to £2,178 per annum according to qualifications and experience. Applications should be received not later than June 1, 1974 by The Registrar, The University, P.O. Box 147, Liverpool L69 3BX from whom further particulars may be obtained. Quote ref. RV/N/276007. (1800)

UNIVERSITY COLLEGE LONDON

Applications are invited for the following positions:

1. **RESEARCH BIOCHEMIST** with postdoctoral or equivalent experience.
2. **RESEARCH STUDENTS**, preferably biochemists, one a C.A.S.E. award.
3. **RESEARCH TECHNICIAN** (Grade 3).

The successful applicants will join the interdisciplinary Enzyme Technology Group which has recently been awarded £90,000 by the Science Research Council for a three-year programme on the Technology of Lipophilic Enzymes. The fundamental biochemical aspects of this project will be particularly challenging.

Applications should be made to the Secretary (N), Enzyme Technology Group, Department of Chemical Engineering, Torrington Place, London WC1E 7JE. (1806)

QUEEN MARY COLLEGE
(University of London)
Mile End Road, E1 4NS
POLYMER RESEARCH: EXPERIMENTAL OFFICER

An Experimental Officer with experience in polymer science or in plastics or analytical chemistry is needed to join a research group of academic and technician staff supported by an S.R.C. Major Centre Special Grant. The successful applicant will be expected to develop methods for the characterisation of chain-extended single crystal polymers produced in the Department of Physics. This will also involve collaboration with the Departments of Chemistry and Materials. Applicants should have an H.N.C. or degree in a physical science, or equivalent qualification or experience. The appointment will be for two years in the first instance on the salary scale (Technician Grade 6) £2,370 by £84 to £2,874 p.a. plus £175 p.a. London Weighting. Five weeks (in total) annual leave. Five day week. Further details of the project can be obtained from Dr. D. Bloor of the Physics Department or write to Assistant Secretary (Establishment) EO/DB. (1808)

EAST MALLING RESEARCH STATION
BIOCHEMIST FOR FRUIT RESEARCH

An experienced biochemist with a good honours degree to study changes in fruit, particularly apples, during storage.

Appointment as Higher Scientific Officer (£2,221 to £2,854) or Senior Scientific Officer (£2,798 to £3,895) according to experience. F.S.S.U. with 4½% non-pensionable allowance to offset personal contributions.

Application forms and further details from the Acting Secretary, East Malling Research Station, East Malling, Maidstone, Kent ME19 6BJ. (1809)

Find your place in British Gas

ELECTRON MICROSCOPIST

London Research Station provides a central analytical service for the Gas Industry involving the development of new methods and the use of specialised techniques. Currently we need a scientist for non-routine electron microscope work.

Duties would involve specimen preparation, microscope operation, interpretation and presentation of results; materials studied include catalysts, special alloys, abraded surfaces, corrosion products, bacteria and plastics.

Applicants should have at least H.N.C. or equivalent with a good physics background and the ability to do delicate manual work. Previous experience of either scanning or transmission electron microscopy would be an advantage.

Starting salary will be according to qualifications and experience on a scale rising to £2,661 or exceptionally £2,913.

For further details and application form, contact the Research Secretary, British Gas Corporation, London Research Station, Michael Road, London SW6 2AD, quoting reference 4000/11/N. (1810)

BRITISH GAS

South African Atomic Energy Board

Pelindaba
Theoretical Plasma Physicist

The Atomic Energy Board is starting a plasma research programme based on a medium sized Tokamak device. Applications are invited from suitably qualified theoreticians to take the part in the research programme. A Ph.D. is preferred. Interest in MHD instabilities will be a recommendation. The commencing salary will be determined according to qualifications and experience.

Fringe benefits include: Medical Benefit Scheme, Assisted Housing loan scheme, housing subsidy scheme, Pension and Group Insurance Scheme, Annual Leave Bonus, ample leave privileges and free transport between Pretoria and the Board's site. A person appointed from overseas will receive financial assistance in regard to travelling expenses and the cost of transporting his furniture and effects including a motor car. He is required to make use of all assistance given by the South African Department of Immigration, the amount received from the said Department will be deducted from the financial assistance offered by the Board. He is also required to serve the Board for two years in consideration of the financial assistance received. A non-South African appointee must undertake to apply for South African Citizenship as soon as he qualifies therefore.

Written applications, giving full details of qualifications, experience, Employer and salary, etc., should be addressed to:

South African Embassy,
Office of the Scientific Counsellor,
Chichester House,
278 High Holborn,
London WC1V 7HE.
(Telephone 01-242 9641). (1804)

ANALYST FOR PHARMACOKINETICS

DEPARTMENT OF METABOLISM AND PHARMACOKINETICS

The Huntingdon Research Centre is the recognised international leader in contract research in the life sciences. As a result of our continuing expansion, a new vacancy exists for a Graduate in our Department of Metabolism and Pharmacokinetics.

The work will mainly involve studies of the metabolic fate and pharmacokinetics of drugs. Candidates should possess an appropriate qualification and should have received formal training in pharmacokinetics, but extensive experience is not essential. Practical ability, particularly in drug analysis, is of prime importance.

This vacancy will provide an ideal opportunity to join one of the leading drug metabolism research groups in the U.K. Attractive career opportunities exist based on merit.

The Centre is located near Huntingdon within easy reach of Cambridge with good access to London by road and rail. We offer excellent working conditions together with progressive general benefits, a competitive salary and the usual pension and other fringe benefits.

Please write or telephone for an application form to Mrs. Nancy McCree, Personnel Officer, Huntingdon Research Centre, Huntingdon PE18 6ES; Tel: Woolley (STD 048 086) 431; quoting reference number 259. (1754)

THE HOSPITAL FOR SICK CHILDREN

TECHNICIAN (biochemical)

Interesting varied cardiac research. Salary: £1,200 to £1,500. Opportunity for higher degree not excluded. Application to Consultant-in-Charge, Department of Clinical Physiology, Hospital for Sick Children, London, W.C.1. (01-405 9200, ext. 126). (1751)

FELLOWSHIPS AND STUDENTSHIPS

LAURA DE SALICETO STUDENTSHIP

UNIVERSITY OF LONDON

Application is invited for the Laura de Saliceto Studentship (part-time) for the Advancement of Cancer Research, value £150 a year for not less than two years. Applications should reach the University not later than June 1, 1974. Further particulars may be obtained from the Secretary to the Scholarships Committee, University of London, Senate House, London WC1E 7HU. (1796)

UNIVERSITY OF NOTTINGHAM

RESEARCH IN ENVIRONMENTAL STUDIES

Applications are invited for a **RESEARCH STUDENTSHIP** and a **RESEARCH ASSISTANTSHIP** in the Environmental Physics Unit, Department of Physiology and Environmental Studies, School of Agriculture.

The **N.E.R.C. RESEARCH STUDENTSHIP** is to study solar and atmospheric radiation on sloping surfaces. Applicants should have, or expect to obtain, a good honours degree in Physics or Engineering. An interest in meteorology and/or biology is desirable.

The **RESEARCH ASSISTANTSHIP** supported by the A.R.C. is to investigate the effects of sulphur dioxide on the gas exchange of crop plants. Applicants should have an honours degree preferably in agricultural or biological science.

Both posts are tenable for three years. The successful applicant for the studentship will be expected to register for a higher degree; the grant is £695 p.a. plus fees. The salary for the research assistant will be on the scale £1,422 to £1,569 (under review). Alternatively a suitable applicant may register for a higher degree at an appropriate salary.

Further details of both posts and an application form may be obtained from Dr. M. H. Unsworth, Environmental Physics Unit, University of Nottingham, School of Agriculture, Sutton Bonington, Loughborough, Leics. (1748)

THE UNIVERSITY OF SHEFFIELD DEPARTMENT OF CHEMISTRY

Applications are invited from first or second class honours graduates in Chemistry or Biochemistry for an Agricultural Research Council Studentship. The appointment will be for 3 years commencing October 1, 1974 and the successful candidate will study aspects of the biosynthesis and metabolism of plant proanthocyanidins. It is expected that the person appointed will register for a higher degree, for which fees are paid. Students about to graduate in Chemistry or Biochemistry will be considered. Applications as soon as possible to Dr. E. Haslam, Reader in Organic Chemistry, Department of Chemistry, University of Sheffield, Sheffield S3 7HF. Quote Ref. R.66/G. (1761)

Commonwealth of Australia

QUEEN'S FELLOWSHIPS *in Marine Science*

To commemorate the Royal Visit to Australia in 1970, the Australian Government established the Queen's Fellowships Scheme. Under this scheme up to five fellowships may be awarded each year for full-time research by young scientists of exceptional promise and proved capacity for original work. These awards are tenable at the Australian Institute of Marine Science, Townsville, an Australian university of approved research institution normally for two years. Tenure of a Fellowship is expected to commence within nine months of the announcement of the award.

QUALIFICATIONS Queen's Fellows should have a Ph.D., or equivalent qualifications, or equivalent research or professional experience, in a discipline applicable in marine science.

Awards will, in general, be restricted to applicants who are not more than 30 years of age on the date when applications close.

STIPEND $9,500 (Australian) per annum—increased to $10,250 per annum at age 28 years.

ALLOWANCES are payable in respect of a Fellow's wife ($500 p.a.), each dependent child ($200), superannuation payments (up to 10 per cent of stipend), appropriate insurance coverage and necessary travel expenses. Host institutions are paid an allowance towards the cost of setting up the fellow and his research work.

For those taking up fellowships at Townsville or Perth a travel grant ($500 p.a.) will also be paid to assist consultation with other research workers in Australia.

APPLICATIONS Persons interested in applying for the above fellowships should obtain application forms and a statement of the conditions of award from the Secretary, Queen's Fellowships Committee, Department of Science, P.O. Box 449, Woden, A.C.T. 2606, Australia; Education Liaison Officer, Canberra House, Strand, London, WC2R 3EH, England; or The Consul General, Australian Consulate General, 636 Fifth Avenue, New York, 20, N.Y. 10020, U.S.A. Applications for the award, which will be announced in DECEMBER 1974 close at the Canberra address on 1 July 1974.

FELLOWSHIPS WYE COLLEGE

(University of London)

POSTGRADUATE AGRICULTURAL RESEARCH STUDENTSHIPS 1974

Applications are invited from graduates or from those due to graduate in agriculture, horticulture or related subjects in the summer of 1974 for Ministry of Agriculture, Fisheries and Food postgraduate studentships tenable from October 1, 1974. A first or Upper 2nd Class Honours degree will be expected.

Department of Agriculture

One award for (a) Minimum cultivation systems; or (b) Pig behaviour in relation to nutrition; or (c) Ruminant behaviour in relation to nutriton.

One award for (a) Tractor utilisation and fuel economy; or (b) Technique of dryeration for grain maize.

Department of Horticulture

One award for "Production of spikelets in the ear of cereals".

Department of Biological Sciences

One award in the field of plant breeding in relation to disease resistance.

Value of Award: £695 per annum plus appropriate allowances and expenses.

Applications to the Registrar, Wye College, Wye, Ashford, Kent TN25 5AH, as soon as possible, and in any case not later than June 15, 1974. (1724)

UNIVERSITY OF CALIFORNIA BERKELEY

POSTDOCTORAL FELLOWSHIP IN LIMNOLOGY

Position vacant immediately for a Limnologist to assist in high-quality basic research on causes of blue-green algal blooms in Clear Lake, California, and applications to control nuisance blooms. Knowledge of modern limnological techniques required. Send curriculum vitae and references to Prof. A. J. Horne, Division of Sanitary Engineering, University of California, Berkeley, CA 94720. (1769)

The University of Leeds DEPARTMENT OF PLANT SCIENCES

A N.E.R.C. RESEARCH STUDENTSHIP is available for up to 3 years from October 1, 1974 for a biology graduate with or about to obtain a good honours degree, preferably including some chemistry or biochemistry. The successful candidate will read for a Ph.D. on aspects of cellular heavy metal localisation in marine algae under the supervision of Dr. L. V. Evans.

Applications, with the names of two academic referees, to Dr. L. V. Evans, Department of Plant Sciences, The University, Leeds LS2 9JT. (1778)

UNIVERSITY OF DUNDEE
DEPARTMENT OF BIOCHEMISTRY
Applications are invited for the post of
POST-DOCTORAL RESEARCH FELLOW

to work with Dr. D. A. Stansfield in investigations of the role of phosphorylation of ribosomal proteins in the cyclic AMP mediated increase in progesterone synthesis in corpus luteum. The project, which is supported by a M.R.C. grant, is to start in October, 1974 and the position is tenable for 3 years from that date.

The person appointed will be working within a small research group currently investigating various aspects of cyclic AMP biochemistry in luteal tissue.

Salary scale £1,929 to £2,058 to £2,223 p.a.

Informal enquiries may be made to Dr. Stansfield in the Department of Biochemistry. Applications, quoting Ref. Est/34/74J and including the names of 2 referees, should reach The Secretary, The University, Dundee DD1 4HN, by May 24, 1974. (1740)

UNIVERSITY OF AUCKLAND
NEW ZEALAND
RESEARCH FELLOWSHIP
MARINE RESEARCH LABORATORY, LEIGH

Applications are invited for a Research Fellowship tenable at the Marine Research Laboratory, Leigh. The Laboratory acts as a research and advanced training facility for the Departments of Botany, Geology, Physics and Zoology. It offers rapid access to a wide variety of unspoilt coastal habitats and has a high quality experimental seawater system and facilities for direct environmental monitoring. Opportunities are particularly good for interdisciplinary environmental studies and work involving close connection between field and laboratory studies. Applicants should have a Ph.D. degree and research experience in some branch of Marine Science.

In the first instance the appointment will be for a period of three years, in the last year of which it will be reviewed by the University Council. Depending on circumstances the Fellowship could be continued for a further period of up to a maximum of five more years.

Salary will be established according to qualifications and experience within the scale for University Lecturers. The present scale for a Lecturer is NZ$6,753 per annum rising to $8,568 per annum by six annual increments.

The Research Fellow will have the opportunity to join the Government Superannuation Fund.

An allowance within approved limits will be made towards travel expenses.

Conditions of appointment and method of application are available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close on **June 16, 1974.** (1755)

UNIVERSITY OF EXETER
DEPARTMENT OF CHEMISTRY

Applications are invited for two Post-Doctoral Fellowships for work on the nature of the action of (a) glutaraldehyde and (b) penicillins on proteins. The work will involve the isolation of the reaction products by chromatography and gel filtration and the elucidation of the side-chain modifications resulting from the reactions.

The appointments will be for one year in the first instance, from October 1, 1974. (Salary: £2,118 per annum plus F.S.S.U.), and are renewable for a second year (Salary: £2,247 per annum). Applications, with the names of two referees, should be sent to Professor H. N. Rydon, Department of Chemistry, University of Exeter, Stocker Road, Exeter EX4 4QD, by July 12, 1974. Please quote **Ref. 1/12/7070** in all correspondence. (1746)

UNIVERSITY OF EXETER
N.E.R.C. RESEARCH ASSISTANTSHIPS

Applications are invited from candidates with good Honours degrees in Zoology or Biology for two Research Assistantships in the Department of Biological Sciences. The posts are each available from October 1, 1974 and are tenable for three years.

The successful applicants will be required to work under the supervision of Dr. C. R. Kennedy on the Management of the Coars Fish in a Freshwater Lake, and to register for a higher degree in this University. The investigation will be carried out in association with Mr. R. B. Troake at Slapton Ley Field Centre, and is supported by the N.E.R.C.

The Assistantships have a nett value of £695 per annum, plus fees.

Further particulars may be obtained from the Secretary of the University, Northcote House, The Queen's Drive, Exeter EX4 4QJ, to whom applications (5 copies, overseas candidates 1 copy), should be sent to arrive not later than May 30, 1974. Please quote Ref. No. 1/12/7069 in all correspondence. (1747)

THE NEW UNIVERSITY OF ULSTER
AND
DEPARTMENT OF EDUCATION FOR NORTHERN IRELAND
POSTGRADUATE STUDENTSHIPS

For the academic year 1974–75 the Department has allocated a number of quota studentships for postgraduate research leading to the degrees of M.Phil./D.Phil. and for advanced courses leading to the degrees of M.Sc./M.A.

Applications are invited in the following subject areas:

1. **M.Phil./D.Phil.**

School of Physical Sciences
Chemistry—Mathematics—Physics

School of Biological and Environmental Studies
Biology—Geography and Environmental Studies—Psychology

School of Social Sciences
Economics—Human Ecology—Social Administration—Social Anthropology and Sociology.

School of Humanities
Classics—English—French—German—History—Irish Studies—Linguistics—Philosophy—Russian—Spanish—West European Studies

Education Centre
Education

2. **M.Sc./M.A.**

M.Sc.—Economics

M.A.—Classics; Education; English; Contemporary International History; General Linguistics; Russian.

To be eligible for an award an applicant must normally be a British subject ordinarily resident in Northern Ireland and should hodl a good honours degree. Applications may be submitted in anticipation of the results of final examinations for honours degrees.

Application forms and further particulars should be obtained from The Registrar, The New University of Ulster, Coleraine, Co. Londonderry, Northern Ireland (quoting Ref. 74/55) to whom completed applications, including the names and addresses of two referees, should be returned not later than May 27, 1974. (1731)

THE UNIVERSITY OF LEEDS
DEPARTMENT OF ORGANIC CHEMISTRY

Applications are invited for the post of RESEARCH FELLOW to work in collaboration with Dr. G. A. Morrison on the synthesis of steroids of potential pharmacological value. The appointment, from October 1, 1974, will be for one year in the first instance, but may be extended. Salary scale £1,929 to £2,223 (under review), depending upon age and experience.

Forms of application and further particulars from the Registrar, The University, Leeds LS2 9JT (please quote 25/16). Applications should be received as soon as possible. (1762)

LINCOLN UNIVERSITY COLLEGE OF AGRICULTURE
NEW ZEALAND
POST-DOCTORAL FELLOWSHIP

The Council of Lincoln College invites applications for a Post-doctoral Fellowship, tenable in any of the undermentioned Departments.

Agricultural Economics and Marketing; Agricultural Engineering; Agricultural Microbiology; Animal Science; Biochemistry; Entomology; Farm Management; Horticulture; Plant Science; Range Management; Soil Science; Veterinary Science; Wool Science.

The object of the award is to contribute to the research programme at Lincoln College. In addition to carrying out research, the successful applicant will be expected to participate in limited teaching activities without further emolument.

The Fellowship shall have a total value of NZ$7,600, part of which will be available for fares. The salary portion of the emolument is taxable in New Zealand unless a double tax agreement operates between New Zealand and the country of the successful applicant. Tenure will be for one year in the first instance, but may be extended at the discretion of the Council.

Conditions of appointment giving further details of this position at Lincoln College can be obtained from the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the College in Canterbury, New Zealand.

Applications close on May 31, 1974. (1678)

ROYAL HOLLOWAY COLLEGE
(UNIVERSITY OF LONDON)
Egham Hill, Egham, Surrey
CHEMISTRY DEPARTMENT
RESEARCH STUDENTSHIP AND RESEARCH TECHNICIAN

The following research posts, supported by the S.R.C. and sunpervised by Dr. Paul Powell, are available from October 1, 1974.

1. S.R.C.(CASE) postgraduate studentship for work on synthesis of terpenoid compounds using transition metal complexes as catalysts, in collaboration with Bush Boake Allen Ltd.
2. Technician Grade 3 with experience in the routine synthesis of organic compounds to work on mechanisms of reactions of nucleophiles with organic ligands co-ordinated to transition elements. Salary £1,650 by £54 to £1,758 a year, plus superannuation.

Applications together with the names and addresses of two referees should be sent to the Personnel Officer (N) as soon as possible, from whom further details may be obtained. (1759)

THE UNIVERSITY OF LANCASTER
DEPARTMENT OF BIOLOGICAL SCIENCES

Applications are invited for two research studentships, supported by N.E.R.C.,

(a) to study the interractions between lumbricid earthworms and soil micro-organisms;

(b) to examine the effect of insecticides on the physiology of fish muscle.

Applicants are expected to have, or to obtain, a first or upper second class honours degree in a relevant subject and should be prepared to commence study in October, 1974.

Further information is available from The Administrative Officer, Department of Biological Sciences, University of Lancaster, LA1 4YQ, to whom applications, including a brief curriculum vitae and the names of two referees should be forwarded by July 15, 1974. (1735)

ULSTER COLLEGE
THE NORTHERN IRELAND POLYTECHNIC

RESEARCH STUDENTSHIPS

Applications are invited for the award of studentships tenable from 1 October 1974 for research in the following fields of study:-

CHEMISTRY
Photochemistry
Polymer Chemistry
Co-ordination Chemistry (2)
Electrochemistry

BIOLOGY
Microbiology/Bacteriology
Plant Tissue Culture

EARTH SCIENCES
Geology and Mineralogy of Ore Deposits
Geomorphology

The conditions for the award and tenure of the studentships are identical to those of the Research Councils. Further particulars and application forms are available from:-

Academic Registrar,
Ulster College,
The Northern Ireland Polytechnic,
Jordanstown,
Newtownabbey.
Co. Antrim, BT37 0QB. (1732)

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF BOTANY
S.R.C./C.A.S.E. RESEARCH STUDENTSHIP

Applications are invited from graduates with a first or upper second class Honours degree in Botany, Biochemistry or other relevant subject, or those graduating this year, for one S.R.C./C.A.S.E. Research Studentship, tenable from Otcober 1, 1974.

The research topic is isoenzyme analysis in assessing the purity of commercially important **Brassica** seeds. In addition to work in the Botany Department, seed testing will also be carried out at Asmer Seeds Ltd.

Applications, together with details of academic background and names of two referees, should be received not later than May 30, 1974 by the Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote ref. RV/N/276001. (1766)

FELLOWSHIPS
THE UNIVERSITY OF MANCHESTER
RESEARCH STUDENTSHIPS IN THEORETICAL COLLOIDS

Applications are invited for two studentships (CASE awards), tenable for up to 3 years from October 1, from candidates with good honours degrees in applied mathematics or in physics or chemistry with a background of mathematics. Successful candidates will register for Ph.D. and will work on (a) theory or absorption of polyelectrolytes; (b) colloid stability theory in hydrocarbon media. They will spend some time in the research laboratory of the co-operating industrial firm: (a) Unilevers; (b) Tioxide International (expenses paid). Applications, giving names of two referees, should be sent as soon as possible to Dr. S. Levine, Department of Mathematics, The University, Manchester M13 9PL. (1728)

AUSTRALIAN CANCER SOCIETY
CANCER RESEARCH FELLOWSHIP

The Australian Cancer Society invites applications for the Postdoctoral Research Fellowship.

The Australian Cancer Society has initiated a Research Fellowship Program. The fellowships are directed at the postdoctoral level and are intended to assist a research worker at this level to become established in Australia.

The Fellowships is tenable in any approved institute in Australia and normally is for one year.

A salary up to $8,500 p.a. is payable, together with $1,000 equipment grant and $500 travel.

Closing date for applications is the first day of August in the year of application.

Application forms and the Conditions of Award may be obtained from:

The Executive Director,
Australian Cancer Society,
P.O. Box 25,
KOGARAH, N.S.W. 2217. (1739)

UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF BIOCHEMISTRY AND DEPARTMENT OF EXPERIMENTAL PATHOLOGY
RESEARCH FELLOW/ RESEARCH ASSOCIATE

Applications are invited for a post of Research Fellow or Research Associate/Senior Research Associate. The successful applicant will be a member of a small research group working on the immunopathology of muscle under the direction of Professors S. V. Perry (Department of Biochemistry) and P. G. H. Gell (Department of Experimental Pathology). The initial aims of the research will be to isolate and characterise proteins from human muscle, study their immunochemistry and evaluate the role of antibodies to them in the aetiology and diagnosis of muscle disease. The successful applicant will undertake the biochemical part of the investigation which will be carried out in close association wth a larger research group engaged in a fundamental study of muscle biochemistry that is located in the Department of Biochemistry.

Applicants should be of post-doctoral status and have had experience in protein chemistry and fractionation procedures; experience of immunochemistry would be an advantage but not essential. The appointment is supported by a five-year grant from the Muscular Dystrophy Group of Great Britain and the salary will be in the range £1,929 to £2,223 (Research Fellow) or £1,569 to £3,048 (Research Associate/Senior Research Associate) plus F.S.S.U. Further particulars from the Assistant Registrar(S), Universoity of Birmingham, P.O. Box 363, Birmingham B15 2TT, to whom applications, including names of two referees, should be submitted by May 31, 1974. (1765)

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Further particulars and application forms may be obtained from:

The Staff Secretary,
West Theatre,
Trinity College,
Dublin 2.

who will receive completed applications until Monday, May 27, 1974. (1805)

UNIVERSITY OF ADELAIDE

Applications are invited for the following appointments:

LECTURER IN GEOGRAPHY (BIOGEOGRAPHY). Preference will be given to candidates interested in plant and animal geography, ecosystem structure and function, and ecology related to resource management. From January 1, 1975 (31.5.74).

LECTURER IN GEOGRAPHY. Preference will be given to applicants qualified to teach geomorphology and soil geography, with particular reference to Australia. From January 1, 1975. (31.5.74).

Salary scale: Lecturer $A8,698 by $A470(1) by $A469(6) to $A11,982; with superannuation on the F.S.S.U. basis. Initial salary will be fixed within the scale in accordance with qualifications and experience.

Further particulars about these posts and the conditions of appointment and other information sought will be supplied on request to the Registrar of the University, or to the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London, WC1H 0PF.

Applications should be sent in duplicate and giving the information listed in the Statement that will be supplied, to the Registrar, the University of Adelaide, North Terrace, Adelaide, South Australia, 5001. (1807)

LIVERPOOL POLYTECHNIC
DEPARTMENT OF BIOLOGY
LECTURER II IN ANIMAL ECOLOGY

Applications are invited for the post of Lecturer II in Animal Ecology. Applicants should have a special interest in Fresh Water or Marine Biology and have pursued a research problem, the level of Ph.D.

Salary £2,700 to £3,474 p.a.

Details available from Staff Office, Dept. N, Liverpool Polytechnic, Richmond House, 1 Rumford Place, Liverpool L3 9RH. (1803)

GRANTS AND SCHOLARSHIPS

DUBLIN INSTITUTE FOR ADVANCED STUDIES

SCHOOL OF COSMIC PHYSICS

The Governing Board of the School of Cosmic Physics invites applications for post-doctoral RESEARCH SCHOLARSHIPS tenable in the ASTRONOMICAL and GEOPHYSICAL Sections of the School during the academic year 1974–75 commencing October 1, 1974. A limited number of stipends within the range £1,480 to £1,680 per annum, depending on experience, will be available. Awards will be made for one year in the first instance and may be renewable for one further year.

For further particulars and Forms of Application candidates should apply immediately to **The Registrar, Dublin Institute for Advanced Studies, 10 Burlington Road, Dublin 4, Ireland.** Completed applications should reach the Registrar on or before 10th June, 1974. (1777)

UNIVERSITY OF SYDNEY

F. H. LOXTON POST-GRADUATE STUDENTSHIPS OR SCHOLARSHIPS IN CHEMICAL ENGINEERING

Applications for the abovementioned awards are invited from male graduates of any age and of any Australian or overseas university.

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Further information and application forms may be obtained from the Registrar, The University of Sydney, N.S.W. 2006, Australia, with whom applications close on **May 24, 1974.** (1727)

UNIVERSITY OF SYDNEY

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Applications are invited for the above-mentioned award from suitably qualified graduates who propose to undertake postgraduate study and research (normally for a higher degree) in the Faculty of Agriculture at the University of Sydney.

The Scholarship is tenable for one year, and may be renewed from year to year if the holder makes satisfactory progress. The present value is $A3,050 per annum, with allowances.

Applications close on **May 24, 1974** with the Registrar, University of Sydney, N.S.W. 2006, Australia, from whom application forms and full information about the Scholarships are available. (1726)

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[1]*Nature, New Biology*, **245**, 193 (1973)

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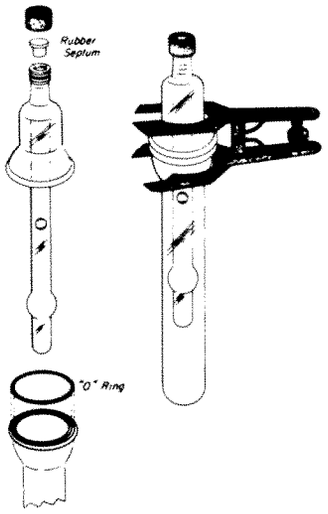


Figure 1. Apparatus for preparing diazomethane • **Z10,100-1 and Z10,102-8**

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The standard preparation of diazomethane involves adding MNNG to a cold 50% aqueous potassium hydroxide solution covered with ether, and then codistilling the diazomethane with the ether. Diazomethane has been reported to be explosive, particularly on contact with ground glass joints, and so generations of chemists have watched this codistillation with trepidation. We now offer two sets of apparatus which allow safe, more convenient generation of diazomethane either with or without distillation.

The first of these enables the preparation of diazomethane from MNNG **without distillation.**[3] Thus, MNNG is placed in the inner chamber and cold ether is introduced into the outer chamber of the apparatus (fig. 1, millimole or micromole size). 5*N* sodium hydroxide is then injected through the silicon septum onto the MNNG and diazomethane collects in the ether, ready for use. Higher diazoalkanes can be prepared from our other *N*-alkyl-*N*′-nitro-*N*-nitrosoguanidines (ethyl, propyl, butyl, are available), as can their deuterated and tritiated analogs.[4,5] Our Diazald® Kit for the generation of diazomethane from Diazald® features smooth Clear-Seal® joints which avoid the hazards associated with ground glass joints. Deuterated diazomethane is easily prepared using our Deutero Diazald® Prep Set which contains instructions and chemicals necessary for generating 50 mmoles of deuterated diazomethane.

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† Denotes molar unit

References

1) A.F. McKay, *J.Amer. Chem. Soc.*, **70**, 1974 (1948).
2) J.D. Mandell and J. Greenberg, *Biochem. Biophys. Res. Commun.*, **3**, 575 (1960); E.A. Adelberg, M. Mandel and G.C.C. Chen, *ibid.*, **18**, 788 (1965).
3) H.M. Fales, T.M. Janouni and J.F. Babashak, *Anal. Chem.*, **45**, 2302 (1973).
4) W.B. Denmore, N.D. Pritchard and N. Davidson, *J. Amer. Chem. Soc.*, **81**, 5874 (1959).
5) J.R. Campbell, *Chem. and Ind.*, 540 (1972).

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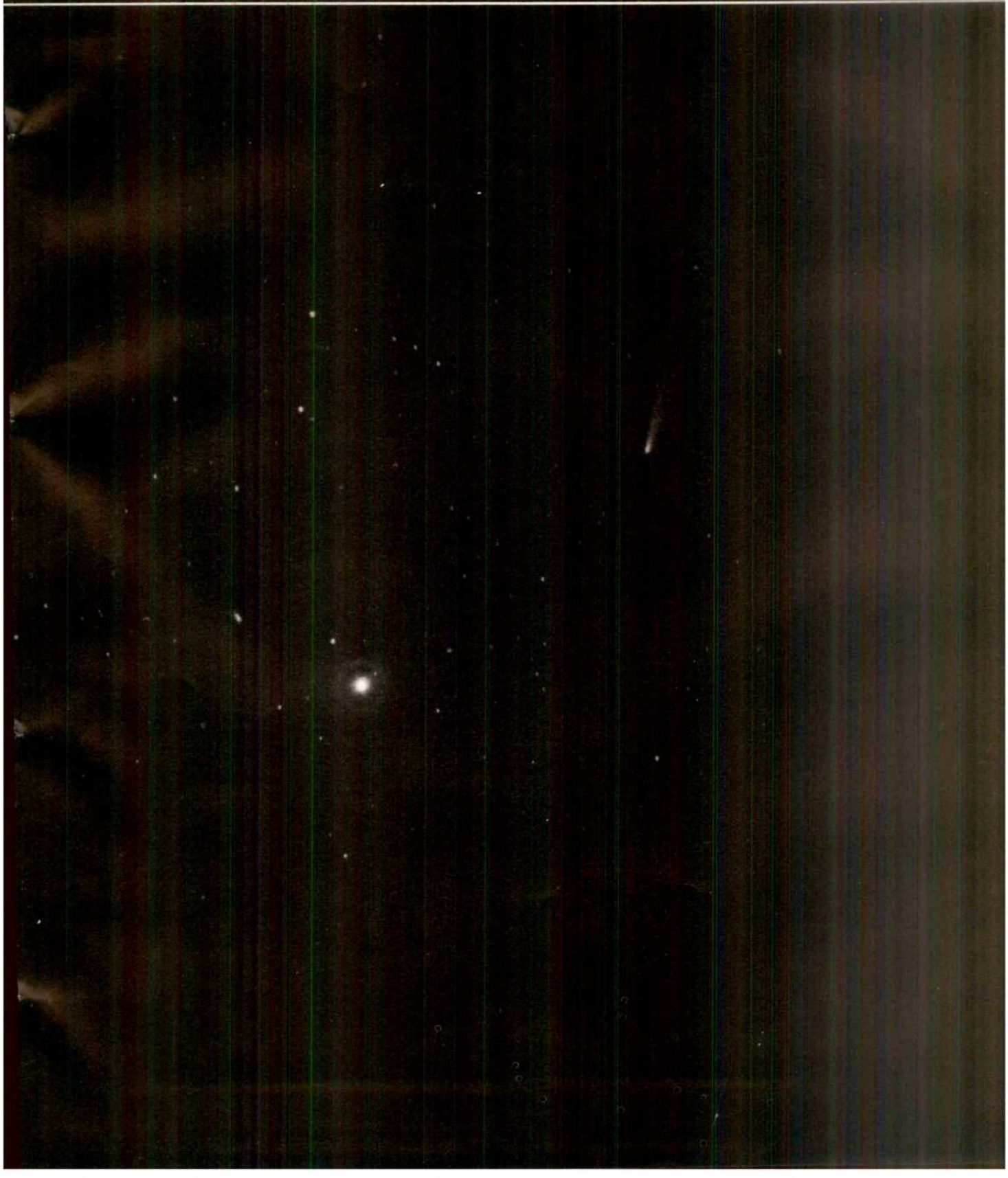
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Comet Kohoutek immersed in the Zodiacal Light, photographed from the Observatorio del Teide on January 10, 1974, by observers from the University of Manchester. The bright object to the left is Jupiter. For more about the comet see page 233.

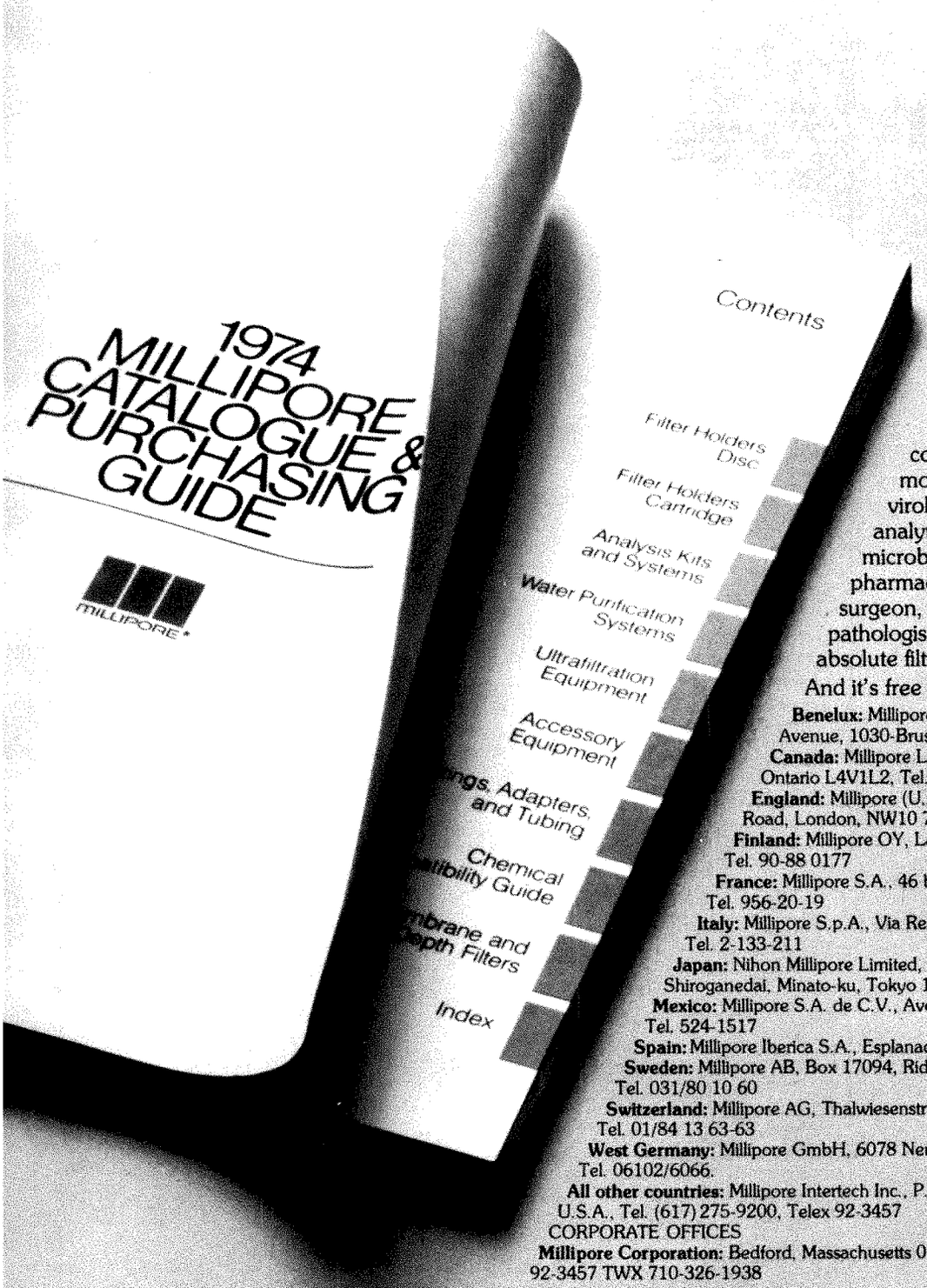
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nature
Volume 249 May 17, 1974

ESF—low budget but higher profile

A EUROPEAN SCIENCE FOUNDATION (ESF) moves closer to reality with the recent meeting in Stockholm of representatives of almost all western European countries at which, after strong competition from Dublin and Leiden, Strasbourg was chosen as the site of the foundation's offices. The general outlines of the statutes were also approved and the next meeting of administrators will be in November at which time the constitution should have been drafted. Membership now seems to be resolving itself—only Portugal (an understandable and presumably temporary absence) was missing from western Europe, Finland sent an observer and Yugoslavia has had second thoughts and dropped out. "The door is open", one delegate said, "for eastern Europe to join, and it would be my hope that within a few years the membership would be truly pan-European". Within a year a President and a Secretary-General should be installed and a secretariat of about ten is anticipated in Strasbourg.

The ESF has had an unusual history and it bears a deceptive name. It was created out of a desire to pre-empt, rather than in obvious response to an immediate need. Administrators throughout Europe saw the growing danger, a few years ago, of a deadening hand from the European Economic Community (EEC) seizing science policy and attempting to run science from Brussels. This caused alarm for two reasons: nobody had much stomach for Brussels bureaucracy and international science policy making cannot stop at the frontiers of the Common Market. The ESF thus emerged to pre-empt the EEC—it is freely admitted that the driving force was fear that the community would otherwise do something of its own. The title is somewhat of a misnomer since, at least in English-speaking countries, ESF suggests a European version of the American National Science Foundation. This it is not. Science has its more continental meaning of learning (the British Academy and the Royal Society are both involved), and it is not planned that the ESF should hand out grants for research.

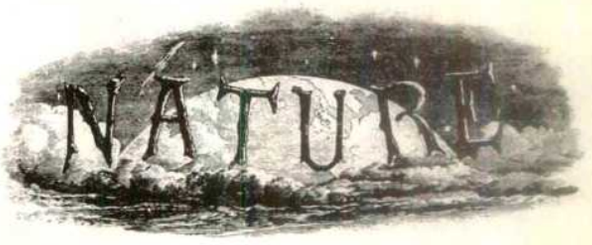
Is all of this a good thing? Scientists in general display little interest in supranational schemes, whether international or regional, until they can see concrete examples of benefit to themselves that can be obtained no other way. Multinational ventures in providing expensive facilities are obviously approved by many, but international associations to cover specialisations are more ambiguously viewed and the ESF will fall into this category. To the extent that they organise grand conferences, publish bulletins and attempt to impose standards they are often ineffectual and even disliked. Their lack of funds for the support of research writes them off for many for whom the only organisations worth respecting are fund-giving organisations. And yet they fulfil one purpose which alone justifies their existence—that of representation direct to governments. An international union may mean nothing to a scientist in an affluent and enlightened country, but to one in less happy circumstances it may mean the moral support that leads to a government grant, permission to work abroad for a spell or even such mundanities as the price of an airline ticket.

It is to this mode of working that the ESF should try to restrict itself. If it is seen to be an inexpensive operation (with a budget remaining below the half-million dollar mark), which can act quickly as a lobby for scientists when rational intervention is needed and which can act as a matrix on to which multinational schemes can be assembled but off which they can rapidly be removed, then it deserves support. If it attempts to become more grandiose, and a hunting ground for the power brokers and bureaucrats of science, it must be stopped by scientists themselves taking action.

Between now and November the administrators have one important job to do—to increase scientists' awareness of the existence of the ESF. A press release about the selection of Strasbourg had yet to reach Britain, a week after the meeting ended. When Sir Brian Flowers (Flora Europea as one wag described him) announced the selection to a dinnerful of physicists the stirring of coffee cups continued unabated. There is no plan that the articles of the constitution should be open for any public discussion before November. This last is particularly unfortunate. Although the point is made that they will in the main be boring, they must inevitably contain a description of what the foundation can and cannot do. Too often scientists exert themselves with organisations only to find themselves up against administrative answers —"we have no charter to do this", "we would like to help but our constitution forbids" and so on. It would be good to think that here was one organisation which was understood by European scientists before it began.

Nobody yet knows what the ESF will do. It will do nothing at all unless scientists can be persuaded to see its value and to use it intelligently. This means a higher profile in the next few months.

100 years ago



THE President of the Institution of Civil Engineers and Mrs. Harrison held a reception on Tuesday evening in the western gallery of the International Exhibition, at which over two thousand guests were present. In addition to the picture galleries and rooms containing machinery in motion, the west quadrant was open, and in it were placed illustrations of recent scientific inventions specially lent for the evening. With the exception of Mr. Crook's experiments showing attraction and repulsion accompanying radiation, and Tisley and Spiller's compound pendulum apparatus, all were applications of scientific inventions to the wants of life, if wicked war may be included among our wants, for Sir W. Armstrong, and other firms, sent models of appliances for the hydraulic mounting of large guns, whereby they can be placed in position with ease.

From *Nature*, **10**, 54 May 21, 1874.

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The significance of the invisible millionaire

Peter J. Smith investigates Mr. Hughes's increasing interest in the ocean floor.



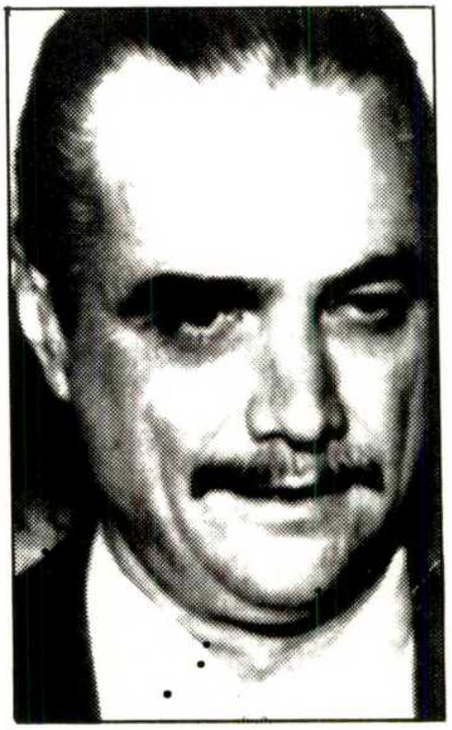
Glomar Challenger: played a part in the discovery of minerals.

HOWARD HUGHES, invisible millionaire and founder of Trans World Airlines and much else besides, is a celebrity; his comings and goings, his doings and undoings, and his deeds and alleged misdeeds seem to be a source of fascination to many in the western world. But earth scientists in general, and oceanographers in particular, should be taking a much more serious interest in the activities of Mr Hughes, some of which are likely to have severe repercussions for everyone who values the freedom to pursue research on, in and under the world's oceans. That such interest is apparently nil, or at least remains unexpressed, must be counted as one of the most remarkable examples of scientific hypocrisy since the hearts of the physicists who had worked on the Manhattan Project bled over the annihilation of Hiroshima.

The problem arises because Mr Hughes has recently sent to sea the most advanced marine mining vessel in the world, the Glomar Explorer, designed and built in secrecy in Los Angeles by the firm of Global Marine Inc. And joining this ship off the coast of Nicaragua will be a large submersible barge constructed in equal secrecy by the Ocean Systems Division of Lockheed. Naturally, few details of these craft are available, although the submersible is known to be a 90 m x 30 m roofed structure which will be held in place beneath Glomar Explorer by docking legs and tubular stability controls 45-m long. A steel pipe passing through the ship's bottom will then be connected by divers to a dredgehead 15 m wide on the barge, whence a dredge pipe weighing about 4×10^6 kg will be lowered several thousand metres to the ocean floor. At the bottom of the dredge pipe will be equipment to skim the material off the surface of the ocean floor rather like a giant vacuum cleaner.

The object of this paraphernalia is, of course, to collect the copper, manganese, nickel and cobalt-rich nodules which lie on the floor of the Pacific; and although the Hughes equipment is still at the trial stage, this particular project is believed to be the most advanced of several undergoing development with similar aims. The rewards of success are likely to be high, which is presumably why Mr Hughes has been willing to invest so much hard cash in what seems at first sight to be such a risky venture. But the rewards of being first in the field will be what a rival of Mr Hughes has described as "phenomenal", for first-comers will have the joy of being able to undercut (some believe drastically) the high and ever-rising world prices for minerals.

Hughes: first in the field



It must be recognised, however, that any success that Mr Hughes might have in mining the ocean floor has important implications for anyone whose interest in the oceans is purely scientific. For one thing, it is already clear that Mr Hughes must have profited greatly from Global Marine's experience in the building, equipping and operating of the Glomar Challenger, the research vessel that has been used extensively by oceanographers to drill the oceanic crust in the search for evidence to support the new global tectonics. This is true not only of the technology involved but also insofar as the Glomar Challenger has played some part in the discovery of the minerals themselves. So one question which needs to be asked is whether it is right that an individual or company with purely commercial interests should benefit so directly and obviously from a project financed from public funds. The issue is an important one, even though it may not be possible in the end to reach a consensus on the right policy to adopt.

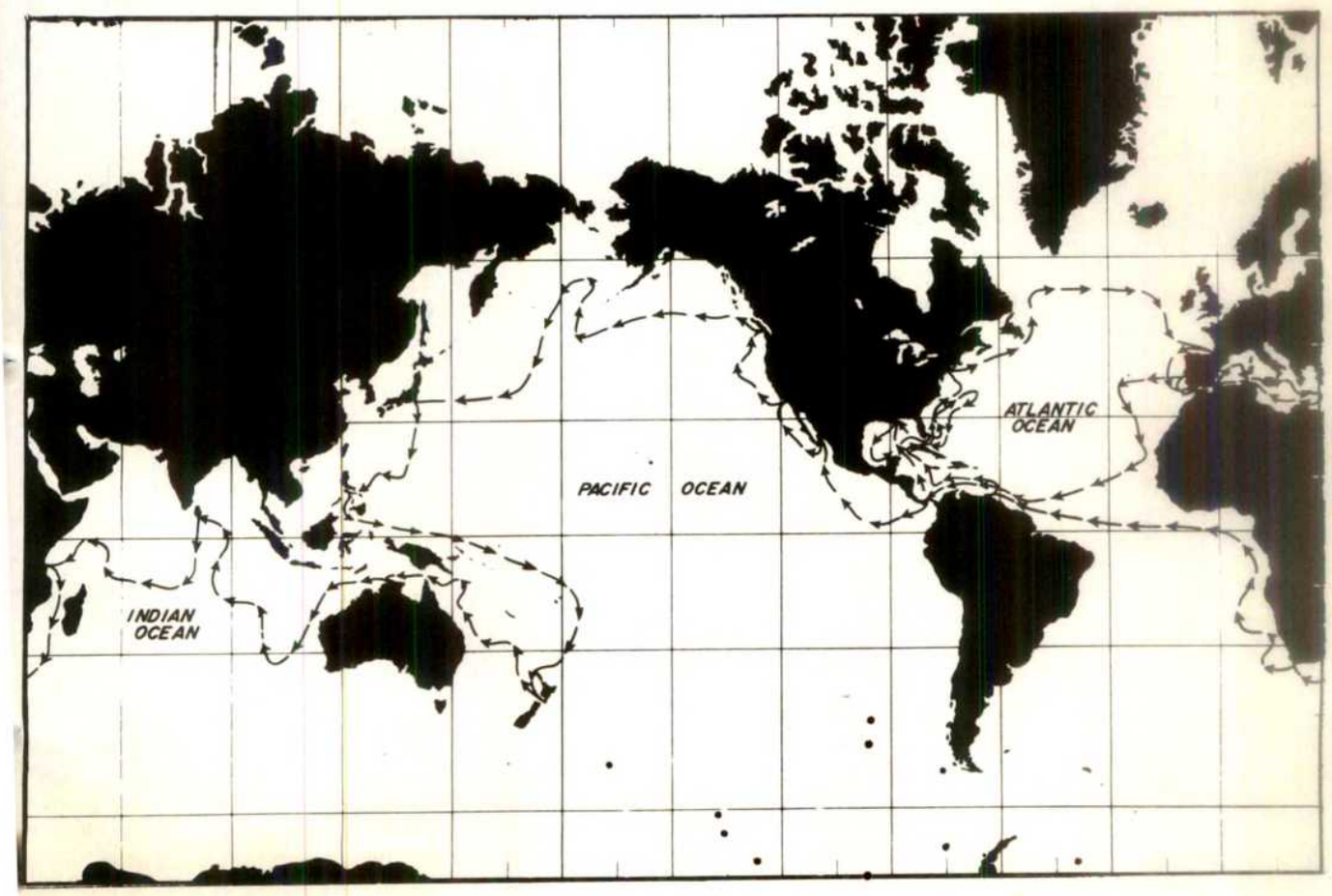
To the extent that the principal participants are American, it may also be thought to be an issue with political ramifications largely internal to the United States. But it is not quite as simple as that. The fact is that much of the mineral wealth of the ocean floor that is likely to be mined lies below what, for want of a better phrase, are called international waters; and some companies with interests in this field have been reported as saying that they will move into such waters whatever

anyone else may think, say or do. One of the practical advantages of so doing, of course, is that profits will be enhanced by the lack of mining taxes payable. But more important, any such move into international waters by a private company, or even by a single nation for that matter, would be contrary to a United Nations resolution declaring the riches of the ocean a common heritage to be shared by all mankind. If this resolution is contravened, and if the forthcoming Law of the Sea Conference fails to agree on a policy to control ocean exploration for the common good (or even if the conference agrees a policy which is later contravened), then as Lord Ritchie-Calder has pointed out, "it isn't hard to see armed conflicts between nations as a result". Moreover, Ritchie-Calder notes that "Hughes may have found a new place to hide—the bottom of the sea—but he won't long be there alone. These industrial privateers are going in for a system much like opencast [strip] mining. Some will do their primary refining at sea, dumping acid and alkaline wastes and tailings overboard. Nobody knows what effect this will have on ocean ecology".

None of this may come to pass, of course; but it affects earth scientists and oceanographers nonetheless, simply because it is possible. And this is where the hypocrisy comes in. During the past year or so several oceanographers have gone out of their way to deplore publicly the threat to basic research from ever expanding national claims to sovereignty over what are now regarded as international waters. The threat is real, but those writers who publicise it disingenuously avoid saying anything about why it exists. One of the reasons is precisely the fear and suspicion of being exploited by more powerful nations—fear and suspicion which are fed by the activities of Howard Hughes and those with similar aims. Oceanographers, and oceanographers in the United States in particular, really should not underestimate the strength of feeling on this issue, not only in the so-called underdeveloped countries but also in those industrialised nations unable to afford the technology for undersea mining.

The Glomar Challenger has played some part in the discovery of the minerals themselves. So one question which needs to be asked is whether it is right that an individual or company with purely commercial interests should benefit so directly and obviously from a project financed from public funds.

There is no doubt a real danger that certain countries will seek to prevent or restrict basic research work by outsiders within several hundred kilometres of their shores, and this may be a blow to science. But if this is to be avoided, these oceonographers who deplore the danger must be equally outspoken against those who feed the fear of exploitation; and the earth science community as a whole must make it clear where it stands on the "common good" resolution passed by the United Nations.



OCEAN drilling routes. For 30 months during the extension period of the Deep Sea Drilling Project, the Glomar Challenger followed the routes indicated below in three oceans and the Mediterranean Sea. The extension drilling was planned for 15 legs of duration two months each, starting in the Gulf of Mexico in February 1970. Other legs followed in the Atlantic Ocean, Mediterranean Sea, the Pacific Ocean, Indian Ocean and ending again in the Atlantic Ocean. These routes followed closely areas expected to yield information concerning the interactions of the movements of continental and oceanic crustal areas. Scripps Institution of Oceanography was managing institution for the project under contract to the National Science Foundation. Builders of the vessel were Global Marine Inc., of Los Angeles.

international news

Concerted criticism of breeder reactors

Colin Norman, Washington

DURING the past couple of weeks, the Atomic Energy Commission (AEC) has been taking a savage public battering over its plans to develop the liquid metal fast breeder reactor (LMFBR) as the keystone to the entire nuclear power programme in the 1990s and beyond. Charges of biased statements, suppression of information, outright deception and other underhand dealings, which are not unusual in Washington these days, have been levelled at the AEC while it has been scrambling to meet a court-ordered deadline of June 14 to produce a final detailed analysis of the environmental, economic and social implications of the LMFBR programme.

Although the AEC is by now used to impassioned attacks by environmentalists and nuclear critics, many of whom are bent not just on stopping the LMFBR programme but also on halting the rest of the nuclear power industry, it may have a more difficult time in defending itself against a particularly devastating critique which was delivered early last week, for it came not from avowed nuclear critics but from another agency in the federal government—the Environmental Protection Agency (EPA).

It is not difficult to see why the LMFBR programme has raised passions in the United States, for in the words of Barry Commoner, the outspoken environmentalist and leader of an organisation called the Scientists Institute for Public Information (SIPI), the country is now "at a crossroads" in its energy policies. A decision to push ahead rapidly with the development of the LMFBR would lock the United States into a nuclear-dominated energy supply programme if for no other reason than the huge cost that the LMFBR would entail. On the other hand, if nuclear critics can nip the LMFBR programme in the bud, they argue that the federal government would be forced to pay more attention to the development of renewable energy resources and energy conservation. The LMFBR has thus become a rallying point for a powerful coalition of environmentalist and scientific groups.

The debate is centred on a landmark decision of the federal appeals court which last year forced the AEC to obey the dictates of the National Environmental Policy Act (NEPA) by producing a broad and detailed analysis of the environmental impact of the commercial application of several hundred LMFBRs. The decision forced the AEC to examine the advantages and disadvantages of the entire LMFBR programme in relation to other methods of producing energy, and the impact statement should therefore form the basis for a public examination of the United States energy policies.

The AEC produced a draft of the NEPA impact statement on March 14 and it is legally empowered to put the statement into final form by June 14. It is the draft statement which is the basis of the AEC's public mauling. The Natural Resources Defense Council (NRDC), a public interest law group, has charged that the draft statement is "a deliberate attempt to mislead the public" and has demanded that it be withdrawn and rewritten. Similarly, the SIPI has castigated the AEC for producing an impact statement which is "so grossly inadequate as to prevent rather than enhance public discussion of the AEC decision to proceed with the programme". And finally, although using rather more restrained language, the EPA has given the AEC's draft statement its lowest possible rating and said that it "does not adequately detail the potential environmental impact" of the programme. In fact, the EPA said that the document was so deficient that it does not provide enough information to allow anybody "to reach the conclusion that the present LMFBR programme is preferable to other energy options".

The EPA's critique has placed the AEC in an unenviable position, for it has hit a number of the most sensitive points in the AEC's justification for the LMFBR programme. If the AEC does alter its statement accordingly, a number of its chief arguments could well disappear, whereas if it ignores the EPA's comments it will lay itself wide open to a court suit by environmentalist groups.

One of the AEC's chief justifications for the LMFBR programme is that breeder reactors are much more efficient than conventional light water reactors (LWRs) in their use of scarce uranium resources. The LMFBR can take uranium-238, which is unsuitable for use in LWRs, and convert it to plutonium which can then be used to fuel both conventional and breeder reactors—it breeds fuel, in other words. Since the AEC predicts that the United States has only enough uranium left to fuel conventional reactors for another 25–50 years, this characteristic of the breeder reactor will be essential for stretching out uranium resources, and it will make the LMFBR the keystone of the nuclear power programme.

The AEC's draft statement estimated that if all goes well the breeder reactor will be introduced in 1987, that 400 of them will be operating by the year 2000 and producing 400,000 MW of electricity and that by the year 2020 LMFBRs will be generating 2.2 million MW. The commission argued that there is no other technology on the horizon which seems capable of supplying that much energy and that to abandon the LMFBR programme now could well plunge the United States into a severe energy crisis towards the end of the century.

The AEC paints this picture of what will happen if the LMFBR is not introduced: as uranium resources dwindle, the cost of mining uranium ore will increase, and this in turn will increase the cost of generating electricity by LWRs and drive up electricity prices. A central theme is that the later the breeder is introduced, the smaller will be the benefits it produces, and therefore a strong commitment must be made as early as possible to the LMFBR programme.

But the NRDC has pulled the AEC's cost-benefit analysis to pieces, suggesting that it is founded on dubious assumptions, unrealistic projections and misleading accounting procedures. And the EPA has backed up most of the NRDC's complaints, suggesting that if the cost-benefit analysis is corrected, it would "preclude acceptance at this time of the AEC contention that deferral of the present LMFBR programme would be intolerably costly". The critique goes on to suggest that there is no reason to rush into the LMFBR programme because "there is sufficient time to consider alternative uranium-saving nuclear technologies and study and resolve some of the issues of environmental risk".

Those statements and the accompany-

ing documentation in the EPA's 94-page critique of the impact statement are likely to provide environmentalist groups with powerful ammunition if they eventually go to court to get the LMFBR programme stopped and, according to one EPA source, if the AEC does amend its impact statement by incorporating some of EPA's criticisms, the economic justification for the breeder could be shot to pieces.

As for the question of plutonium toxicity, that is likely to provide a considerable headache for the AEC for a long time to come. The draft NEPA statement concludes that the entire breeder reactor programme is likely to cause only 2.2 deaths in the United States through radiation from power plants, fuel reprocessing plants and waste disposal facilities. This statement was made in spite of acknowledgments elsewhere in the AEC's document that there is a good deal of uncertainty about the health effects associated with plutonium.

One of the chief uncertainties is the question of potential damage to the lungs from tiny particles of plutonium which have been inhaled, a topic which has recently been thrust into the public spotlight by two nuclear experts working for the NRDC, Dr Arthur Tamplin and Dr Thomas Cochran. Even before the draft impact statement was published, Tamplin and Cochran launched a vigorous attack on the AEC's radiation standards, contending that they are based on an unrealistic assumption that radiation from inhaled plutonium particles is evenly distributed throughout the lungs. They argue that tissue immediately surrounding a particle of plutonium is getting a radiation dose of about 4,000 rems a year, but if the dose is averaged out over the entire lung, it amounts to only 0.003 rems per year. They therefore argue that the AEC should modify its regulations governing the release of plutonium into the atmosphere by a factor of 115,000.

Although the EPA critique takes up no position on the validity of Tamplin and Cochran's theory, it says that "a scientific basis for estimating health effects from plutonium particulates is not available at this time". The EPA therefore recommends that the AEC's final environmental impact statement should indicate what steps the commission would take if the Tamplin–Cochran thesis turns out to be correct, and it also suggests that "as a final alternative, the commission may want to examine what tradeoffs exist in delaying the implementation of a full-scale commercial LMFBR programme until the risks from particulate doses, as compared to those from a uniform lung dose, are on a more firm scientific basis so that meaningful public judgment on the risks and benefits of the programme is possible".

As for the safety of the LMFBR, the EPA critique of the draft impact statement says that the AEC has not been sufficiently qualitative in its assessment for the EPA examiners "to conclude what safety approaches will be taken in many important areas". It is suggested that the AEC should include in its final impact statement a comparison of the risks of the LMFBR and the LWRs.

In view of the serious deficiencies which have been unearthed in the AEC's draft impact statement, the EPA has recommended that AEC lawyers should go to court to get an extension of the June 14 deadline for producing the final impact statement, while the SIPI and the NRDC have suggested the more drastic step of writing another draft statement and following that up with a final document.

Whether or not the AEC sticks to the present deadline, it can be certain that its final impact statement will be challenged in the courts if it fails to meet the objections raised by EPA or other critics

Commoner lays into AEC

ONE of the most stinging criticisms of the Atomic Energy Commission's draft environmental impact statement on the liquid metal fast breeder reactor (LMFBR) programme has come from Barry Commoner, an outspoken champion of the environmentalist movement. Commoner has charged in two press conferences that the Atomic Energy Commission (AEC) has been deliberately suppressing information on the potential of solar energy in order to make its case for the breeder reactor look good.

The accusation stung, for AEC chairman Dixie Lee Ray immediately put out a statement labelling the charge "totally false" and has steadfastly maintained that the commission is not trying to suppress any information relating to the LMFBR programme.

The nub of the dispute is this. Commoner, who is chairman of the Scientists' Institute for Public Information (SIPI) maintains that the AEC's draft impact statement fails to take into account a very optimistic report on the potential for solar power which was prepared for Dr Ray last year. The report, prepared by a panel of experts which assisted Ray in putting together her recommendations for a five-year energy research and development programme, concluded that solar power technologies could be developed to produce about 21% of the electrical energy needs of the United States by the end of this century.

The AEC's draft statement, however, said that "the outlook appears to be that solar energy has little potential as an economic, major source of electricity for several decades" and it went on to suggest that "the use of solar energy will not materially reduce the need for alternative electrical energy sources in the foreseeable future". The impact statement makes no reference at all to the panel's report.

The dispute about solar energy is central to the debate about the need for the LMFBR programme because, if the panel's projections prove to be correct, solar energy would be able to supply almost as much power as the breeder reactor by the year 2000 (see Table 1). And that would shoot to pieces the AEC's contention that the breeder reactor is desperately needed to avoid an energy crunch toward the end of the century. Commoner contended in his first press conference on the matter that "the AEC may be involved in another Washington coverup —this time an attempt to cover up the Sun".

According to the SIPI, which together with the Natural Resources Defense Council brought the suit which forced the AEC to produce the impact statement on the LMFBR programme, the statement's failure to consider solar energy as a viable alternative to the breeder programme is "a gross dereliction of the AEC's duty to the demands of NEPA [the National Environmental Policy Act] and the courts".

Table 1 Summary produced by SIPI of contributions to projected United States electrical demand (year 2000)

Energy source	Generating capacity (millions kw)	Percentage of projected demand
Solar technologies		
Photovoltaic	140	7
Solar thermal	40	2
Heating and cooling of buildings	35	2
Wind	170	9
Ocean thermal	Not available	Not available
Bioconversion	25	1
Total, solar technologies	410	21
Geothermal	80	4
Conservation of electricity	236 (minimum)	12 (minimum)
Grand total	765	37
LMFBR programme	435	23

Alkali Inspectorate still at first base

Eleanor Lawrence

HER Majesty's Alkali and Clean Air Inspectorate comes under strong criticism in a recent report for its attitude to the problems of pollution control which, says the report, is now out of date and often ineffective. (*The Alkali Inspectorate*, Social Audit, 9 Poland Street, London W1V 3DG, £1).

The report, prepared by Maurice Fraenkel for Social Audit, criticises the inspectorate for not publishing the detailed data it collects and for general secrecy and non-cooperation towards members of the public and local authorities who make complaints against specific works. Also, says the report, it seems unwilling to put the maximum pressure on factories who do not comply even with its standards.

These specific criticisms stem from the way the inspectorate itself sees its role in controlling industrial pollution. In the words of the present Chief Alkali Inspector, Mr F. E. Ireland, the first Chief Inspector, Dr Angus Smith in 1863 "set the policy pattern of the inspectorate which has been followed ever since". This policy involved a sympathetic approach towards the manufacturers which included technical help towards solving their problems which was very welcome and did result in the early days of the inspectorate in a tremendous reduction of pollution. But, says the report, times have changed and this attitude may not be quite so effective nowadays and is in fact blocking the way to a more effective control of pollution.

"We like to help industry", Mr Ireland told the author of the report, "when we visit a works they are actually glad to see us." But from the outside, local authorities and members of the general public have made many complaints that the standards that the inspectorate itself lays down are often not being followed by the factories and that the inspectorate will allow works more than ample time to put their house in order even when this results in serious nuisance to local residents.

Local authorities which, through their own Public Health Inspectors, control pollution from all the factories and processes that are not registered under the Alkali Act, are one of the main critics of the Alkali Inspectorate. Some of the larger authorities have tried to break into the mutual admiration society obtaining between the inspectorate and the industries they monitor by suggesting that they should take over the inspectorate's duties, but this suggestion has been repulsed both by the inspectorate and by the industries themselves, who are seemingly unwilling to leave the inspectorate's protection.

Many of the complaints about inefficiency on the part of the inspectorate can be traced to its staffing which is described in the report as totally inadequate. Thirty-five inspectors monitor the 2,000-odd works registered under the Alkali Act and in these circumstances even the inspectorate admits it is unable to carry out the job as well as it would like.

The report concludes that despite its proud record and the undoubted technical expertise and determination of its staff, the Alkali Inspectorate is particularly ill-suited to the role now expected of it. It suggests that the simplest solution to the problem might be to transfer the responsibility for control of registered works to the local authorities, which nowadays have the technical skills needed to deal with this sort of work.

If the Alkali Inspectorate is to continue as an enforcement agency, then it needs more staff and more frequent inspections; but most of all, says Mr Fraenkel, it must be made accountable and all pollution data other than genuine industrial secrets must be published in a readily available form. The inspectorate's defence of its policy of non-publication is that the public does not understand pollution data.

Azbel's visa

WE have just received news that Professor David Azbel, one of the three Jewish intellectuals who went on hunger strike "taking a step befitting political prisoners" earlier this year (*Nature*, **248**, 2–3 (1974)) has received a visa permitting him to leave the Soviet Union. No visas have as yet been granted to his fellow strikers, Vitalii Rubin and Vladimir Galatskii.

Happy birthday, Soviet style

Vera Rich

As readers will be aware by now, the 250th Anniversary Meeting of the Soviet Academy of Sciences has been postponed. A brief note in the *Pravda* of May 6, 1974 (which appeared on page 2, the page devoted to Party and political news) announced:

"The Academy of Sciences of the USSR with the approval of the Central Committee of the Communist Party of the Soviet Union has resolved to postpone the meeting devoted to the 250th anniversary of its foundation, from May, 1974, until a later date. During this time, celebratory (anniversary) meetings of the Academies of Science in the Union Republics will be held with the participation of Soviet society. Meetings devoted to the anniversary of the Academy of Sciences of the USSR, and to problems of the better implementation of scientific achievements in practice, will also be held in scientific institutions and establishments. It is intended that scientists will speak at industrial enterprises, collective farms and state farms with the aim of popularising scientific knowledge among the workers. The Academy of Sciences will complete its preparations and the publication of fundamental scientific works in honour of the anniversary, and will organise thematic exhibitions under the slogan, 'The Achievements of Science are for the National Economy'."

In these brief words, the preparations and expectations for a grand international celebration are 'postponed'—a word which may be taken in its literal sense, but which under the Stalin bureaucracy was a frequent euphemism for 'scrapped'.

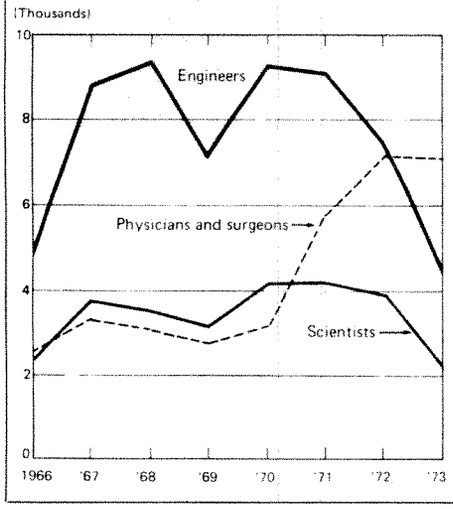
There is of course a certain suggestion of unpreparedness here—that although the resolutions to celebrate the anniversary were published last autumn, the Academy is still in a state of unreadiness. In spite of the Soviet predilection for the fulfilment of norms ahead of schedule, such lack of preparedness for conferences has been known to occur.

Even if there has been some tardiness in preparation, however, this is not the full story, particularly as the date of the 'foundation' of the Academy can be interpreted in more than one way. What it was intended to celebrate this year was the issuing of a decree by Peter I ("the Great") establishing the Academy, on January 28, 1724. Having begotten the Academy, however, Peter died and the inaugural meeting took place nearly two years later, on December 27, 1725, in the presence of Peter's widow and successor Catherine I. On at least one occasion, the 1725 date has been celebrated as the anniversary; in 1945 a 220th jubilee celebration was held and, although under war conditions a 1944 celebration would have been impossible, there was nothing in the official publications about the occasion to suggest that this was in any sense a postponement.

Looking closer at the announcement, however, it is not merely a postponement; it is a change of aim and intention. Instead of a grand international gathering (which has been 'postponed'), there are concrete plans for lectures on the grass roots of Soviet society and a stress on scientific utilitarianism.

But would it have been an international meeting at all? Would the leading scientists of the world really

Brain drain reduced to trickle



THE huge influx of scientists and engineers into the United States during the late 1960s, which became popularly known as the 'brain drain', has been sharply curtailed by restrictive immigration regulations imposed by the United States Department of Labor. At the same time, however, physicians and surgeons are flocking to the New World in record numbers.

According to statistics published by the National Science Foundation, some 6,600 scientists and engineers entered the United States between July 1972 and June 1973—the first year in which the full force of the new regulations was felt. This compares with an annual influx of from 10,300 to 13,300 between 1967 and 1972. As for physicians and surgeons, 7,100 were admitted to the United States last year, more than twice the number corresponding in the late 1960s.

Designed to protect the jobs of American citizens against competition from cheap foreign labour, the new immigration regulations specify that an alien can work in the United States only if he has been offered a job which cannot be filled by a suitably qualified indigenous worker. Since there has been considerable unemployment among scientists and engineers stemming chiefly from cutbacks in expenditure on aerospace activities in the late 1960s, non-American citizens from those professions have a tough job getting round the regulations. By the same token, however, because there is a shortage of physicians and surgeons in the United States, qualified doctors are flooding into the country in increasing numbers.

The sharpest reduction in immigrant scientists and engineers between 1972 and 1973 was in the number of qualified Asians allowed to enter.

One sad irony is that scientists and engineers from India, for example, are being refused entry to the United States although there is massive unemployment among college graduates in India. At the same time, physicians and surgeons are flooding into the United States when they are badly needed in their home countries.

have gathered in Moscow to honour the Soviet Academy as a member of the international fraternity of science. With Academician Sakharov pilloried in the Soviet press as unworthy of the dignity of a Soviet scientist? With Jewish scientists who dare to apply for a visa for Israel submitted to all forms of harassment, from dismissal from their posts to petty and often puerile vexation, such as the ostentatious shadowing for no reason but to annoy? With an "opposition" seminar planned to be held in July in the Moscow apartment of one such dismissed scientist, Professor Aleksandr Voronel? With the British Royal College of Psychiatrists demanding in no uncertain terms last November an "impartial commission of enquiry by a broadly based group of psychiatrists of international repute, drawn from a number of countries", to investigate "the reports it has received about the alleged abuse of psychiatry in the management of individuals who take up a position of political dissent" in the Soviet Union?

In such circumstances, an international gathering might well prove too explosive a celebration. But the postponement announcement does not have the air of simply waiting for the tension to cool. The return to the grass roots, to the factories and farms, has the air of a total rethinking of how the event should be celebrated. If the outside world is critical of how the Soviet Union conducts its science, the Academy is willing to go out into the highways and byways and celebrate with the workers and peasants.

Start for UK on radiation

from our Solid State Physics Correspondent

BRITAIN had its first meeting on radiation effects in electronic devices on April 24—a one-day event organised by the Institute of Physics (IOP) at Imperial College, London. The meeting is notable mainly because of the general issues it raised. In the United States there has been an annual three-day meeting on this topic since 1964, the proceedings of which run to 250 pages but ten years later, the United Kingdom has had a mere one-day meeting with no published proceedings. The organiser, Mr K. J. S. Cave of the IOP Electronics Committee, termed the lag "regrettable".

Why are the levels of interest so disparate in the two countries and is this apparent lack of interest within the United Kingdom justified? In answer to the first question, the main difference could derive from differences in the size and philosophy of defence and space programmes in the two countries, rather than from differing extents of use of radiation sources for more mundane purposes (such as nuclear reactors, processing irradiators, ion implantation, radiography or basic research). A fair proportion of the physical investigations has been done in the course of producing 'radiation-hardened' semiconductor devices for the United States Department of Defense and, to a much lesser extent, for the National Aeronautics and Space Administration (NASA). The equivalent bodies in the United Kingdom government are not such expansive customers, although a few exploratory development contracts have been let by the Ministry of Defence. The difference here lies in the fact that United States strategic missiles must withstand nuclear attack, whereas the United Kingdom government has no such requirement. Space agencies in general seem to sponsor less work in this field in proportion to their need, partly because only a certain group of satellite trajectories intersect space radiation belts and perhaps the planners presume that they can avoid these—or fudge a solution.

As to the second question, the measure of justifiable interest for physicists should be the degree of understanding of physical processes which derives from the research. It is widely agreed that the physicist who studies the effect of radiation in an electronic device usually ends up by knowing more about that device than the man who designed it. Thus, we can say that the device physicist should gain benefit from such studies. Furthermore, it is common that investigators have to improve the general level of knowledge of radiation-induced defects in solids in order to formulate improvements in the 'radiation hardness' of a device. Thus, the phycist with an interest in the defect state of solids should find such work a suitable challenge. Similar arguments can be developed for ceramics, metals, plastics and other bulk materials and it can be said that the return in basic

knowledge is rather higher than is usual for such tactical research. On this reasoning, scientists in the United Kingdom may be missing a few tricks by not doing more work on radiation effects on their own behalf.

The planners may also have missed a trick here. Developing a radiation-tolerant material or device takes a very long time. In several advanced technological developments, radiation damage may often provide the limit to the usefulness of system: examples of such systems are fusion reactors and unmanned space vehicles. To obtain true effectiveness in research on radiation effects, the lead time may need to be well over ten years and start long before engineering designs take shape. A surprising amount can be gained by research which prevents device designers from pursuing the goal of radiation resistance in the wrong way. The analogy with preventive medicine is, in fact, strong and probably again extends outside the field of intricate semiconductor devices, with which the meeting in question was concerned. Because there is not, as in the United States, a well developed 'radiation effects community', the need for both underlying and short term research in this speciality may not get pressed as strongly as it should in the United Kingdom—there is no one to give the whole picture to the top level technological planners. In fact, it is notable how the 'reactor people', the 'device people', the 'dosimetricians' and other specialists, who have a lot to gain from each other's experience, very rarely join forces either in conference or Whitehall corridor to express a common interest in two basic processes with which they are all concerned, namely displacement damage and ionisation effects. A happy exception to this is the case of the ion implantation specialists, whose techniques are useful both to reactor and device designers and whose meetings embrace both fields. This suggests that it is time for a professional body to sprout a committee which does the job of bringing together specialists.

correspondence

Referees in print

SIR,—I read the recent letter by H. Fraenkel-Conrat [*Nature*, March 1] concerning anonymity of scientific reviewers with some interest.

I wonder if many readers are acquainted with the compromise system currently being used by the *Journal of Geophysical Research (Space Physics)*, and instituted by my predecessor, W. I. Axford. Each manuscript sent out for review is accompanied by a form stating that the journal acknowledges the assistance of reviewers by naming them at the end of the paper when it finally appears in print. The reviewers are asked to indicate on the form whether they agree to being acknowledged. If they agree, the paper appears with a footnote that states: "The Editor thanks A. Smith and B. Jones for their assistance in evaluating this paper". If one of them does not agree, the statement reads: "The Editor thanks A. Smith and another referee for their assistance in evaluating this paper". If both disagree, of course, no footnote appears, but many such papers are rejected in any case.

This rather simple device has worked very well since its inception; more than 90% of our reviewers do agree to this form of acknowledgment. It not only provides some small tangible acknowledgment of the reviewer, but it also apparently reduces the incidence of irresponsible reviews that are written solely for "the joy of releasing adrenaline with anonymous impunity", as Fraenkel-Conrat describes one such effort.

To my knowledge, no other journal has yet adopted the system in spite of its demonstrated success and general acceptance among scientists in the field of space physics. It does carry with it certain corollaries that may not be acceptable to many editors—for instance, reviewers' reports must be transmitted to authors in a more or less unadulterated form, since it would hardly be fair to acknowledge a reviewer publicly for a report that had been translated and heavily doctored by the editor himself.

Yours faithfully,

GEORGE C. REID

Aeronomy Laboratory,
National Oceanic and Atmospheric Administration,
Boulder, Colorado 80302

Soviet scientists

SIR,—Professor Burhop states in *Nature*, April 12:

> " . . . In Western society too much concern for problems of peaceful coexistence, disarmament and the abolition of nuclear weapons is regarded as at best naive and at worst subversive. In Soviet society these things are highly regarded."

It is reassuring to think that the crowds assembled in Moscow's Red Square on May 1 and November 3 are there to protest against the parade of intercontinental missiles with nuclear warheads that we see in the news photographs. Their protest is made impressive by the fact that it is expressed as applause, which makes it more orderly.

Professor Burhop also says that Soviet scientists who wish to emigrate to Israel bear a resemblance to Oswald Mosley in 1939. This lacks verisimilitude. The British Fascisti (the BBF of Aldous Huxley's *Point Counterpoint*) did not try to *leave*, even though many people wished they would.

Yours faithfully,

THOMAS H. JUKES

University of California,
Berkeley, California 94720

Lead in petrol

SIR,—In the second paragraph of the article "Lead in the Environment" (*Nature*, April 5, 1974). the writer states that in 1972 about 90,000 tonnes of lead were added to petrol in the United Kingdom alone. No reference is made to the source of this information but you may wish to correct the figure quoted which is grossly in error.

The Associated Octel Co. Ltd is the sole manufacturer and supplier of lead anti-knock compounds in the United Kingdom. The total production of lead alkyl compounds in 1972 was 101,000 tonnes, 27,600 tonnes of which were supplied to refiners in the United Kingdom and Eire, the remainder being exported worldwide. Since the lead metal content of anti-knock compound is approximately 40% w/w, the total weight of lead supplied to refiners in the United Kingdom and Eire was therefore around 11,000 tonnes in 1972.

Yours faithfully,

R. G. AICKIN

The Associated Octel Co. Ltd,
London W1X 6DT

Our figure for United Kingdom lead additives in petrol should have been 9,000 tonnes. Its appearance as 90,000 tonnes was a printing error—Editor.

news and views

Selectivity of nature

THE rate of evolution, and the varieties of adaptation open to a population depend on the degree of genetic variation which exists within it. The problem of measuring this crucial parameter seemed to be resolved with the use of the electrophoretic mobility of enzymes as markers for different alleles. This technique was first used to assess allele frequencies and heterozygosity in *Drosophila pseudoobscura* (Hubby and Lewontin, *Genetics*, **54**, 577; 1966) and has since been applied to numerous other species from armadillos to thrips. The results obtained from these vastly different species have been quite consistent. Between 10% and 30% of the gene loci sampled in a species are polymorphic, and 5%–15% of those in an individual are heterozygous. This seemed to provide abundant genetic variation on which selective pressure could act, until Kimura (*Nature*, **317**, 624; 1968) proposed that the different alleles at a given locus produce the same phenotype, so that most of the genetic variation revealed by enzyme electrophoresis is probably selectively neutral.

In that case, there would be comparatively little genetic variation which could be affected by evolutionary processes other than genetic drift. Kimura's argument for neutral mutation is based on the substitution rates of alleles producing macromolecules as the α and β chains of haemoglobin and cytochrome *c*, which have been roughly constant through evolutionary time. That would be the result if genetic drift were acting on neutral alleles, but not if there were alleles at a selective advantage or disadvantage. Kimura has further argued that the variation in fitness necessary to maintain so much genetic variability by selection would represent a "genetic load" so great as to result in the extinction of the population.

But heritable variability in virtually all characters has been the foundation of Darwinian evolution, and finding it difficult to believe that much of the genetic variability does not manifest itself in phenotypic variation, population geneticists have challenged Kimura's hypothesis on a number of grounds. Most of the parameters (effective population size, selection coefficients, mutation rates, and so on) necessary for population genetics theory are, however, unknown. This has meant working from 'reasonable' values to explore the consequences of the theory and see if plausible conclusions are reached; and what seems reasonable to one worker may not seem so to another.

Some more recent challenges have adopted a new approach, questioning Kimura's theory on its internal consistency in explaining certain kinds of data. There are several levels of attack, all based on the idea that even though relevant parameters such as population size are unknown, they have the same values for different loci of a species or for different alleles at a locus. Although, therefore, it is impossible to determine the selection acting on a specific locus or allele, it is possible, in some cases, to determine that there is selection on some of a group of loci or on some alleles at a locus. These consistency arguments, when properly applied, have been able to show that selection is acting on at least some of the loci which can be studied with electrophoretic techniques.

For example, if genetic drift is the only factor affecting gene frequency, each locus should be affected in the same way, regardless of the breeding structure—that is the population size, migration and inbreeding patterns—which are always unknown for natural populations. By sampling from several populations, it is possible to compute for each locus an effective inbreeding coefficient, F, which is nearly 1 when genetic drift is weak and nearly 0 when genetic drift is very strong. The observed F values for different loci can then be compared to find whether they are too heterogenous to be consistent with neutral mutation theory. This procedure was first suggested by Cavalli-Sforza (*Proc. R. Soc.*, **B164**, 362; 1966) with data from human blood groups and later used by Lewontin and Krakauer (*Genetics*, **47**, 175; 1973) on the fly *Dacus*. The most complete study of this type is that of Nevo (*Nature*, **244**, 575; 1973), who sampled thirty-one loci in eleven populations of *Theomomys talalpoides* (a pocket gopher). He argues that his discovery of F values ranging between 0 and 0.8 reveals too much heterogeneity to be consistent with neutral mutation theory.

Another approach, introduced by Johnson (*Nature*, **237**, 170; 1972) and analysed in detail by Johnson and Feldman (*Theor. pop. Biol.*, **4**, 209; 1973) is based on the evenness of the distribution of allele frequencies at different loci. If all of a given number of alleles at one locus are assumed to be neutral, then their frequencies are not expected to be either too similar or dissimilar. Each locus with a given number of alleles is an independent trial. Johnson and Feldman analyse previously published data on several *Drosophila* species and show that the frequencies are too even to be consistent with the neutral mutation theory. As with the distributions of inbreeding coefficients, one cannot determine which of the alleles are selected but only that there must be some factors, other than mutation and genetic drift which are affecting the gene frequencies.

Measures of the similarity of allele frequencies in different geographic regions forced Bulmer to the same conclusion (*Nature*, **241**, 199; 1973). Bulmer claimed that geographically separated natural populations of *Drosophila* are too similar in genetic composition for genetic drift, alone, to be acting. Maruyama and Kimura (*Nature*, **249**, 30; 1974) point out that Bulmer's argument depends on a theory which assumes implicitly that there are an infinite number of subpopulations and hence an infinite number of individuals in the species. Although mathematically correct, Bulmer's application was inappropriate. Maruyama and Kimura apply the theory of genetic drift in a finite number of subpopulations, first worked out by Maruyama (*Genetics*, **69**, 377; 1972), and conclude that the data are, in fact, consistent with the neutral theory. Although Maruyama and Kimura are forced to assume 'reasonable' values for several unknown parameters, they do show that, contrary to Bulmer's conclusion, the existing data on geographic variation are not inconsistent with the neutral theory. They also show, surprisingly, that even a very small amount of migration between subpopulations can maintain genetic uniformity in unselected loci over very large distances, on the same scale as the total range of the species. The next logical step will be to examine geographic variation in a single species in the light of Maruyama's theory in order to distinguish the selected from the neutral loci.

The recent challenges to the neutral mutation theory indicate a maturation of population genetics theory. Much of

the previous work in the subject has been directed to what might happen in natural populations in different sets of hypothetical circumstances. The apparent goal was to show that Darwinian evolution could be accounted for by changes in gene frequencies. Much of the recent theory takes that for granted and is motivated by and directed to observations of gene frequencies in natural populations. The neutral mutation hypothesis of Kimura has been one focus of such efforts. Although the studies of Nevo and Johnson show that some selection must be acting, their tone indicates the extent to which the neutral theory has been accepted. The question is no longer whether all of the genetic variation is selected or not but what fraction is selected. The recent work has shown that a significant fraction is selected, but it would be difficult to argue that the fraction approaches one.

From a Correspondent

How special is the Universe?

AN examination of the delicacy of advanced life forms and the requirement of highly special conditions for their existence would seem to support the long-standing tradition that the Universe has been created in a singularly convenient form for the presence of intelligent life.

It is truly remarkable that modern theoretical physics can make a contribution to this philosophical question. For example, it is sometimes pointed out how various naturally occurring numbers must be restricted in their values in order to be consistent with the existence of living matter. Dyson has remarked (*Scient. Am.*, **225**, 51-59; 1971) that an increase in the coupling constant of the strong interaction by only a few percent would have resulted in a catastrophic synthesis of most of the hydrogen in the Universe during the early stages of the big-bang, thereby depriving stable stars such as the Sun of their energy source, making life seemingly impossible. Another important naturally occurring number is the ratio between the electrical and gravitational forces between an electron and a proton (a staggering 10^{39}). Strangely, this number coincides with the age of the Universe measured in atomic units of time. Carter has argued (unpublished work) that this coincidence is necessary for the existence of intelligent life, on the basis that the lifetime of stable stars depends on the first number, and that this must be somewhere near the time required for intelligent life to evolve, which is of course just equal to the present age of the Universe. Thus the approximate equality of the two numbers is not a coincidence, but a direct result of our own existence.

The application of this philosophy to the initial state of the Universe rather than to the values of the constants of nature has been made by Collins and Hawking (*Astrophys. J.*, **180**, 317–334; 1973) who addressed themselves to one of the outstanding mysteries of modern cosmology. Why, out of all the possible distributions of matter, are we living in a Universe in which this distribution is so accurately isotropic? For it is a remarkable fact that whichever direction we look, the 3 K background radiation has the same intensity to one part in 1,000. This fact is all the more puzzling when it is realised that such widely separated regions of the Universe cannot have even been casually connected when this radiation was emitted, owing to the existence of a so-called particle horizon across which no information can pass. Collins and Hawking have pointed out, however, that although the Universe appears isotropic now, it need not always have been so. Restricting their discussion to homogeneous cosmological models, they show that there is a class of cosmologies which become isotropic as they expand from the initial big-bang. The most relevant discovery of their work is that the set of initial states of the Universe for this to happen is of measure zero in the set of all possible initial conditions. In layman's language, there can be no deviation at all from these special initial states if the Universe is to become isotropic. Moreover, it is just this same class of cosmological models which permits the growth of galaxies and stars by ordinary gravitational condensation, and hence permits the existence of life. So Collins and Hawking conjecture that man's own existence is a consequence of the isotropy of the Universe (see *Nature*, **242**, 304; 1973).

It must not be supposed that the adoption of the philosophy that the existence of life imposes severe constraints on the structure of the Universe implies a 'purpose-built' world with man as the end product. Most subscribers to the former viewpoint prefer to conjecture that there is not just one single Universe, but a whole ensemble, all with widely varying properties. In most of these universes life would be impossible because the very restrictive conditions would not be met. But, in some like our own, intelligent life is able to evolve and become conscious of its surroundings—in a nutshell, the world we live in is the world we live in. In answer to the question—where are these other universes?—we have two choices. Wheeler has suggested that our own Universe may not continue to expand forever, but may be returned eventually to a very dense condition where it will be 'reprocessed' and emerge into another cycle of expansion and recontraction. These cycles are supposed to continue indefinitely, passing through an infinite variety of structure and conditions with the 'constants' of nature, and perhaps even the laws of physics changing from one cycle to the next. Only very rarely does a cycle permit conscious organisms to evolve. Another possibility has been suggested by Everett in his interpretation of the laws of quantum mechanics. It is well known that quantum theory cannot predict precisely the outcome of any realistic experiment, but at best can only give the relative probabilities of the various possible outcomes. Conventionally, it is supposed that the real world selects from all the choices one particular result at random, but Everett has developed a theory in which all possible quantum alternatives presented are in fact realised, by the entire world splitting continuously into a stupendous number of 'parallel worlds'. Once again, only a small subset of such worlds would permit the existence of life.

Curiously enough, this 'bioselection' philosophy is usually accredited to modern cosmologists such as Dicke, Carter and Wheeler. In fact it was also used as long ago as the last century by Boltzmann in an attempt to explain why the Universe was in such a very remarkable state, rather than the much more probable condition of thermodynamic equilibrium. Boltzmann proposed that the Universe indeed spent nearly all its time in equilibrium, but on exceedingly rare occasions produced a vast fluctuation from equilibrium, thereby explaining its present (unstable) condition. The reason why we happen to be here to observe this extraordinarily unlikely occurrence because it is itself a necessary precondition for life, which requires continual thermodynamic disequilibrium for its existence. Boltzmann's fluctuations, like Wheeler's cycles, were supposed to occur repeatedly, though only after enormous durations.

Popular though this bizarre philosophy may be, there exists a counter philosophy the proponents of which are no less distinguished. According to this latter point of view, the Universe is not at all a remarkable place, but the almost inevitable outcome of a very wide range of initial conditions. Thus, for example, it has been claimed that there are various dissipative effects which would be present in the early Universe, and have the effect of damping out any anisotropies which may have been present initially, thereby leading to the high degree of isotropy now observed. In particular, Misner has examined the effects of neutrino viscosity, and recent exciting work by Zel'dovich and Starobinskii in the Soviet Union has shown that the tidal

gravitational effects present in an initially anisotropic Universe would give rise to creation of particle pairs out of the vacuum. The back reaction of this particle production on the evolving Universe is such as to remove some of the isotropy. Whether or not processes like these can really explain the isotropy of the Universe is not yet known, and it is in any case hard to see how sufficient damping could work for any initial conditions.

The most recent contribution to this fascinating debate is published by Bonnor in the latest *Monthly Notices of the Royal Astronomical Society* (**167**, 55–61; 1974). One of the restrictions of the Collins–Hawking result was that they treated only homogeneous cosmological models, on the supposition that any inhomogeneity would be expected to impede rather than assist the growth of isotropy. Bonnor claims, however, that this supposition may be unfounded. By removing the assumption of homogeneity and investigating the evolution of cosmological models which are initially inhomogeneous, he finds that under a wide range of initial conditions these models eventually approach both homogeneity and isotropy, that is, the Robertson-Walker type universes. Unfortunately, it is not clear from Bonnor's work just how general this result is, for his model is also restricted by being spherically symmetric about a privileged point in space, and therefore does not obey the usual cosmological principle.

The significance of Bonnor's result for the philosophical debate is that our present isotropic Universe may well have evolved from one of a wide variety of initial states of varying inhomogeneity, rather than from the exactly determined class of Collins–Hawking models. Whatever the outcome of this continuing debate, it is bound to have an influence on the way in which man relates himself to the Universe.

P. C. W. DAVIES

Changing views of the Moon's magnetism

BEFORE 1959 there was no direct way of telling whether the Moon possesses a magnetic field or not. But in that year prospects for the experimental determination of any possible lunar field improved markedly when Luna 2 took a magnetometer down to within 55 km of the Moon's surface. No field was actually observed, which, taking into account the sensitivity of the magnetometer, put an upper limit of 6×10^{21} gauss cm^3 on the Moon's global magnetic moment—a maximum value some four orders of magnitude lower than the present dipole moment of the Earth. This apparent lack of any significant global magnetic field on the Moon was confirmed a few years later by Luna 10, and again in 1967 by Explorer 35 which enabled the maximum moment for a dipole aligned along the lunar axis to be reduced to 6×10^{20} gauss cm^3 (Ness *et al.*, *J. geophys. Res.*, **72**, 5769; 1967 and **73**, 3421; 1968). A more detailed analysis of the Explorer 35 data, carried out a little later by Behannon (*J. geophys. Res.*, **73**, 7257; 1968), yielded a still lower value of 1×10^{20} gauss cm^3, and the most recent estimate of the maximum lunar dipole moment (based on Apollo subsatellite magnetometer results) is 6×10^{19} gauss cm^3.

Thus for more than a decade from the first attempt to determine the lunar magnetic field directly, most (but not quite all) indications were that the Moon is magnetically inert—a result entirely in accord with preconceived ideas about the nature of magnetism in planetary bodies in general and about the nature of the Moon in particular. Because the only viable theory for the origin of the Earth's highly variable magnetic field involves dynamo action in the molten core, it seemed natural to consider extraterrestrial bodies in similar terms. In other words, the absence of a global field on the Moon was seen as the result of the lack of any dynamo process in the lunar interior; and the absence of a lunar dynamo was to be expected, partly because the existence of molten metallic core of appreciable size seems to be precluded and partly because the Moon's rate of rotation is low. So few people were surprised at the inability to detect a lunar global field. It is true that before the end of the 1960s some of the Explorer 35 data had also been interpreted as indicating the existence of small scale (10–100 km) magnetic anomalies on the Moon, mainly in the highlands. But although the presence of anomalies has since been confirmed, the original interpretation was indirect and attracted comparatively little attention. It is thus historically quite correct to suggest, as Fuller (*Rev. Geophys. Space Phys.*, **12**, 23; 1974) does in his excellent review of the subject, that before the Apollo programme the Moon was generally regarded as 'magnetically uninteresting'.

Against this background of assumption and self-consistency, it apparently came as quite a shock to many geophysicists to discover that some of the returned Apollo 11 samples contained stable natural remanent magnetisations (NRMs) acquired before leaving the Moon—a discovery repeated with samples from subsequent Apollo missions. Moreover, magnetometers landed on the Moon by Apollo 12 onwards recorded surface magnetic fields in magnitude from about a gamma to hundreds of gammas; and there can be no doubt that these fields arise from the magnetisations of rocks in the lunar surface. Understandably enough, there was again a tendency to see these new and unexpected results in terms of processes comparable to those occurring on Earth. Thus for one thing it seemed reasonable to suppose that the NRMs in at least the lunar igneous rocks were thermoremanent magnetisations (TRMs) acquired as the rocks cooled through their Curie points. Since the time span by the magnetic lunar rocks represents at least several hundred million years, the implication then is that the magnetising field must also have lasted that long; and the field source most compatible with a long-term existence is, of course, the dynamo. In other words, although the Moon now has a dipole moment, if any, of less than about 10^{20} gauss cm^3 and apparently does not support a dynamo, for an extended period over 3,000 million years ago it must have acted as a dynamo giving rise to a dipole moment much greater than 10^{20} gauss cm^3.

Clearly this chain of argument contains its own inbuilt assumptions, the most obvious of which is that during its early history the Moon must have possessed a molten core with properties compatible with dynamo action. Presumably the lunar interior then cooled or the Moon's rate of rotation decreased, or both, so that at some time during the past 3,000 million years or so the dynamo ceased. All this is quite possible, although as Strangway and Sharpe point out on page 227 of this issue of *Nature*, as a theory it is rapidly going out of fashion in favour of one involving a Moon which accreted cold and subsequently heated up. This rules out an early lunar dynamo, but as Runcorn and Urey (*Science*, **180**, 636; 1973) noted last year, opens up the possibility, long rejected in the case of the Earth, of a magnetising field derived from permanent magnetism. In other words, although rocks such as the mare basalts would still have acquired their magnetisations thermally, they would have done so not in a field of dynamo origin but in the field from a 'permanently' magnetic lunar interior. As this permanent magnetism evidently does not exist now, it was presumably destroyed thermally as the Moon gradually heated.

By what process and in what type of field the permanent magnetism itself formed are not known. There is no shortage of fields suitable in principle (a much stronger solar field, a much stronger solar wind field, or the terrestrial field when the Moon was much closer to the Earth, for example), although it is difficult to see how the one which actually

magnetised the Moon can ever be identified. As far as the lunar magnetisation process is concerned, Runcorn and Urey rejected TRM because the required sequence of temperature changes is difficult to envisage and rejected viscous magnetisation because of its fundamental instability. Instead, they favoured depositional magnetisation involving magnetised particles settling nonturbulently through the accreting gas sphere in the external magnetic field, a process consistent with the existence of low temperatures. In this issue of *Nature*, Strangway and Sharpe now investigate a fourth possibility—isothermal magnetism acquired in a steady external field—in the light of the Moon's thermal evolution.

PETER J. SMITH

Dimensions of the possible

David Davies

THE "Recognition of Alien Life", a symposium organised by N. W. Pirie (Rothamsted Experimental Station) at the Royal Society on May 2, could as well have been called the Carl Sagan Symposium. Sagan—from Cornell University—jetted in to London, talked to the press, gave the first lecture, was never far from other speakers' minds for the rest of the day, interjected wittily, moved on to lecture elsewhere and jetted out. And with good cause was he at the centre, for nobody can have thought more than he about life elsewhere.

Alien life may be intelligent or not; Sagan considered the intelligent sort. At present, he said, nothing is known about extraterrestrial intelligence, but the technology is now available for the analysis of signals from stars and the scanning of planetary surfaces. Critical experiments, in his view, are coming up soon. The best data with which to start are observations of the Earth such as, say, Martians could make (not that Sagan has much time for Martian intelligence). All depends on resolution, as a collection of satellite photographs show. At 1 km resolution he could find no sign of life on Earth—except at Cochran, Ontario! True, cities appear as dirty smudges, but they show no sign of reworking. A useful guide is 'departure from thermodynamic equilibrium' such as is shown by geometrisation of Earth features. At 100 m resolution, this begins to show up in, for instance, large field patterns. The nocturnal light of cities is obvious, but an observer would have doubts, as many large cities are on waterfronts; maybe some chemical process could generate the light.

Eavesdroppers at stellar distances off radio emissions from Earth would be struck by the enormous energy density around metre wavelengths from radio, radar and television. These signals would be detectable at tens of light years and intelligent terrestrial life would rapidly be inferred from their highly ordered nature. With radio telescopes the range could be a thousand times greater, though given limited facilities, it is better, Sagan believes, to receive than transmit as man is likely to be the backward partner. The art of anticryptography—devising self evident messages for other intelligent life—is growing.

C. H. Waddington (University of Edinburgh) also considered intelligent alien life in trying to pin down what tells human beings that an artefact is just that. Unless one stumbles across a mechanical device, it is probable that repetition of shapes is as good a guide as any to intelligence. P. H. Gregory (Rothamsted) stressed that in talking about the recognition of life at the microscopic level the decision is largely an aesthetic one; on Earth man is still inadequately aware of the realms of possibility. He proposed an atlas of terrestrial microscopic objects as a valuable step towards improving methodology.

At this stage the meeting became philosophical as the discussion circled inconclusively round the question from the floor, "What is life?", reminding me of Gluck, my neighbour of Shelley and Sir Peter Medawar of flogging a dead horse.

The next contribution, by J. E. Lovelock (University of Reading), was in many ways the most fascinating. What could astronomical observations reveal about the probable existence of a biosphere? Thermodynamic disequilibrium in the atmosphere could be a good guide to the existence of life, and on Earth gross departures from equilibrium in nitrogen, hydrogen and methane point to life being present, a conclusion which multiplex spectrometry does not endorse for other planets.

The remainder of the meeting was devoted to questions of life on Earth, its past record, its energy relationships and its evolution. I. R. Kaplan (University of California, Los Angeles) reviewed stable isotope analysis, particularly of hydrogen, carbon, oxygen, nitrogen and sulphur. Variations in concentration ratios by biological enrichment are helpful in understanding past terrestrial environments. This scheme was taken further by P. C. Sylvester-Bradley (University of Leicester) for the Precambrian geological record. The origin of life is placed at about 4,000 million years ago (a review of this field appeared in *Nature*, **248**, 730; 1974). J. De Ley (State University, Gent) discussed energy sources and storage and remarked on the adenine-based nature of life on Earth through the dominance of ATP as an energy carrier. Finally, A. G. Cairns-Smith (University of Glasgow) speculated on the role of clays as possible catalysts or frameworks for the evolution of biochemicals from inorganic origins.

Medawar summed up characteristically: "The heterogeneity of life and the varieties of metabolism and modes of behaviour are so enormous that nothing should surprise us about alien life. All we can do is attempt to sketch out the dimensions of the possible".

Alloy glasses and beyond

from a Correspondent

AT the eighth International Thin Films Conference held at the University of Sussex from April 8 to 10 under the sponsorship of the Thin Films and Surfaces group of the Institute of Physics, the most noticeable feature was the diversity of topics discussed: many of the phenomena of solid state physics have now been reproduced in thin film form, and for good measure some novel phenomena have been discovered.

The growth in the technology of electron-optical instruments continues to provide new and valuable aids to the structural investigation of thin films. According to M. Prutton (University of York), it seems that the scanning Auger spectroscope may soon become a viable research tool, and already it can be used to analyse the composition of a 10 Å-thick surface layer. In the scanning mode of operation, simultaneous composition analyses can be obtained from a large number of positions on the surface of a given specimen.

J. G. Wright and P. K. Leung (University of East Anglia) used *in situ* electron diffraction methods to observe thin films of amorphous Co, Fe, Mn and Cr prepared by evaporation under ultra-high vacuum onto a substrate at 4 K. This work should provide final confirmation that amorphous metallic films are not caused simply by impurities in vacuum systems. In a similar vein, B. Cantor and R. W. Cahn (University of Sussex) described the structures of highly extended solid solutions in binary metal alloy films.

A large section of the conference was devoted to various electrical and magnetic properties of thin films. C. H. L. Goodman (Standard Telephone Laboratories, Harlow) pointed out that previous claims that chalcogenide and metal oxide glasses represent the biggest breakthrough since the transistor

are exaggerated, and he was not very confident of their potential usefulness except in relatively limited areas such as addressed display systems. In a stimulating paper, N. Boonthanom and M. White (Brighton Polytechnic) described the co-evaporation of metal/polymer mixtures to form a structure of conducting metal islands in an insulating polymer matrix. In these films teh resistivity changes by seven orders of magnitude over a temperature range of only 200 K.

The final session was concerned with the problems of fabricating magnetic bubble materials. It is a sign of the rate of progress in thin film physics that in such a recent development, the fundamental physics is already well established and the current problems are technological in nature.

More muonless neutrino events

from a Correspondent

LAST year the users of the European bubble chamber Gargamelle, at CERN Geneva, reported that they had observed neutrino interactions without outgoing muon tracks (*Phys. Lett.*, **46B**, 138; 1973; see also *Nature* **245**, 119; 1973). The simplest explanation for these events is that the weak interaction has a 'neutral current' component. If the effect is genuine then the implications are profound. The existence of a neutral weak current makes it possible to construct a rigorous theory combining both the weak and the electromagnetic interactions. Such theories have been proposed by Weinberg (*Phys. Rev.*, **D5**, 1412; 1972), and others.

When the Gargamelle result was announced there were unofficial reports that an American group had seen similar events in an experiment at the National Accelerator Laboratory (NAL) near Chicago. Now, after almost a year of careful checking, these data have been published (Benvenuti *et al.*, *Phys. Rev. Lett.*, **32**, 800; 1974) by a combined team from Harvard, University of Pennsylvania, Wisconsin and NAL. The groups used scintillation-counter and spark-chamber techniques to detect the events, to measure the energy of the reaction products and to identify muon tracks. Their neutrinos were produced from primary proton beams of 300 and 400 GeV, much higher than the 30 GeV energy of the CERN proton beam.

The team saw 76 events which must have been caused by neutrino interactions, but did not have a muon track: 93 neutrino events were seen with an identification muon. A number of different techniques have been used to calculate how many muon events had been wrongly identified, in particular, how many muons missed the muon detector. All versions of this calculation fail to account for the whole of the observed signal of muonless events. About 36 genuine muonless events remain, giving a corected ratio of muonless events to muon events of 0.23 ± 0.09.

Since this ratio is almost the same as that seen by Gargamelle, even though the detection technique is totally different and the beam energies are ten times larger, there can now be no doubt that muonless neutrino events do occur. Whether they are caused by a neutral weak current is not yet completely certain, but other explanations are becoming increasingly implausible.

Nutrient supply and ecosystem growth

from Peter D. Moore,
Plant Ecology Correspondent

THE term 'oceanicity' traditionally has conveyed to ecologists the impression of a climate with a low amplitude fluctuation in seasonal temperature and a high annual precipitation. There is now evidence, however, that other aspects of maritime environments—for example, nutrient cycling—may be of equal or greater ecological importance.

Nutrient elements in terrestrial ecosystems are cycled constantly between non-living and living components. There are gains and losses: gaseous elements are gained and lost through atmospheric movements; non-gaseous elements enter terrestrial ecosystems largely by means of the weathering of rock particles in the soil or as solutions or particulate fragments from the atmosphere, arriving as aerosol or dust. In some rather exceptional types of ecosystem other transport mechanisms may be important, such as inwash of detritus by rivers or sea, or the immigration of animals to roost, nest or defaecate. Non-gaseous nutrient elements are lost from ecosystems largely as a result of soil leaching (see Bormann and Likens, *Science*, **155**, 424; 1967).

In a stable, climax ecosystem nutrient gains are balanced by nutrient losses, whereas in seral ecosystems, still developing, gains exceed losses. The excess of gain over loss is normally associated with an increase in biomass. The final constraint which limits ecosystem development, however, is normally a climatic one rather than nutrient availability, although in some climax ecosystems (for example, ombrotrophic mires) nutrients may be limiting (Firbas, *Veröff. geobot. Inst. Zurich*, **25**, 177; 1952). It is possible, within the general climatic restrictions, that availability of nutrients in an ecosystem could influence the rate at which succession takes place and equilibrium is attained.

In the light of this knowledge of nutrient cycling in ecosystems, it is rather surprising that nobody has yet attempted to measure the rates of successions in maritime regions where aerial nutrient input can be expected to be high and to compare these with areas of similar edaphic and climatic qualities far removed from the sea. Art, Bormann, Voigt and Woodwell (*Science*, **184**, 60; 1974) have now produced data from a maritime climax forest ecosystem which makes such a comparison even more imperative.

Fire Island, the research site, is a barrier island off Long Island, New York. It is fringed by sand dunes and salt marshes and contains a forest ecosystem in the lea of mature dunes. Art and his colleagues have attempted to determine the source of incoming nutrients to this forest and, by a process of elimination, they have come to the conclusion that aerosols and airborne particles constitute most of the nutrient input. They consider weathering an unimportant source of nutrients because the substrate is 98% quartz sand and the remainder (magnetite, garnet and tourmaline) is also unlikely to be a major source of ions. Animal inputs, mainly seabird guano, are only locally important and are of no significance within the study area. The soaking of seawater into the ecosystem from below does not occur because of the peculiar hydrology of the island; a high precipitation: evaporation ratio leads to a downward movement of freshwater to a depth of about 40 m below sea level in the centre of the island, thus excluding salt water. There remains the income from airborne sources.

Art *et al.* divide the airborne material into three types—a rain component (collected in rain gauges open only during rain), a dry fallout component (from gauges open all the time, less the rain component) and an impaction component (by measuring the difference between above canopy and below canopy composition of rainfall and determining the excess beneath canopy). Total annual figures of meteorological input obtained were (in g m^{-2}): 0.73 K; 14.15 Na; 0.98 Ca; and 1.91 Mg. These figures compare very closely with those obtained in other oceanic situations; for example, the Shetland Isles (see

Gore, *J. Ecol.*, **56**, 483; 1968) 0.55 K; 13.3 Na; 0.67 Ca; and 1.92 Mg and on the Lizard Peninsula in Cornwall (Malloch, *J. Ecol.*, **60**, 103; 1972) 3.8 K; 19.8 Na; 0.9 Ca; and 2.0 Mg.

The annual uptake of nutrients by the vegetation of the Fire Island forest was determined by measuring the nutrient content of the net primary production in midsummer. The loss of nutrients by litterfall and canopy leaching was found to balance this uptake for most elements, and it would thus seem that the forest is in a climax, equilibrium state. The limitations upon growth in this ecosystem seem to be quite complex. Increased nutrient input would, theoretically, result from a larger tree canopy (that is, greater above-ground-biomass) because this would enhance the impaction component, but this is made impossible by the destruction of emergent shoots by high intensity, salt-laden winds. Vegetative proliferation is possible only by low-level lateral branches in the canopy and this seems now to have reached maximum development; as a result a climax has been attained.

In terms of biomass (17,000 g m^{-2}) and net primary productivity (1,100 g m^{-2}) the Fire Island forest compares well with the figures for temperate forest collected by Rodin and Basilevich (in *Functioning of Terrestrial Ecosystems at the Primary Production Level* (edit. by Eckardt), Unesco, Paris, 1968). This is despite the extreme nutrient poverty of the sandy substrate. A comparison of nutrient input figures from the Fire Island forest with those from other forest ecosystems shows that the meteorological input alone of several important elements (for example, K and Ca) equals the total (meteorological plus weathering) input of these elements in forests further from the sea. Thus, if nutrient availability is arresting the succession, it is not surprising that the biomass of this 'subclimax' falls little short of that found in climatically limited forests.

Finally, to return to the relation between nutrient availability and the rate of successions, it is interesting to note that the Fire Island forest is considered, on the basis of radiocarbon dating and geological evidence (Sirkin, *Bull. Torrey bot. Club*, **94**, 131; 1972), to be only 200–300 years old. The dune systems around the inland lake Michigan are considered to have taken thousands of years to attain a forested state, but there the low nutrient availability from weathering is accompanied, one presumes, by a low aerial nutrient input. Much more data are needed from such inland dune systems in terms of their nutrient balance, but it seems that there is a strong case for supposing that overall nutrient availability is the factor controlling the rate of succession on those sand dunes. Such a knowledge will undoubtedly provide ammunition for those ecologists who consider that the limiting effects of nutrient supply upon the developmental rate of ecosystems is a general feature of successions (see *Nature*, **240**, 443; 1972).

Repressors at the molecular level

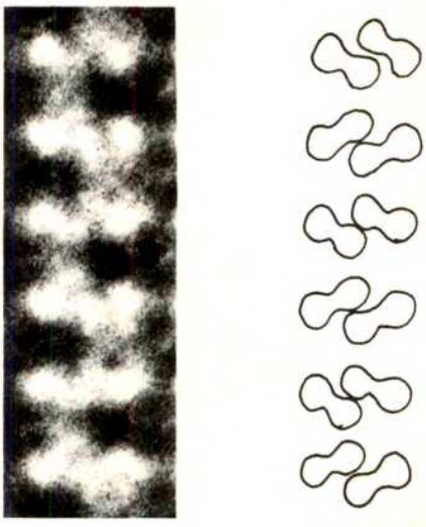
from Stephen Neidle

THE cluster of three genes responsible for lactose metabolism in *Escherichia coli* have been perhaps the most intensively studied of all bacterial chromosome systems. Indeed advances in knowledge of this cluster, known as the *lac* operon, have often tended to be hailed as landmarks in molecular genetics generally. It is now some 13 years since Jacob and Monod first proposed that the control of protein synthesis in bacteria involves certain agents that can repress the relevant genes, and hence inhibit specific messenger RNA synthesis. The first of these substances to be isolated and characterised was the lactose (*lac*) repressor, which is a tetrameric protein of molecular weight 150,000 (Gilbert and Müller-Hill, *Proc. natn. Acad. Sci. U.S.A.*, **56**, 1891; 1966). These workers followed this considerable technical achievement by demonstrating convincingly that the repressor does indeed act on the *lac* operator locus of a DNA molecule (*ibid.*, **58**, 2415; 1967).

Detailed knowledge of how repressor proteins turn off DNA at the molecular level, however, is still almost entirely lacking, although some interaction and control models have been proposed, notably by Sobell, Müller-Hill and Gierer. The amino-acid sequence of the *E. coli lac* repressor, as well as the nucleotide sequence of its target on the chromosome, the *lac* operator, have both been recently determined (*ibid.*, **70**, 3576; 1973). Thus, it is not altogether unexpected that protein crystallographers have begun to take an interest in repressor proteins, especially since modern techniques can now provide structural information about such large molecules. Steitz and his colleagues at Yale (*ibid.*, **71**, 593; 1974) have succeeded in crystallising the *lac* repressor, but have obtained disappointingly small microcrystals. Although these are not large enough for even preliminary single-crystal studies, they have revealed a measure of useful information. A combination of powder X-ray diffraction data and electron micrographs has given a good indication of the size and possible symmetry of the unit cell of the protein, as well as that of the tetrameric unit itself. There are four of these tetramers in each orthorhombic repeat, and they appear to be asymmetric and dumbbell in shape, with a length of about 135 Å. It seems most likely that the subunits of the repressor molecule are related by D_2 point-group symmetry, rather than the four-fold symmetry proposed by Sobell (*ibid.*, **69**, 2483; 1972).

This necessarily meagre amount of data has nonetheless enabled Steitz *et al.* to both reject several of the proposed repressor–DNA operator interaction models, and to suggest one of their own. Any such model has to take into account the two-fold symmetry regions found in the operator nucleotide sequence by Gilbert and Maxam. Rather than binding to either single stranded DNA or four-leaved cloverleaf DNA (both suggested arrangements for the operator locus), the new suggestion is that the dumbbell repressor binds in a manner such that its long axis and the double-helical polynucleotide axis are parallel. The arrangement is described as being similar to a "hotdog in a hotdog bun", which analogy is perhaps clearest to those most familiar with the finer points of the American cuisine. The model has, however, several attractive features, notably that it does not contravene accepted dogma regarding protein–substrate and protein–protein interaction. Also, it has two-fold symmetrical binding grooves, which comply rather well with the operator sequence data.

Until there are more detailed structural studies of both the repressor protein itself, and maybe even complexed with the *lac* operator fragment of the genome, there will doubtless be numerous more suggested models.



Steitz *et al.*'s enlargement of part of the translationally averaged election micrograph with, on the right, their interpretation of the molecular outline. Each dumbbell is one repressor tetramer and there are four tetramers per unit cell.

Roundtable on neuroendocrinology

from Barend ter Haar

DURING the weekend April 6-7 recent neuroendocrinological findings of most of the leading laboratories in Europe were discussed informally at Magdalen College, Oxford in an effort to establish the most fruitful course of future research, and to sow the seeds for greater international collaboration. The sessions were divided under the general headings of hypothalamic releasing hormones, secretion of the hormones of the posterior pituitary, monoamine systems affecting endocrine function, rhythms involved in neural control of reproductive function and processes involved in sexual differentiation and puberty.

It had been hoped that with the elucidation of the amino acid sequence of luteinising hormone releasing hormone (LHRH) and its subsequent chemical synthesis, antibodies prepared against this material could be used to identify, by immunofluorimetric techniques, the neurosecretory cells for LHRH in the brain. Though J. Barry (University of Lille) has been able to demonstrate a distribution of such neurones throughout certain areas of the anterior and medial hypothalamus of the guinea pig, he has as yet been relatively unsuccessful in other species: there is still much progress to be made in this field. It was noted with interest that Barry has also found labelled axons extending from the hypothalamus to the limbic system: it is now becoming more obvious that classical concepts of unidirectional flow of neuronal information from the limbic to the anterior hypothalamus and then to the median eminence have to be altered.

To add to the complexity, M. C. Harris (University of Birmingham) and G. B. Makara (Institute for Experimental Medicine, Budapest) indicated that they had also found not only cells in the arcuate nucleus and median eminence with axonal terminations in the anterior hypothalamus, but also hypothalamic cells which appeared to project both forwards, to the paraventricular nucleus, and also caudally, to the median eminence. Of further interest concerning efferents from the hypothalamus to other brain areas, and in the context of recent observations of effects of thyrotropin releasing hormone (TRH) on certain psychiatric disorders, were the observations presented by R. Dyer (University of Bristol) that both TRH and LHRH can modify electrical activity in the cerebral cortex in a similar way to their observations in the hypothalamus.

It is clear that though radioimmunoassay techniques have greatly helped to advance neuroendocrine knowledge, great care is still needed in the interpretation of results, the principal difficulty being that immunoreactive sites on a molecule do not necessarily relate to biologically active sites. Furthermore, such assay techniques are of little use in the detection of brief hormonal discharges such as the 2-second discharge of oxytocin demonstrated by D. W. Lincoln (University of Bristol) by means of transient changes in mammary pressure. For the release of posterior pituitary hormones, N. A. Thorn (University of Copenhagen) suggested a similar involvement of calcium in hormone discharge as in the mechanisms of muscle contraction.

In discussing the effect of circadian rhythms on reproductive function, B. K. Follett (University College of North Wales, Bangor) described how in the sparrow photosensitivity seems to vary in a circadian fashion in that a limited period of sensitivity occurs about 12 h after dawn. He has found that if light is present at this time reproductive activity is promoted so that sexual activity becomes linked with maximum day length. P. C. B. MacKinnon (University of Oxford) described how the circadian rhythm of cerebral protein metabolism in the rat is sexually differentiated, and put forward the hypothesis that the 'female' rhythm is involved in the synchronisation of internal and external signals to bring about reproductive activity in the most suitable conditions.

In considering the processes involved in the onset of puberty, F. Döcke (Humboldt University, Berlin) indicated that the limbic system is involved in controlling sexual development, and H. Moll (Erasmus University, Rotterdam) suggested that the search for only one control process may be misguided, and that puberty may be brought about by the coordinated maturation of several processes.

Obviously much more was discussed than there is space here to relate. The organisational efforts of B. T. Donovan (Institute of Psychiatry, London), C. Kordon (INSERM, Paris), and G. P. van Rees (University of Leiden) in getting together so many authorities on various branches of neuroendocrinology were well rewarded by the informal exchange of ideas that was achieved.

Correction

IN the News and Views article "Beyond systems ecology" (*Nature*, **248**, 637; 1974), the authorship of the two publications cited was misattributed. The article in *Proc. N.Y. Acad. Sci.* is by R. Levins, and the book *Stability and Complexity in Model Ecosystems* is by R. M. May.

Characterising the stringent system

from Benjamin Lewin
Molecular Genetics Correspondent

POSSIBLE relationships between protein synthesis and transcription have attracted attention for more than a decade, and in recent months this concentration of interest has been justified by results which may lead to the definition of the components of the stringent system of *Escherichia coli*. Genetic characterisation of this system has so far led to the isolation of two classes of mutant. As was first shown by Cashel and Gallant (*Nature*, **221**, 838; 1969) and Cashel (*J. biol. Chem.*, **244**, 3133; 1969) relaxed (*rel*$^-$) mutants do not restrain ribosomal RNA synthesis during starvation for required amino acids because (unlike the wild type stringent cells) they cannot increase their levels of the 'magic spot' compounds ppGpp and pppGpp in response to the deprivation. Cells of the spotless (*spoT*$^-$) mutant, isolated more recently by Laffler and Gallant (*Cell*, **1**, 27; 1974), cannot produce pppGpp during amino acid starvation, but overproduce ppGpp.

Although it is clear that accumulation of ppGpp and pppGpp is responsible for inhibiting RNA synthesis, the mechanism of formation and action of these two compounds has only more recently come under investigation. By following the abilities of *rel*$^+$ and *rel*$^-$ strains to form magic spots and respond to changes in growth conditions, Lazzarini, Cashel and Gallant (*J. biol. Chem.*, **246**, 4381; 1971) found that *rel*$^-$ cells, although unable to respond to amino acid starvation, show the same response as *rel*$^+$ cells to other changes, such as shifts in growth rate. The *rel* gene must therefore code for a protein implicated in forming ppGpp and pppGpp specifically in response to lack of amino acids, a reaction which has proved to be mediated by the consequent lack of aminoacyl-tRNA. By showing that the stability of ppGpp is much enhanced in *spotT*$^-$ cells, Stamminger and Lazzarini (*Cell*, **1**, 85; 1974) have come to the conclusion that the *spoT* gene product may be responsible for converting ppGpp to pppGpp. Clearly, many other genes may also be part of this system and the levels of the magic spots may be controlled at degradation as well as synthesis.

The concept originally suggested some time ago that an 'idling reaction' of protein synthesis may be responsible for formation of magic spots has recently been substantiated by the use of *in vitro* systems. Haseltine *et al.* (*Nature*, **238**, 381; 1972) found that ribosomes from stringent cells can synthesise ppGpp and pppGpp *in vitro*

from substrates of GTP and ATP; and ribosomes of relaxed cells are unable to perform this reaction, but can do so when extracts are supplemented with a 'stringent factor' extracted from wild type cells. Formation of the magic spot compounds takes place *in vitro* when the A site of the ribosome is occupied by an uncharged transfer RNA; this evidently acts as a signal to the ribosome that protein synthesis is limited and allows the cell to titrate its amount of uncharged tRNA.

The protein responsible for responding to the blocked A site has been studied by Ramagopal and Davis (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 820; 1974). Examining *rel*$^+$ ribosomes showed that the protein is easily extracted from them and so may be only loosely bound to the ribosome. Using an assay in which protein extracted from *rel*$^+$ ribosomes was measured for its ability to stimulate the reaction *in vitro* of unwashed *rel*$^-$ ribosomes (the lack of washing means that these ribosomes possess all other necessary components), Ramagopal and Davis found that 50S subunits, 70S ribosomes of polysomes, and 70S ribosomes which have runoff polysomes all possess *rel*$^+$ protein activity. The protein activity is not present on 30S subunits. These results suggest that the stringent factor is a ribosomal protein as such, rather than a factor which associates with the ribosome during only part of its protein synthetic cycle, and is a loosely bound component of the 50S subunit. Since it is present on both free and polysomal 70S ribosomes, these results cast no light on the conditions necessary for formation of magic spots. But they offer the prospect that further characterisation of the protein may allow both its stringent activity and also whatever other functions it may have to be defined.

Although both genetic analysis and biochemical characterisation of the stringent system suggest the outlines of its operation, many important questions remain to be answered. The first class concerns the formation of ppGpp and pppGpp. Clearly, they are formed on the ribosome in response to inhibition of protein synthesis by entry of uncharged tRNA to the A site. Although the exact mechanisms of this reaction remain to be elucidated, the *rel*$^+$ protein seems to mediate this response. One discrepancy which remains to be resolved is that ppGpp can be formed *in vitro* from GDP and ATP, whereas pppGpp can be synthesised from GTP and ATP; but *in vivo* the *spoT* gene product has been implicated in converting ppGpp to pppGpp. Another critical reaction, so far investigated scarcely at all, is how these components are formed in response to conditions other than amino acid starvation—isolation of mutants in these processes must provide an essential first step in analysis.

The second set of problems, which at the present seems more difficult to approach, concerns the reactions by which ppGpp and pppGpp mediate the inhibition of rRNA synthesis. One particularly interesting problem is why there are two magic spot compounds and what difference there may be in their functions. But attempts to show *in vitro* that ppGpp influences rRNA synthesis at the level of RNA polymerase activity have not so far proved very satisfactory. Yet the nature of this general coordinating system may prove to throw an important new light on the control of transcription in bacteria.

Control of 3T3 cell growth

from Brigid Hogan

THE mouse 3T3 line—so-called because it was derived from embryo cells transferred every 3 days at an inoculum of 3×10^5—since it was established in 1963 has been widely used in studies of the control of proliferation of mammalian cells in culture. As long as the strict transfer regime is maintained, the cells consistently show distinct growth properties. First, they are very sensitive to a reduction in serum concentration in the medium and divide about five times slower in 1% than in 10% serum. Second, even if the cells are regularly replenished with 10% serum medium, they only grow very slowly after reaching a monolayer density of about 5×10^4 cells per cm^2. Finally, they will not grow in suspension in agar or methylcellulose. It is, however, well established that 3T3 cells transformed by the oncogenic viruses SV40 or polyoma exhibit very different properties; they divide rapidly in 1% serum, reach far higher densities and form colonies in agar.

During the past few years Pollack's group in Cold Spring Harbor has been investigating the complex interrelation between these three parameters and the way in which they are modified by tumour viruses, by selecting sublines of SV3T3 which have reverted back to more normal behaviour. The first lines were selected for a reduction in growth at high cell density by exposing dense cultures to 5-fluorodeoxyuridine for 2 days so that cells synthesising DNA were killed. Clones which survived this treatment had a much more 'normal' morphology and were therefore called "flat" revertants, and reached a saturation density of only about 8×10^4 per cm^2, although the shutoff in division at this density is not as complete as in normal cells.

More recently similar 'density revertants' have been selected by killing growing cells with 5-bromodeoxyuridine or colchicine (Vogel *et al.*, *J. cell. Physiol.*, **82**, 181-188; 1974). When their other properties were tested, the density revertants fell into two classes; one which grew well in 1% serum and one which did not, but neither class would grow in agar. Using similar techniques Pollack's group have also selected 'serum revertants' which do not grow rapidly in low serum (Vogel and Pollack, *ibid.*, 189–198); these also show low saturation density but may or may not grow in suspension. Thus it has been possible to select variants of SV3T3 which have reverted in either of two, or in all three aspects of transformation. All classes of revertants have more chromosomes per cell than the parent, synthesise SV40 Ts antigen, and in some cases have been shown to contain intact virus genomes, so that their altered behaviour is likely to arise from a change in the expression of some cellular gene(s).

Pollack's group have not been slow to take advantage of the properties of their mutants to throw light on the role of cyclic AMP in the control of 3T3 cell proliferation (Oey *et al.*, *Proc. natn. Acad. Sci., USA.*, **71**, 694–698; 1974). In agreement with many other groups they found that sparse cultures of 3T3 in 10% serum contain twice as much intracellular cyclic AMP as SV3T3s. In the same conditions most revertant lines returned to having the higher levels of cyclic AMP characteristic of 3T3s, but two retained the low concentration found in SV3T3s. These two are the only revertants able to form colonies in methylcellulose so that there may be a correlation between low amounts of cyclic AMP and ability to grow in suspension. Only those revertants which are unable to grow in low serum share the property with 3T3s of rapidly and extensively increasing their concentrations of cyclic AMP when the serum concentration is reduced to 1%, and density revertants which can grow in low serum maintain the lower concentrations of cyclic AMP found in SV3T3s.

Finally, Oey *et al.* allowed normal 3T3s and two serum revertants to attain their low saturation density in conditions in which the 10% serum medium was regularly replaced, even after cell growth had slowed down. In all three lines they failed to find an increase in cyclic AMP as the cells reached their saturation densities. Taken together with the other findings, this suggests that density restriction and serum restriction of growth are not identical phenomena, as some workers have postulated, but are mediated by two different, but probably interrelated, mechanisms.

Radioactivity of tobacco trichomes and insoluble cigarette smoke particles

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Airborne ^{210}Pb is concentrated on small Aitken particles which accumulate on tobacco trichomes. Tobacco curing and the combustion of trichomes in burning cigarettes produce insoluble particles of high ^{210}Pb radioactivity which are inhaled and deposited in the bronchi of smokers. The subsequent ingrowth of ^{210}Po results in high local α irradiation which may account for bronchial cancer among smokers.

EXPOSURE to α radiation from the short-lived radon daughters, ^{218}Po and ^{214}Po, is generally considered the cause of bronchial cancer in uranium miners[1]. The presence of the α emitter ^{210}Po in the bronchi of smokers in excess of ^{210}Po levels of non-smokers[2,3] thus warrants serious consideration as the possible agent of bronchial cancer among smokers. Both ^{210}Pb and ^{210}Po have been observed in tobacco, in cigarette smoke and in smokers' lungs[2–7].

Radford and Hunt[4] pointed out that volatile ^{210}Po compounds are inhaled by smokers and suggested that ^{210}Po was bound strongly to the surfaces of smoke particles, thereby entering the bronchi. But, as Holtzman[8] and others pointed out, it is likely that the volatile compounds of ^{210}Po which are inhaled are widely dispersed and cleared rapidly from their deposition sites in the lung. Little *et al.*[2], however, found high local tissue concentrations of ^{210}Po corresponding to an estimated maximum dose of ~200 rem per 25 yr to the bronchial epithelium of lower-lobe bifurcations of cigarette smokers[3]. This can be explained by the accumulation of insoluble ^{210}Pb particles in the bronchial bifurcations. The ^{210}Pb activity of insoluble smoke particles is sufficiently high to give rise to α doses much higher than 200 rem per 25 yr in regions of high particle concentration.

^{210}Pb on tobacco trichomes

Reports on the origin and distribution of ^{210}Pb and ^{210}Po in tobacco plants are contradictory. Tso *et al.*[9], conclude that the principal mechanism involves uptake into roots from soils and fertilisers, whereas Francis *et al.*[10] suggest deposition in rainfall. The properties and distribution of trichomes (hairs) on tobacco leaf surfaces[11,12], however, suggest that they are effective collectors of small Aitken particles by means of diffusive deposition. Most commercial varieties of tobacco have 300 to 900 trichomes cm^{-2} on each side of mature leaves. The distribution of trichomes on cured tobacco is illustrated in Fig. 1. About 85% of tobacco trichomes have glandular heads, coated with a sticky exudate mixture of organic compounds. Because of the hydrophobic nature of this exudate it is unlikely that even soluble, ionic substances are washed off by precipitation. Thus, trichomes may retain the small atmospheric particles which are deposited on their glandular heads throughout the period of plant growth.

The thick distribution of trichomes maintains a stagnant air layer over the leaf surface with only the glandular heads of trichomes protruding. For the low air flow rates in the vicinity of trichome heads, the only effective mechanism for airborne particle capture is likely to be diffusive deposition due to Brownian motion of the particles. The efficiency of this process varies inversely with particle size and flow rate and thus may result in the selective deposition of particles of ~0.1 μm diameter and smaller. Radon daughters in air are concentrated on small Aitken particles[13,14]. Impactor measurements (my unpublished results for radon daughter aerosol properties, with H. E. Moore and S. E. Poet) show that ~90% of airborne ^{210}Pb resides on particles $\leq$0.3 μm diameter at concentrations between 2×10^3 and 10^4 pCi g^{-1}. The ^{210}Pb concentration increases markedly with decreasing particle size[14]. Thus Brownian capture should give rise to high ^{210}Pb concentrations on tobacco trichomes.

Preliminary measurements of ^{210}Pb on cured tobacco leaves by analysis of surface particles removed by brushing and by sonic cleaning, showed ^{210}Pb concentrations of leaf surfaces up to 30 times that of the bulk leaf per g ash. Microscopy showed that trichomes were only a minor constituent of this surface debris. Subsequently, 2,000 trichomes were removed from North Carolina flue-cured tobacco and assayed for ^{210}Po by α spectroscopy[15]. The average ^{210}Pb activity per trichome, based on this ^{210}Po measurement and the ^{210}Po:^{210}Pb ratio of the tobacco sample, was $(3.2 \pm 0.6) \times 10^{-6}$ pCi per trichome. Turkish tobacco and Kentucky burley showed ~1/2 and ~1/4 as much activity per trichome, respectively. These results indicate the accumulation and retention of tens of thousands of Aitken particles on individual trichomes, presumably on the exudate-coated glandular heads.

The solubility of separated trichomes from cured tobacco was examined. Although exudate is soluble in ether and other organic solvents before the tobacco is cured[12], the trichome heads for cured tobacco seem to be insoluble in ether, acetone and other common organic solvents as well as in concentrated HCl, HNO_3 and HCl-HNO_3 mixtures. This result is attributed to the polymerisation of exudate compounds during the curing period.

The trichomes and their glandular heads have an open, cellular structure of very low density. These properties are illustrated by the collapse of trichomes when subjected to high vacuum. ^{210}Pb activity on trichomes is probably largely associated with the exudate coatings of trichome heads. When a smoker draws on a burning cigarette, the trichomes on the tobacco fibre surfaces are subjected to temperatures from 650° C to more than 800° C[16]. The pyrolysis of glandular heads of trichomes at these high temperatures should yield smoke particles with specific activities of ^{210}Pb which approach or exceed an estimated 10^5 pCi g^{-1}.

^{210}Pb in smoke particles

Published radiochemical data for inhaled mainstream smoke show an average ^{210}Po content of ~0.036 pCi per cigarette or ~2.6 pCi ^{210} Po per g smoke[17], with a ^{210}Pb:^{210}Po ratio of 0.66 ± 0.23[6]. The smokers' intake of ^{210}Po and ^{210}Pb within several days is equivalent to the observed excess burden of 3–10 pCi of ^{210}Pb and ^{210}Po in smokers' lungs[6,7]. Thus most of

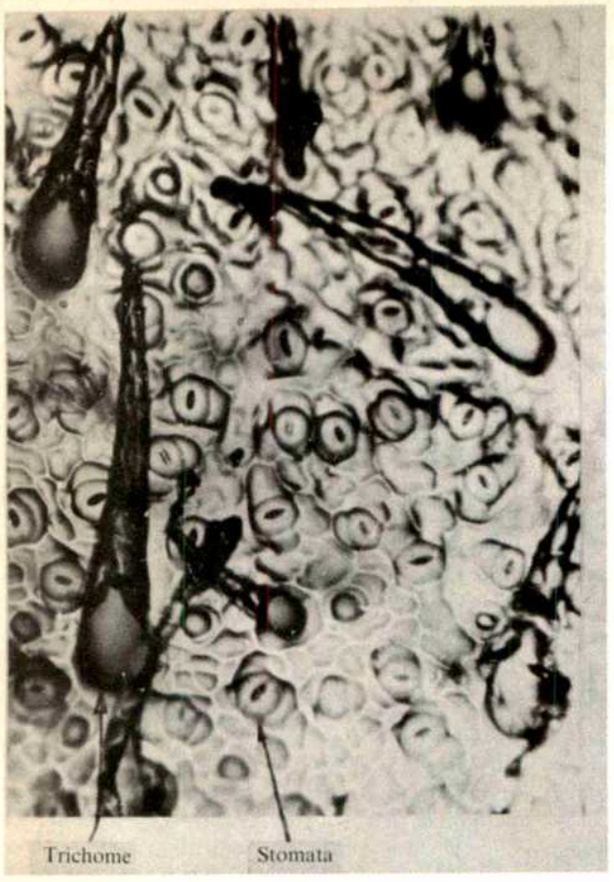


FIG. 1 Microphotograph of flue-cured tobacco, Hicks variety, bottom surface, showing trichome and stomata distribution (leaf section 550 μm × 835 μm).

the ^{210}Pb and ^{210}Po deposited in the smoker's lung must be cleared rapidly.

There is no published evidence about the physical and chemical properties of the ^{210}Pb content of insoluble smoke particles which may persist in the lung. To investigate this question, I used samples of whole condensate, prepared by standard techniques and procedures[18]. University of Kentucky 1R1 reference cigarettes (a non-filtered cigarette of standard US blended mixture) and Players cigarettes (all flue-cured tobacco) and large whole smoke condensate samples of each, were used.

Condensate samples were heated to drive off volatile constituents. The ^{210}Po was volatilised almost completely at temperatures between 600° C and 650° C, whereas all the ^{210}Pb remained in the residue, indicating that ^{210}Po is inhaled in volatile form and ^{210}Pb is associated with the particulate fraction of mainstream smoke.

Preliminary radiochemical results for 1R1 tobacco showed that the ^{210}Pb content was 0.40 pCi per g dry tobacco, 0.81 pCi per g of dry whole smoke condensate, and 130 pCi per g of the non-volatile residue after slow distillation of the volatiles up to 650° C. Similarly, for Players tobacco ^{210}Pb contents were 0.48 pCi per g dry tobacco, 1.46 pCi per g dry whole smoke condensate, and 52 pCi per g of non-volatile residue after distillation of condensate up to 600° C. The condensate residue was largely water soluble, and most of the ^{210}Pb was associated with the insoluble fraction, at a concentration of $\sim 1 \times 10^4$ pCi per g of residue. Preliminary microscopic examination of the non-volatile, water-insoluble residue of the condensate revealed small irregular particles resembling combustion products, with a maximum diameter of ~ 4 μm. Successive extractions of the residue with dilute and concentrated HCl and HNO_3 resulted in the incomplete solution of the particles and their ^{210}Pb content. After acid extraction, translucent orange particles with the appearance of cured trichome heads were prominent in the residue.

Thus ^{210}Pb is highly enriched in the insoluble particulate fraction of inhaled mainstream smoke. Presumably the volatile smoke constituents are dispersed widely and removed rapidly from lung surfaces. Water-soluble constituents also will be quickly removed from their sites of deposition. The insoluble particles will be further fractionated during deposition and clearance, depending on their physical properties and solubility in lung fluid. The preliminary results indicate that the type of insoluble smoke particles that will persist and accumulate in the bronchi of smokers is between $\sim 10^4$ pCi per g, the activity of water-insoluble smoke particles, and $\sim 10^5$ pCi per g, the estimated activity of pyrolysed trichome heads.

Bronchial epithelium dose estimates

Based on the measured concentrations of ^{210}Po in epithelial samples, Little and Radford estimated a maximum dose of $\sim$200 rem per 25 yr to lower-lobe bifurcations[3]. Holtzman's[8] questions regarding this estimate are answered by the presence of ^{210}Pb in insoluble particles of high specific activity. The insoluble particles should, at least in part, persist sufficiently long to allow ingrowth of the ^{210}Po daughter activity. This possibility can be assessed by analysis of ^{210}Pb and ^{210}Po in small bronchial bifurcation specimens from deceased cigarette smokers.

For a single insoluble particle with 3×10^{-6} pCi ^{210}Pb (the average for North Carolina flue-cured tobacco trichomes) and a ^{210}Po:^{210}Pb ratio of 0.8 (corresponding to particle residence time of 10^3 d in the bronchi) the α radiation dose to cells within the α range ($\sim$6 cell diameters) would be about 0.5 rad or 5 rem per year. However, it has been reported[20] that the pulmonary accumulation of insoluble dust particles involves a sequestering tissue reaction which results in high local dust concentrations in plaques or nodules. The cumulative α radiation dose to lung cells exposed continuously to high concentrations or clusters of insoluble smoke particles will substantially exceed the estimated maximum of 200 rem per 25 yr based on ^{210}Po tissue measurements[3]. ^{210}Po and ^{210}Pb measurements of small bronchial epithelium tissue specimens from deceased smokers, as well as α autoradiography of thin tissue sections, will be needed to assess the actual α dose distribution.

The presence of insoluble particles of high specific ^{210}Pb activity in mainstream cigarette smoke should lead to their accumulation in the bronchi of smokers with residence times sufficient to allow ingrowth of the ^{210}Po daughter radioactivity. This sequence of events must explain the high local concentrations of ^{210}Po observed[2] in the bronchial epithelium bifurcations of smokers which indicate a maximum α dose of some 200 rem per 25 yr[3]. High concentrations or clusters of these insoluble smoke particles may give rise to a much higher dose in small volumes of tissue.

A significant excess incidence of lung cancer has been observed in all groups of uranium miners exposed to more than 120 working level months (WLM) of radon daughter exposure[20]. The corresponding α radiation dose, about 0.5 to 1.0 rad per WLM[21], is 60 to 120 rad, or 600 to 1,200 rem. Correspondingly high α radiation doses in smokers seem to be restricted to relatively small volumes of tissue, thereby suggesting a reduced risk of carcinogenesis. The results for internal emitters reported by Cember[22], however, indicate that the risk for a given cumulative radiation dose may increase significantly at lower dose rates and longer periods of exposure, suggesting a higher risk for smokers.

Asbestos workers who smoke cigarettes have a very much

higher incidence of bronchial cancers than cigarette smokers[23], whereas no excess incidence of bronchial cancers was observed for asbestos workers who do not smoke. It was also reported[24] that insoluble dust accumulations in the bronchi contribute to lymphatic stagnation and thus to a larger accumulation of metabolic products. On this basis it is possible that the higher bronchial cancer risk among asbestos workers who are smokers may be due to the accumulation of a larger burden of insoluble ^{210}Pb smoke particles.

The case for insoluble α emitting particles as the primary agent of bronchial cancer among smokers is reinforced by other lines of evidence. The carcinogenicity of the chemical carcinogens in cigarette smoke has not been demonstrated for the low concentrations which occur in smoke. Insoluble radioactive particles rather than volatile constituents of smoke would seem to be the more logical agent for excess cancers in smokers on the basis of the enhanced cancer risk among smokers who inhale deeply and smoke unfiltered cigarettes, the slow decrease in the residual risk with time following cessation of cigarette smoking, and the occurrence of excess cancers in other organs of the respiratory and gastrointestinal tracts of smokers[25]. Additionally the specific α radioactivity of insoluble smoke particles approaches that of ThO_2 which was widely used as a contrast medium in human radiography and gave rise to carcinomas of the liver and other soft tissues without involvement of chemical carcinogens[26].

Thus, it seems that α radiation from ^{210}Po in insoluble smoke particles may be the primary agent of bronchial cancer in smokers. If so, a significant risk of cancer can be attributed to chronic exposure to low levels of insoluble α emitting particles which give rise to whole organ burdens of only a few pCi of α activity, but involve very high concentrations locally in small volumes of tissue.

I thank S. E. Poet for measurements of ^{210}Pb and ^{210}Po and Art Fong and John Gardner for collecting tobacco trichomes. I also thank Ashok Taori who directed my attention to the nature and distribution of tobacco trichomes. Tobacco and condensed smoke samples were supplied by John Benner and J. H. Smiley of the University of Kentucky and J. A. Weybrew of the University of North Carolina.

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1 Lundin, F. E., jun, Wagoner, J. K., and Archer, V. E., *Radon daughter exposure and respiratory cancer: quantitative and temporal aspects*, NIOSH-NIEHS Joint Monograph No. 1 (US Department of Health, Education and Welfare, 1971).
2 Little, J. B., Radford, jun., E. P., McCombs, H. L., and Hunt, V. R., *New Engl. J. Med.*, **273**, 1343 (1965).
3 Little, J. B., and Radford, jun., E. P., *Science*, **155**, 606 (1967).
4 Radford, jun., E. P., and Hunt, V. R., *Science*, **143**, 247 (1964).
5 Hill, C. R., *Nature*, **208**, 423 (1965).
6 Holtzman, R. B., and Ilcewicz, F. H., *Science*, **153**, 1259 (1966).
7 Blanchard, R. L., *Health Phys.*, **13**, 625 (1967).
8 Holtzman, R. B., *Science*, **155**, 607 (1967).
9 Tso, T. C., Harley, N., and Alexander, L. T., *Science*, **153**, 880 (1966).
10 Francis, C. W., Chesters, G., and Erhardt, W. H., *Envir. Sci. Tech.*, **2**, 690 (1968).
11 Barrera, R., and Werusman, E. W., *Tobacco Sci.*, **10**, 157 (1966).
12 Chakraborty, M. K., and Weybrew, J. A., *Tobacco Sci.*, **7**, 122 (1963).
13 Lassen, L., and Rau, G., *Z. Phys.*, **160**, 504 (1960).
14 Junge, C. E., *Air chemistry and radioactivity* (Academic Press, New York, 1963).
15 Poet, S. E., Moore, H. E., and Martell, E. A., *J. geophys. Res.*, **77**, 6515 (1972).
16 Wakeham, H., in *The chemistry of tobacco and tobacco smoke*, 1–20 (Plenum, New York, London, 1972).
17 Kelley, T. F., *Science*, **149**, 537 (1965).
18 Benner, J. F., and Keene, C. K., in Proc. University of Kentucky, Tobacco and Health Research Institute, Conference Report 3, 185–192 (1972).
19 Hatch, T. F., and Gross, P., *Pulmonary deposition and retention of inhaled aerosols* (Academic Press, New York, 1964).
20 Archer, V. E., and Wagoner, J. K., *Health Phys.*, **25**, 351 (1973).
21 *BEIR Rep. (Biological effects of ionizing radiation)*, 145–157 (National Academy of Sciences, Washington, DC, 1972).
22 Cember, H., in *Progress in experimental tumor research*, **4**, 251–303, (Karger, Basel, New York, 1964).
23 Selikoff, I. J., Hammond, E. C., and Chrug, J., *J. Am. med. Ass.*, **204**, 106 (1968).
24 McDonald, J. C., Biggs, G. W., Manfreda, J., and White, F. M. M., in *Multiple factors in the causation of environmentally induced disease*, (edit. by Lee, D. H. K., and Kotin, P.,), Ch. 12 (Academic Press, New York, 1972).
25 *The health consequences of smoking*, a report to the Surgeon General (US Department of Health, Education and Welfare, 1971).
26 Proc. conf. distribution, retention and late effects of thorium oxide, *Ann. N. Y. Acad. Sci.*, **145**, 523–858 (1967).

Class of promoter sites for *Escherichia coli* DNA-dependent RNA polymerase

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Some promoters for E. coli *RNA polymerase contain either of two nucleotide sequences recognised by Hind endonucleases, GTCGAC or GTTAAC. Such promoters are found not only in the DNA of bacteriophages (λ and T7) but also in that of mammalian viruses, including the simian virus 40 and the human adenovirus 2.*

PROMOTER sites for *Escherichia coli* DNA-dependent RNA polymerase have been defined genetically by mutations that abolish the initiation of transcription. Attempts to define these sites biochemically have shown that, *in vitro*, strong complexes form between *E. coli* RNA polymerase and specific sites on Lac[1], T7[2,3], λ[4] and fd[5,6] DNA. It seemed likely that these strong binding sites for RNA polymerase were the genetically defined promoter sites, although there was no direct evidence. The first such evidence came from the observation by Maniatis *et al.*[7] that polymerase binding protects the early leftward promoter (p_L) in λ DNA from cleavage by the endonucleases prepared from *Hemophilus influenzae*. We report here results that confirm and extend these data.

The *E. coli* RNA polymerase recognises many promoters

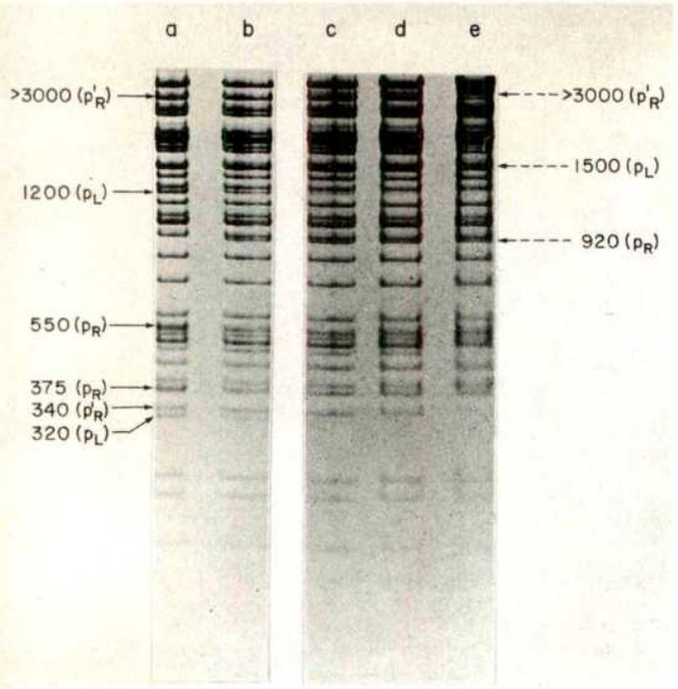


FIG. 1 Polymerase protection of Hind cleavage sites in λ DNA. RNA polymerase and λcI857S7 DNA were prepared as described previously[14]. λcI857S7 DNA (10 μg) in 250 μl (final volume) of 6.6 mM Tris-Hcl, *p*H 7.5, 6.6 mM $Mg(CH_3COO)_2$, 6.6 mM β-mercaptoethanol was incubated at 37° C for 40 min with various amounts of polymerase: (a) no addition; (b) 5 μg (molar ratio of enzyme to DNA, 30:1); (c) 10μg; (d) 12.5 μg; and (e) 50 μg. A 5 μl sample of the Hind endonucleases[12] was added to each sample, and incubation was continued at 37° C for 12 h. The reaction mixture was chilled to 4° C and extracted with phenol, previously saturated with 10 mM Tris-HCl, *p*H 8.0, 1 mM EDTA, and the aqueous phase was precipitated with two volumes of ethanol at −20° C. The samples were submitted to polyacrylamide gel electrophoresis, and the bands were stained with methylene blue as described previously[12]. Fragments containing DNA sequences from the promoter sites p_L, p_R, and p_R' are shown by the solid arrows. The dashed arrows point to fused fragments, which arise as a consequence of the protection of Hind cleavage sites by RNA polymerase. The fused fragments include the indicated promoter sites, and their sizes are expressed in base pairs, as described previously[12].

in bacterial and phage DNAs, and there is evidence that it also transcribes *in vitro* the DNA from mammalian viruses such as SV40[8] and adenovirus-2[9]. *A priori*, these facts suggest that either (1) the various promoters share a nucleotide sequence specifically recognised by the *E. coli* RNA polymerase or (2) the promoters vary in primary structure but display, in their secondary or tertiary structures, similarities which give them a specific affinity for the polymerase.

Our results suggest that several promoters, recognised by the *E. coli* RNA polymerase, are identical in at least part of their nucleotide sequence. Such promoters are found not only in the phage genomes but also in the DNA of two mammalian viruses, SV40 and adenovirus-2. These conclusions are based on the observation that the specific binding of the *E. coli* RNA polymerase protects these promoters from cleavage by an endonuclease prepared from *Haemophilus influenzae*.

The restriction endonuclease of *H. influenzas* Rd contains at least two activities, Hind II and Hind III, separable by phosphocellulose[10] or DEAE chromatography and each recognising two hexanucleotide sequences. It is not known, however, whether Hind II and Hind III each contain but a single enzyme of dual specificity or whether the fractions Hind II and Hind III themselves contain two or more different enzymes. Furthermore, *H. parainfluenzae* contains a restriction endonuclease, Hpa I, believed to recognise one of the two sequences recognised by the enzyme Hind II, as judged by its action on SV40 DNA[10,11]. This assumption is strengthened by our observation that digestion of λ DNA with the combination Hind II/Hind III in the presence or absence of Hpa I leads to an identical pattern of cleavage products[12]. Smith and Wilcox found that the Hind endonucleases recognise two palindromes of six bases on T7 DNA[13]. Because purified Hind III does not cleave T7 DNA (our unpublished result) these palindromes represent the nucleotide sequences recognised by Hind II, which are GTTAAC and GTCGAC, differing only at the two internal bases. It is not known which sequence is recognised by Hpa I.

We showed earlier that in λ DNA the early promoters p_L and p_R and the late promoter p_R' are cleaved by the Hind endonucleases, because the specific binding of *E. coli* RNA polymerase resulted in the fusion of pairs of fragments which flank these sites. In addition, for two mutants with altered p_L, *sex*1 and *sex*3, cleavage of the promoter was abolished even in the absence of polymerase. However, a mutant with a defect in p_R, *x*13, did not abolish the corresponding Hind cleavage site in the absence of polymerase. We therefore concluded that the Hind sextuplet was only part of the nucleotide sequence responsible for polymerase recognition. Finally, it was suggested that p_L, p_R, and p_R' contain one of the two sequences recognised by Hind II, that sequence which is not cleaved by Hpa I.

When increasing amounts of RNA polymerase were added to λ DNA the protection from Hind cleavage proceeded in the order p_R, p_L, p_R' (Fig. 1). Protection by polymerase still occurred in the presence of ATP or the mixture ATP-GTP (Fig. 2), when the enzyme was believed to form an initiation complex at the promoter site. This supports the view that the strong binding sites for polymerase described here coincide with, or at least overlap, the promoter sites. It does not agree with the suggestion[15] that the RNA polymerase binds far from the initiation site of transcription. By contrast, if all four triphosphates were included in the reaction mixture, the protection was abolished, presumably because the polymerase moves away from the promoter site as it transcribes the DNA. The RNA synthesised was heterogeneous in size, and superimposes a diffuse pattern in the lower part of the gel (Fig. 2). In our experiments, the triphosphates were added after the formation of the polymerase–promoter complexes. If rifampicin, an inhibitor of polymerase binding to the first purine nucleoside triphosphate[16], was added together with the triphosphates, the pattern of protection by polymerase was not altered (Fig. 2).

Other promoter sites in λ DNA[14] (for example, in *b*2 and in the *O-P* region, or p_e and p_e' that control transcription of gene *c*I) failed to protect a Hind cleavage site by binding of *E. coli* RNA polymerase. This raises the possibilities that either (1) these promoters bind the polymerase but do not contain the nucleotide sequence recognised by Hind, or (2) these promoters include a Hind II sextuplet but binding of polymerase is not detected in the test situation used. These possibilities have not been investigated.

Promoter sites including a Hind sextuplet were also detected in T7 DNA. Figure 3 shows that when T7 DNA was preincubated with increasing amounts of *E. coli* RNA polymerase (enzyme:DNA molar ratios from 30 to 250), four sites were successively protected from cleavage by the Hind endonucleases. Under the same conditions, but with Hpa I replacing the Hind endonucleases, two sites were protected, but only at the higher polymerase:DNA ratios. The protec-

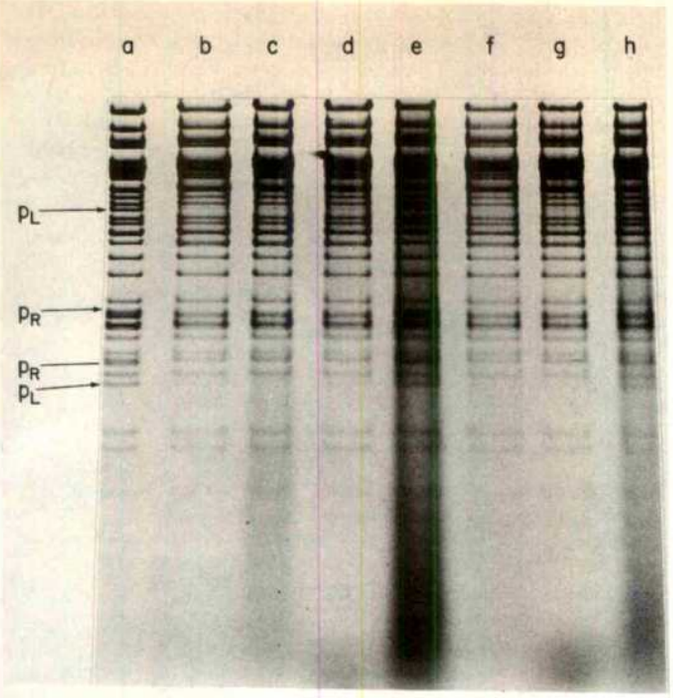


FIG. 2 Effect of ribonucleoside triphosphates on the polymerase protection. λcI857S7 DNA (10 μg) was incubated with RNA polymerase (20 μg) under the conditions shown in the legend to Fig. 1. (Sample (a) is a control without polymerase.) Then various combinations of ribonucleoside triphosphates were added as follows: (a and b) no addition; (c) 60 μg of ATP; (d) 60 μg each of ATP and GTP; (e) 60 μg each of ATP, GTP, CTP, and UTP; (f) 60 μg each of ATP and GTP, and 2 μg of rifampicin; (h) 60 μg each of ATP, GTP, CTP, and UTP, and 2 μg of rifampicin. After incubation at 37° C for 10 min, 5 μl of the Hind endonucleases was added to each sample and incubation was continued at 37° C for 12 h. Extraction with phenol and analysis by polyacrylamide gel electrophoresis were as described in Fig. 1. Fragments containing DNA sequences from the promoter sites p_L, p_R, and p_R' are shown by the solid arrows. The dashed arrows point to fused fragments, which arise as a consequence of the protection of Hind cleavage sites by RNA polymerase.

tion resulted in the appearance of two bands (Fig. 3) missing when preincubation with polymerase was omitted. It seems therefore that in T7 DNA two strong promoters contain the sequence found in p_L, p_R and P_R' in λ DNA, that is, the sequence recognised by Hind II but not by Hpa I. Two weaker promoters were cleaved by Hpa I and differed from the other two. However, because the sextuplets recognised by Hind II differ only by two internal base pairs, as noted above, the promoter sites cleaved by Hind II alone, or cleaved by both Hind II and Hpa I, are closely related in at least part of their nucleotide sequence.

The DNAs from two mammalian viruses, simian virus 40 (SV40) and adenovirus-2 (Ad2) stimulate *in vitro* transcription by the *E. coli* RNA polymerase[8,9]. Interestingly, in each of these viral DNAs, binding of the *E. coli* polymerase prevented cleavage by Hind at one site. In SV40 DNA the Hind cleavage site between fragments B and G (Fig. 5*A*) was protected, at position 18% on the standard map (Fig. 4). In fact, this site was cleaved by Hpa I, and polymerase binding protected one site from cleavage by this endonuclease (Fig. 5*A*). It seems therefore that a promoter for *E. coli* polymerase in SV40 was cleaved by Hpa I, as were two promoters in T7 DNA. Dhar *et al.*[17] have shown that *in vitro* transcription of SV40 DNA is initiated in this region of the SV40 genome and proceeds anticlockwise through the G fragment. Their data show that the G fragment, which is about 350 base pairs long, is cleaved by the restriction endonuclease RII into two fragments. The smaller of these represents approximately one-third of the Hind G fragment, that is, 120 base pairs, and contains the first sequences transcribed. Extensive sequence analysis of this transcript[17] revealed that the recognition site for the RII endonuclease occurs at position 115 from the start of the transcript. This implies that the promoter is extremely close to the Hind recognition site and also close to the start of transcription, in agreement with the results obtained for λ DNA (Fig. 2). Figure 5*B* shows that Ad2 DNA also contains a Hind cleavage site that can be protected by *E. coli* RNA polymerase. The protection was not observed with Hpa I. It is tempting therefore to speculate that this site is a promoter for *E. coli* RNA polymerase *in vitro*, of the same type as the λ and T7 promoters. An estimate of its location in Ad2 DNA can be deduced from the cleavage pattern of the hybrid Ad2-SV40 (Ad2$^+$ ND1) DNA. Ad2$^+$ ND1 derives from Ad2 by two alterations in the viral DNA. (1) A portion of Ad2 DNA has been deleted between the positions 14.0% and 19.4%, measured from one end of the DNA molecule[18]. (2) Instead, a portion of SV40 DNA has been inserted, covering the region between 11% and 28% of the standard SV40 map. Interestingly, Ad2$^+$ ND1 contained the Hpa I cleavage site between SV40 DNA fragments B and G, and Fig. 5*C* shows that two fragments, missing from Ad2 DNA, were fused in the hybrid as a consequence of the polymerase protection. However, the polymerase failed to protect any other Hind cleavage site in the hybrid. Thus it can be deduced that the polymerase binding site that was detected in Ad2 DNA must be in the part of the molecule that is deleted in the hybrid, that is, somewhere between map positions 14.0% and 19.4%.

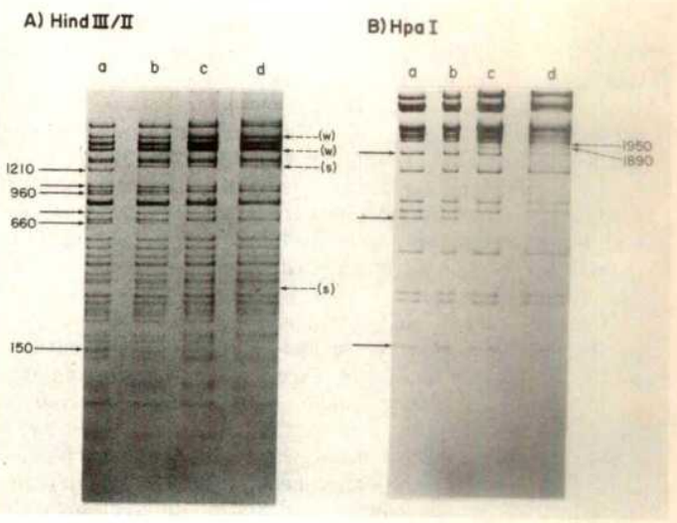


FIG. 3 Polymerase protection of Hind and Hpa I cleavage sites in T7 DNA. T7 DNA (8 μg) was incubated with varying amounts of RNA polymerase as shown in the legend to Fig. 1. (a) no addition; (b) 5 μg; (c) 12.5 μg, and (d) 40 μg. Then 5 μl of the Hind endonucleases (A) or 10 μl of purified Hpa I (gift of J. Sambrook[11]) (B) was added and the incubation was continued at 37° C for 12 h. The samples were prepared and analysed as described in Fig. 1. The dashed arrows point to fused fragments which arise from the protection of cleavage sites. The letters in parentheses indicate whether the fused fragments contain a weak (w) or a strong (s) promoter site, as judged by the amount of polymerase needed to detect their appearance. The solid arrows point to fragments which progressively disappear when increasing amounts of polymerase are tested. The sizes of the fragments are expressed in base pairs (Fig. 1).

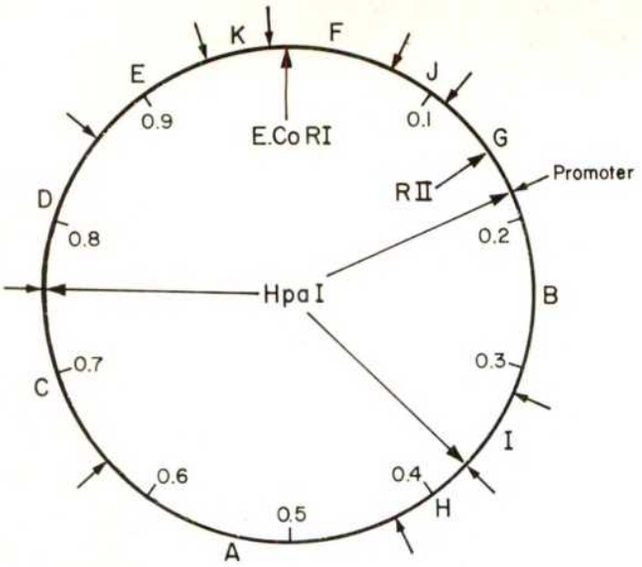


FIG. 4 Physical map of fragments in SV40 DNA. The map was adapted from Danna *et al.*[10] The arrows outside the circle indicate the sites of cleavage by the Hind endonucleases, and the resulting fragments are designated by letters (A to K)[10]. The sites of cleavage by Eco RI, Hpa I, and the site of cleavage by RII within the Hind fragment G are indicated by arrows inside the circle.

Our results support the conclusion that a class of promoters for *E. coli* RNA polymerase shares identical nucleotide sequences, at least those contained in the specific sextuplets recognised by Hind II. The strongest evidence that a part of the Hind sextuplets actually belongs to the promoter sites comes from the fact that two mutations that altered a genetically defined promoter in λ DNA, *sex*1 and *sex*3, also altered the nucleotide sequence recognised by Hind II. Clearly, sequences outside these sextuplets are also involved in specifying promoter sites, because only a small proportion of the Hind II cleavage sites can bind polymerase. The lengths of these additional sequences are unknown, but they presumably could not exceed the 47 to 52 base pairs protected from pancreatic DNase digestion when *E. coli* polymerase binds to promoters[3]. To what extent the promoter sites described here are homogeneous in their DNA sequences outside the Hind II sextuplets has yet to be determined.

It must be noted that, for λ and T7 DNAs at least, the number of RNA polymerase molecules (30–250) needed to protect Hind cleavage sites exceeded by far the number of promoters or strong binding sites (7–10) reported in these DNAs[15,19]. This indicates that either (1) only a fraction of the molecules in the polymerase preparation could bind DNA; or (2) the experiments were performed under conditions of kinetic control and reflect differences in the rate of complex formation; or (3) the experiments were performed under conditions of thermodynamic control, equilibrium was attained, and the differences reflect differential affinities; or (4) several polymerases were bound at single sites. In the latter case, however, the multiple polymerase molecules would probably not have been arrayed linearly, because polymerase binding to promoter sites only protects stretches of 47–52 nucleotide base pairs from digestion by pancreatic DNase[3]. Experiments are in progress to decide among these possibilities.

As we pointed out earlier, several promoter sites are not identified by polymerase protection of a Hind II site. Such promoters may not contain any of the Hind II sextuplets. Alternatively, they may include one of them but not bind the polymerase to a detectable extent under the conditions

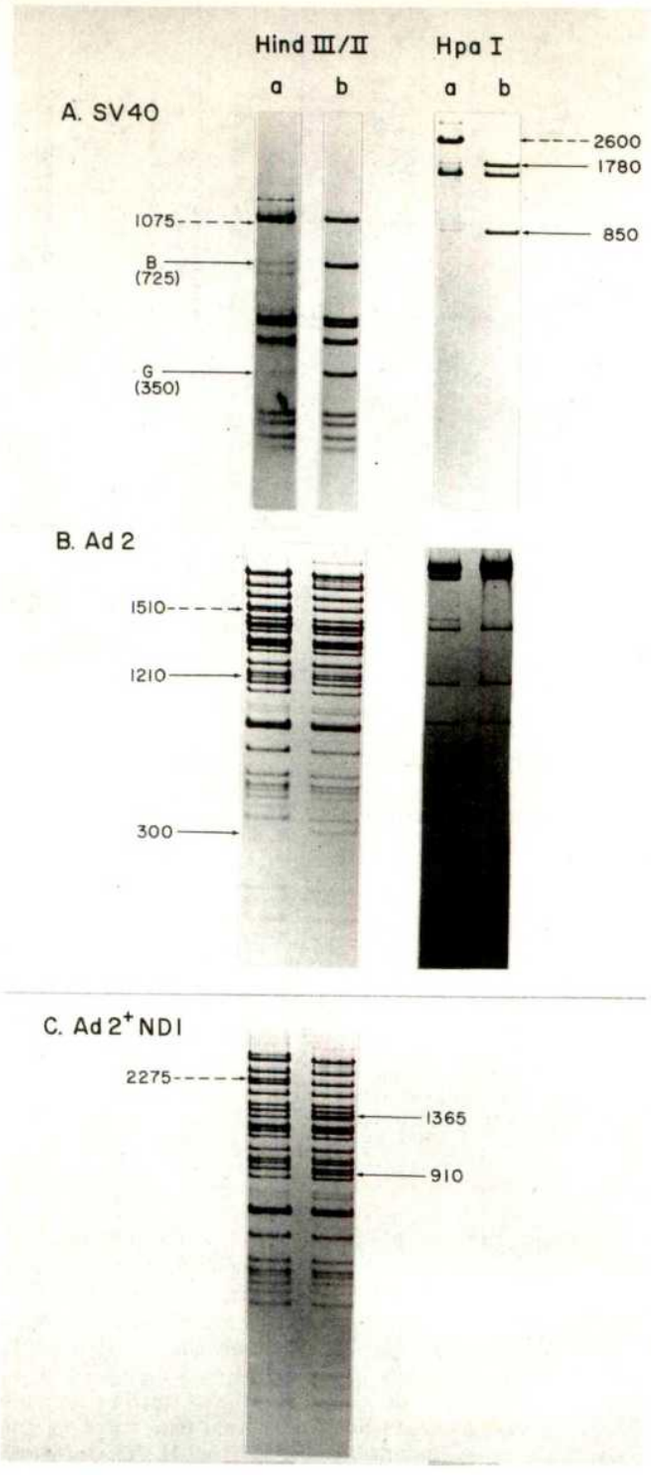


FIG. 5 Polymerase protection of sites cleaved by Hind III/II and Hpa I endonucleases in SV40, Ad2, and Ad2⁺ ND1 DNAs. SV40 (A), Ad2 (B), and Ad2⁺ ND1 (C) DNAs (10 μg) were incubated in the presence (a) or absence (b) of *E. coli* RNA polymerase (20 μg) at 37° C for 40 min as described in the legend to Fig. 1. Then 5 μl of Hind III/II was added and the incubation was continued at 37° C for 12 h. Subsequent extraction and analysis were performed as described in the legend to Fig. 1. In the case of samples subsequently digested with Hpa I (10 μl), 5 μg of each DNA was used with 20 μg of polymerase. The solid arrows indicate fragments that are present when polymerase is omitted from the reaction mixture. The dashed arrows indicate fused fragments arising as a result of polymerase protection. The sizes of the fragments are expressed in base pairs. In the Hind III/II pattern of SV40, the positions of fragments B and G (Fig. 4) are indicated.

used. The first hypothesis was verified in one case at least, that of the wild type promoter for the *lac* operon. This conclusion arose from the mapping data of the Hind II-III fragments generated from *lac* DNA in the well characterised λ*plac*5 DNA[20] (in collaboration with P. Tiollais, unpublished data). The mapping data indicate that there is no Hind cleavage site within the first 500 base pairs in the *lac* DNA, which, in this insertion, extends from the promoter (*p*) through the operator (*o*) to the end of the gene for β-galactosidase (*z*). This is a clear indication that *p* is not cleaved. Thus it seems that the promoter sites analysed in this report represent a subset only of the promoters recognised by *E. coli* RNA polymerase.

The fact that we could identify a strong promoter site for the *E. coli* polymerase in the DNAs from two mammalian viruses, SV40 and Ad2, is intriguing. Clearly, a random base distribution would not be likely to generate a sequence of this presumed complexity in DNA molecules as small as 26 million daltons (Ad2) or 3 million daltons (SV40). Thus it is tempting to believe that these sequences are of biological importance for SV40 and Ad2. In SV40 DNA, the early RNA molecules are synthesised *in vivo* from a promoter located at a site widely distant from the promoter site recognised by the *E. coli* polymerase. It seems therefore that in the early stage of the viral development the mammalian polymerase does not recognise the same promoter site as is recognised by the *E. coli* enzyme. Whether the mammalian enzyme recognises this site at some later stage is unknown. It would be of particular interest to carry out similar experiments with the mammalian polymerase.

Since this report was submitted for publication, Drs S. Weissman and K. Murray, who independently communicated this result to us, have shown that Hpa I recognises the sequence GTTAAC. This means that the sequence GTCGAC is associated with the strong binding sites for *E. coli* RNA polymerase, and the sequence GTTAAC is associated with the weaker binding sites. Furthermore, from the recent sequence analysis communicated by Dr Weissman it is now known that the starting site for transcription in fragment G of SV40 is located 29 base pairs from the Hpa I sextuplets which delimits fragments B and G.

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1 Gilbert, W., and Maxam, A., *Proc. natn. Acad. Sci. U.S.A.* (in the press).
2 Hinkle, D. C., and Chamberlin, M., *Cold Spring Harbor Symp. quant. Biol.*, **35**, 65 (1970).
3 Le Talaer, J. Y., Kermici, M., and Jeanteur, P., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2911 (1973).
4 Nakano, E., *FEBS Lett.*, **22**, 139 (1972).
5 Okamoto, T., Sugiura, M., and Takanami, M., *Nature new Biol.*, **237**, 108 (1972).
6 Heyden, B., Nüsslein, C., and Schaller, H., *Nature new Biol.*, **240**, 9 (1972).
7 Maniatis, T., Ptashne, M., and Maurer, R., *Cold Spring Harbor Symp. quant. Biol.* (in the press).
8 Sambrook, J., Jackson, A., Keller, W., Ozanne, B., Sharp, P. A., and Sugden, B., *Symp. Tumor Virus Host Cell Interaction* (North Holland, in the press).
9 Pettersson, U., Sambrook, J., Delius, H., and Tibbetts, C., *J. molec. Biol.* (in the press).
10 Danna, K. J., Sack, G. H., and Nathans, D., *J. molec. Biol.*, **78**, 363 (1973).
11 Sharp, P. A., Sugden, B., and Sambrook, J., *Biochemistry*, **12**, 3055 (1973).
12 Allet, B., and Solem, R., *J. molec. Biol.* (in the press).
13 Smith, H. O., and Wilcox, K., *J. molec. Biol.*, **51**, 379 (1970).
14 Allet, B., Katagiri, K. J., and Gesteland, R. F., *J. molec. Biol.*, **78**, 589 (1973).
15 Blattner, F. R., and Dahlberg, J. E., *Nature new Biol.*, **237**, 227 (1972).
16 Hinkle, D. C., Mangel, W. F., and Chamberlin, M. J., *J. molec. Biol.*, **70**, 209 (1972).
17 Dhar, R., Zain, S., Weissman, S. M., Pan, J., and Subramanian, K., *Proc. natn. Acad. Sci. U.S.A.* (in the press).
18 Kelly, T. J., and Lewis, jun., A. M., *J. Virol.*, **12**, 643 (1973).
19 Hinkle, D. C., and Chamberlin, M. J., *J. molec. Biol.*, **70**, 157 (1972).
20 Malamy, M. H., Fiandt, M., and Szybalski, W., *Molec. gen. Genet.*, **119**, 207 (1972).

Promoters are in the operators in phage lambda

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E. coli *RNA polymerase binds to a specific site within each λ operator. This DNA site is recognised by three proteins: RNA polymerase, the λ phage repressor, and a restriction endonuclease (Hin).*

EACH of the two operators in bacteriophage λ, O_L and O_R, consists of catenated, nonidentical repressor binding sites[1–3] (Fig. 1). At each operator, repressor first binds to a terminal site, and subsequent molecules add in the direction opposite to that of transcription of the adjacent controlled operons. Moreover, although the two operators are not identical, each contains, between the first and second repressor binding sites, the same short sequence that is cut by a restriction endonuclease (Hin) isolated from *Haemophilus influenzae*. We show here that both operators contain a specific binding site for RNA polymerase (promoter). This operator–promoter site includes the restriction endonuclease target, and we conclude that repressor, RNA polymerase, and Hin recognise shared DNA sequences. A preliminary account of related experiments was presented elsewhere[3]. Experiments similar to some of those recorded here and in ref. 3 are also reported in the accompanying article by Allet *et al.*[4]

Promoter mutations in the operators

A mutation has been described, called *sex*1[5] that decreases transcription *in vivo* and *in vitro* of the operon controlled by

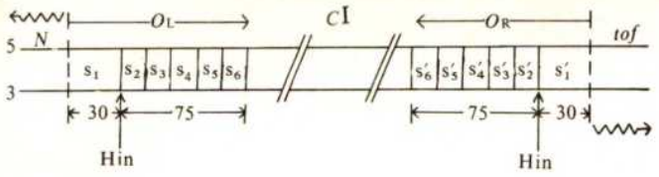


FIG. 1 Schematic representation of the λ operators and adjacent genes. The wavy arrows show the direction of transcription of the repressor-controlled genes *N* and *tof*. Two arrows indicate the order of repressor binding from the first sites (S_1 and S_1') in O_L and O_R. The Hin cutting sites in O_L and O_R are indicated along with the number of base pairs within the operators on either side of each Hin cut. The repressor gene is *cI*.

O_L[6,7]. We have shown[3] by RNA polymerase-DNA binding experiments that *sex*1 decreases the affinity of a DNA fragment bearing the leftward promoter, P_L, for RNA polymerase, and we concluded therefore that the mutation is in that promoter. We now wish to ask, where is *sex*1, and hence the promoter, located in relation to O_L?

The experiment of Fig. 2 shows that *sex*1 is located within O_L, in the sequence recognised by Hin. We compared a Hin digest of whole λ wild-type DNA with that of λ*sex*1 DNA (*a* and *b*). The figure shows that the fragments designated Hin 320 and Hin 1125, generated from λ wild-type DNA, are replaced by a larger fragment about 1,450 base pairs long in the digest of λ*sex*1 DNA. This is the only difference in the two gel patterns. To show that this difference is attributable to the *sex*1 mutation itself, and not to some irrelevant difference between λ wild-type and λ*sex*1 DNA, we isolated revertants with wild-type plaque morphology from λ*sex*1, as described in the legend to Fig. 2. The figure (*c* and *d*) shows Hin digests of examples of the two classes of revertants recovered. In one (*d*) the original Hin cutting site is restored, and in the other this site remains defective. (Three out of five revertants tested, not necessarily representing independent back mutations, lack the original Hin site.) We have shown previously[2,3] that the junction between Hin 320 and Hin 1125 is the Hin cutting site in O_L. We conclude, therefore, that the *sex*1 mutation alters the sequence of the O_L Hin site. Allet and Solem[8] have reported, and we have confirmed (unpublished), that *sex*3, an independently derived leftward promoter mutation (personal communication from M. Gottesman), also alters the O_L Hin site.

We have also found that DNA bearing a rightward promoter mutation, *x*3[9], lacks the O_R Hin site (not shown), in formal analogy to the alteration of the O_L Hin site by *sex*1. Although we have not studied x^+ revertants, this finding is consistent with other evidence, presented below, that the rightward promoter includes the O_R Hin site. (It should be noted that not all so-called x mutations alter the O_R Hin site; *x*13, for example, located to the right of *x*3[9], does not affect this site[8]. As suggested by Ordal and Kaiser[9], who presented evidence for interpenetration of operator and promoter at O_R, the mutation *x*13 may block rightward transcription by action distal to the promoter.)

Binding of RNA polymerase to Hin sites

The experiment of Fig. 3 shows that RNA polymerase binds with special avidity to the two Hin sites located in O_L and O_R. To perform this experiment we digested whole ^{32}P-labelled λ DNA with Hin in the presence of varying amounts of RNA polymerase. Most of the Hin cutting sites were unaffected by the presence of polymerase, but at the lowest ratios of polymerase to DNA used two sites were specifically protected. These two sites are those previously mapped in O_L

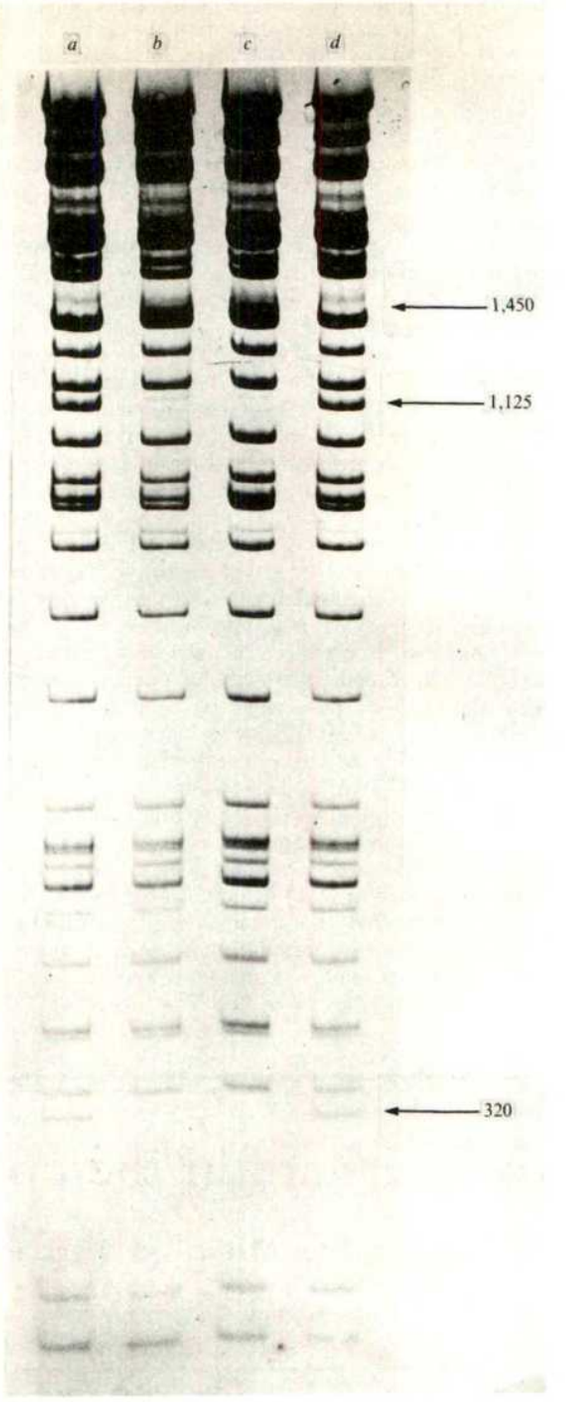


FIG. 2 Hin digestion patterns of DNA from λ, λ*sex*1 and two λ*sex*$^+$ revertants of λ*sex*1. DNA isolated from each phage (100 μg) was digested with Hin as described in ref. 3 except that no additional carrier DNA was added. Hin was isolated from the exonuclease-free strain Rd com^{-10} (ref. 13) as described in ref. 3. The digested DNA was fractionated by electrophoresis in a 3.5% TBE gel[1], and visualised by staining with 0.05% methylene blue. Sex$^+$ revertants of λ*cI*857*sex*1*S*7 were isolated by picking large plaques on an *su*III$^+$ strain, which is permissive for the growth of *S*7 phages. An *su*$^-$ strain was then lysogenised separately with these revertants, and high titre stocks were prepared by thermal induction. *a*, λ Wild type; *b*, λ*sex*1; *c*, λ*sex*$^+$ revertant lacking the O_L Hin site; *d*, λ*sex*$^+$ revertant bearing a restored O_L Hin site. The labels in the figure give the number of base pairs in the indicated fragments, as determined in ref. 3.

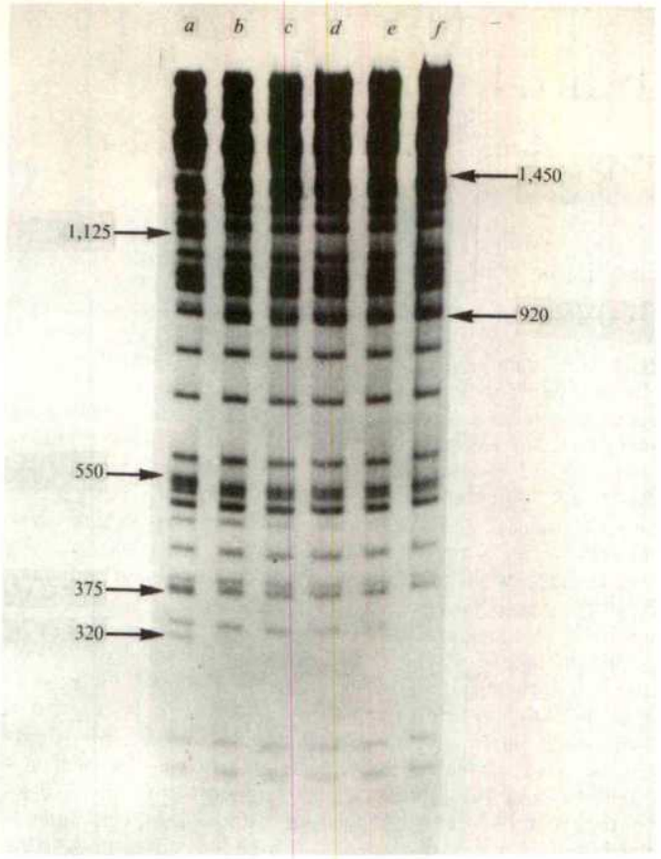


FIG. 3 Autoradiogram of λ DNA digested with Hin in the presence of various amounts of RNA polymerase. 3 μg of ^{32}P-labelled λ DNA (about 10^5 c.p.m. μg^{-1}) was incubated with RNA polymerase for 5 min at 37°C in a solution containing 50 mM KCl, 8 mM $MgCl_2$, 20 mM Tris, *p*H 8.0, 0.1 mM DTT, 5% DMSO and 0.1 mM each rATP and rGTP. Then 50 μl of Hin buffer[2] was added with Hin, and the mixture was incubated for another 60 min at 37°C. After phenol extraction the products were examined as in Fig. 2. *a*, No RNA polymerase (RNP); *b*, 1.5 μg RNP; *c*, 3 μg RNP; *d*, 4.5 μg RNP; *e*, 6 μg RNP; *f*, 7.5 μg RNP.

and O_R. Thus Hin 320 and Hin 1125, the junction of which is in O_L, were replaced by a larger fragment, and, similarly, Hin 375 and Hin 550, the junction of which is in O_R[2], were replaced by a larger fragment. Figure 3 also shows that at ratios of RNA polymerase to DNA higher than that required to protect the Hin sites in O_L and O_R, two additional sites were protected. Allet and Solem[8] suggest a location for one of these additional sites, but the location of the fourth site is as yet unknown.

The RNA polymerase protection of the O_L Hin site in whole λ DNA is consistent with data presented elsewhere showing a similar effect of polymerase on Hin digestion of a DNA fragment bearing an intact O_L[3]. However, the protection of the O_R Hin site, also observed in the experiment of Fig. 3, contradicts our earlier unexplained failure to observe protection of that site when the experiment was performed with a specific fragment bearing an intact O_R. In recent experiments with this fragment we have observed protection of the Hin site in O_R at ratios of polymerase to DNA comparable with those used in the experiments of Fig. 3 (unpublished).

Relationship of promoter to operator

The results of our experiments show that at both O_L and O_R, repressor and RNA polymerase recognise in common a specific DNA base sequence which includes a Hin restriction endonuclease cutting site. Since the Hin site occupies a corresponding position in each operator, the relationship of promoter to operator is apparently the same at O_L and O_R. These findings confirm a specific mechanism of repression at the λ operators: repressor bound to the operator prevents binding of RNA polymerase to the promoter. It is a remarkable fact that mutations in the operators can affect RNA polymerase binding without, apparently[10], affecting repressor binding.

Our results also require a reconsideration of the hypothesis of RNA polymerase 'drift'[11] between promoter and transcription start point. From the following facts we deduce that the O_L Hin site (and hence the RNA polymerase binding site) is within 30–50 base pairs of the start point of transcription of gene *N*. First, there are 30 base pairs between the O_L Hin site and the left terminus of O_L[2], and second, the left terminus of O_L is within 20 base pairs of the start point of transcription[3]. Our deduction is proved directly by DNA sequence analysis which reveals that 33 base pairs separate the O_L Hin site from the start point of transcription (Maniatis *et al.*, in preparation). We do not yet know precisely how many bases on each side of the Hin cutting site are covered by bound RNA polymerase, but since DNA-bound RNA polymerase covers about 40 base pairs[12], it is possible that polymerase bound at the O_L Hin site extends to the transcription start point. In any case it is clear that RNA polymerase 'drift', if it occurs at all, is certainly over a much shorter distance than the 100–300 base pairs previously estimated[11].

We thank Allan Maxam for RNA polymerase, David Friedman and Louis Reichardt for phage strains and Debbie Gernes and Andrea Jeffrey for technical assistance. This work was supported by a grant from the US National Science Foundation.

Note added in proof: A slightly modified interpretation of our findings, which we cannot yet exclude, is that RNA polymerase binds adjacent to (that is within a few bases of) the Hin site rather than directly over it. This scenario would require a more complicated explanation of the effects of the *sex* mutation than the one we have suggested.

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1 Maniatis, T., and Ptashne, M., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1531 (1973).
2 Maniatis, T., and Ptashne, M., *Nature*, **246**, 133 (1973).
3 Maniatis, T., Ptashne, M., and Maurer, R., *Cold Spring Harbor Symp. quant. Biol.*, **38** (in press).
4 Allet, B., Roberts, R. J., Gesteland, R. F., and Solem, R., *Nature*, **249**, 217 (1974).
5 Gottesman, M. E., and Weisberg, R. A., in *The Bacteriophage Lambda* (edit. by Hershey, A. D.), 113–138 (Cold Spring Harbor Laboratory, New York, 1971).
6 Roberts, J. W., *Nature* **224**, 1168 (1969).
7 Nijkamp, H. J., Bovre, K., and Szybalski, W., *J. molec. Biol.*, **54**, 599 (1970).
8 Allet, B., and Solem, R., *J. molec. Biol.* (in the press).
9 Ordal, G. W., and Kaiser, A. D., *J. molec. Biol.*, **79**, 709 (1973).
10 Chadwick, P., Pirrotta, V., Steinberg, R., Hopkins, N., and Ptashne, M., *Cold Spring Harbor Symp. quant. Biol.*, **35**, 283 (1970).
11 Blattner, F. R., Dahlberg, J. E., Boettiger, J. K., Fiandt, M., and Szybalski, W., *Nature new Biol.*, **237**, 232 (1972).
12 Heyden, B., Nüsslein, C., and Schaller, H., *Nature new Biol.*, **240**, 9 (1972).
13 Gromkova, R., Bendler, J., and Goodgal, S., *J. Bact.*, **114**, 1151 (1973).

Clonal cell lines from the rat central nervous system

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Five neuronal and a large collection of putative glial cell lines from the rat central nervous system have been established in clonal cell culture and partially characterised. These cells shed new light on the distribution of neurotransmitter synthesis and brain-specific antigens among nerve and glia.

ALTHOUGH the C1300 mouse neuroblastoma[1,2] has been useful for studies of the differentiation and trophic interactions of nerve cells, many more neuronal cell lines will be required before generalisations can be made about the behaviour of these cells in culture and maximum use made of the clonal culture system. This also applies to the glia and other satellite cells, whose role in the metabolism and physiology of nervous tissue is poorly understood; only a few clonal cell lines of glial origin have been described[3–5].

To establish defined cell lines from the central nervous system (CNS), cells must first be adapted to permanent culture and cloned; at present neoplasms are the only efficient source of dividing cells for this purpose. The established clones must then be sorted into cell types, for example, neurones must be distinguished from glia, and, among the glial cells, astrocytes from oligodendrocytes. We describe here an attempt to establish and characterise several clonal cell lines from the rat CNS. Of approximately 120 cell lines isolated from independently arising tumours so far, some properties of 22 clones from independently arising tumours are described.

Establishment of cell lines

Neoplasms were induced transplacentally with nitrosoethylurea (NEU)[6]. BDIX rats were injected 15 d after conception with 4.0 mg of NEU per 100 g body weight, which reduced the litter size by 50%. Between 4 and 10 months after birth, approximately half the offspring had symptoms of extreme nervous system disorders and were examined for tumours. In 93% of the animals, tumours were found in the CNS; the others had tumours in other areas. Excised tumours were minced finely in modified Eagle's medium containing 20% foetal calf serum[7], cells (including pieces of tissue) were diluted and 1×10^4–1×10^6 cells were plated on Falcon 60 mm tissue culture dishes. Each dish was examined periodically for cell proliferation, and as areas of extensive growth were found, cells were isolated by cloning rings and transferred to another dish. Since the initial explants contained cells of widely different morphologies, the most complex cell types were selected. When these cells reached confluency, they were either cloned or passaged for further study. Only one clonal cell line was initially obtained from each tumour. Of the fourteen cell lines examined, all were near diploid.

Excitability and morphology

Mammalian nerve or muscle can be distinguished from glia and most other cells, as the former have electrically excitable membranes[8]. To assay for membrane excitability, cells were impaled with a microelectrode as described elsewhere[9]. When penetration was achieved, defined as a stable resting potential greater than −30mV, the cells were monitored for electrical excitability by anode-break stimulation. Table 1 shows that at least five of the cell lines (B35, B50, B65, B103 and B104) could produce a regenerative action potential. Cells were scored as negative if there was no evidence of a regenerative response after at least twenty penetrations with resting potentials greater than −30mV. Negative data, however, can be false due to technical difficulties[10]. Although electrical excitability indicates that a minimum of five of the cell lines are of neuronal or muscle origin, on the basis of other evidence discussed later, it is unlikely that any are of muscle origin.

In some nerve and muscle cells the presence of acetylcholine (ACh) receptors is correlated with electrical excitability, and the binding of ^{125}I-α-neurotoxin can be used to detect these receptors. Using established techniques[11], the number of curare-protectable α-neurotoxin-binding sites was determined in several cell lines (Table 1). Of the twelve new lines examined, three cell lines with excitable membranes have detectable ACh receptors. Clones of the C1300 neuroblastoma and the L6 myoblast line also bind toxin, while 3T3 fibroblasts do not.

After cloning, the cells could be classified into six morphological groups (Table 1 and Fig. 1). Four of the five neuronal lines—B35, B50, B65 and B103—fall into a class with clones of C1300 neuroblastoma (group 2); they all

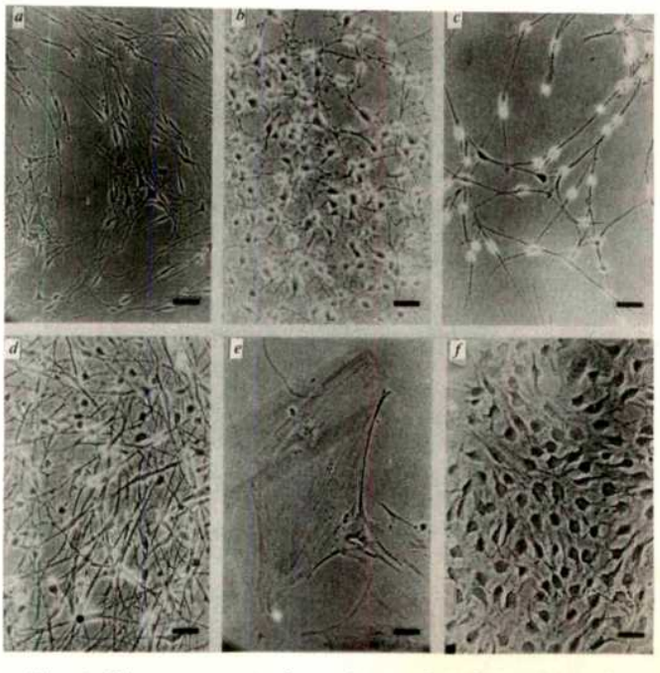


FIG. 1 Phase contrast photomicrographs of representative cell lines. The bar in each photograph represents 50 μm. *a*, B6 (group 1); *b*, B35 (group 2); *c*, B103 (group 2); *d*, B111 (group 3); *e*, B25 (group 5); *f*, B12 (group 6).

TABLE 1 Characteristics of cell lines

Line	Group	Excitable membrane	α-Neuro-toxin	O-Serum	DBcAMP	ChA	TH	GAD	GABA	AChE	BChE	AChE/ BChE	%HP	S100	14-3-2
B1	IV	−		−	−	16				<1	<1	—	4.5	<5	<50
B6	I	−		−	++	31			6.3	16	1	16		41	
B9	III			−	+++	30							4.1	21	230
B11	VI			−	++	15		<20	13	1,200	880	1.4		16	780
B12	VI	−		−	+	12	7.		2.1	540	380	1.4	2.0	49	560
B15	I	−	<1	−	++	41		<20	1.5	100	60	1.7		32	930
B19	I	−		−	±	5	19			51	15	3.4		66	<50
B23	VI		<1	−	+++	36	7	<20	5.6	1.200	860	1.4	2.6	42	330
B25	V			−	±									15	
B27	I			−	±	33				540	320	1.7	4.7	<5	80
B35	II	+	<1	++	++	40	<2	330	7.1	100	60	1.7	0.7	<5	520
B49	IV	−	<1	−	+	<4	<2	<20		1,200	830	1.4	8.2	24	500
B50	II	+	<1	++	++	<4	7	1,350	30	2,300	1,800	1.3	1.8	45	560
B56	I			−	±	<4				38	38	1	2.3	<5	<50
B65	II	+	5.4	++	++	25*	50	1,200	84	130	90	1.4	1.5	28	610
B82	II	−	<1	−	+++	24	11	<20	1.1	310	280	1.1	1.8	86	3,300
B90	V		<1	−	++	38	10	<20	1.4	70	70	1		20	
B92	III	−	<1	−	++	<4	6		2.1	5,300	4,300	1.2	3.6	21	1,120
B103	II	+	7.9	+++	+++	33	<2	1,180	29	270	130	2.1	2.2	10	450
B104	I	+	82	+++	+++	77	6	<20	6.3	160	50	3.2	4.2	17	570
B108	VI	−	<1	−	+++	<4	22	<20	3.4	3	3	1	0.7	20	1,250
B111	III			−	−	<4	8	<20		290	190	1.5	2.6	33	450
C1A	II	+	5	+++	+	15			4.2	1,100	15	76	1.4	<5	500
S20	II	+	1			400			9.4						<50
L6†	IV	+	400	−	−	10	<2	<20	<0.1	12	5	2.4	4.9	<5	<50
Brain						500	450	6,000						350	890
3T3	IV		<1	−	+	<4	<2	<20	<0.1	17	3	5.7	18	<5	<50
Liver						<4									<50
P3				−	−	<4			<0.1	4	4	1	<0.01		

With noted exceptions, stationary phase cells from passages 5 to 10 after cloning were assayed for enzyme activities, proteins and morphologies as described in the text. The amount of S100 and 14-3-2 is expressed as ng specific protein per 100 ng of total soluble protein. Bovine antigens were used in both cases; these cross react extensively with the respective rat proteins (H. Herschman, personal communication). The enzyme-specific activities are expressed as follows: esterase, using either acetylthiocholine or butyrylthiocholine as substrates, ΔA_{412} per min per mg protein; choline acetyltransferase, pmol ACh formed per min per mg protein; tyrosine hydroxylase, pmol $^{14}CO_2$ released per 20 min per mg protein; glutamic acid decarboxylase, pmol $^{14}CO_2$ released per 20 min per mg protein inhibitable by 1 mM aminooxyacetic acid. The neurotransmitter products were identified chromatographically. GABA assays were done on tricholoroacetic acid soluble cell supernatants using an amino acid analyser with expanded resolution (D.S. and W.C., in preparation). The results are expressed as residues of GABA per 1,000 residues of free amino acids. The effects of serum-free medium and 1 mM dibutyryl cyclic AMP on cell morphology were assayed on exponentially dividing cells and range from no change (−) to the greatest change (+++); an effect greater than (+) is considerable. The percentage hydroxyproline is expressed as the percentage of proline found in secreted protein which is hydroxylated (see ref. 33 for discussion and assay procedures). The binding of ^{125}I-α-neutrotoxin is expressed in 10^{-15} mol toxin bound per mg protein. The morphological groups indicate the general morphologies of the cells (see Fig. 1 and text). Electric excitability is scored as positive if regenerative action potentials are observed, and negative if there was no evidence for this event in at least 20 cells examined with resting potentials more negative than −30mV. C1A and S20 were clones of the C1300 mouse neuroblastoma[12,34], L6 was a clonal rat myoblast line[33], 3T3 was a fibroblast cell line, and P3 an immunoglobulin-secreting plasmacytoma. O-serum, the effect of serum-free medium on cell morphology; DBcAMP, the effect of dibutyryl cyclic AMP on cell morphology; ChA, choline acetyltransferase activity; TH, tyrosine hydroxylase activity; GAD, glutamic acid decarboxylase activity; GABA, residues of GABA per 1000 residues of free intracellular amino acids; AChE, esterase activity using acetylthiocholine as substrate; BChE, esterase activity using butyrylthiocholine as substrate; AChE/BChE, the numerical ratio of the two esterase activities; % HP, percentage of the total secreted proline found as 4-hydroxyproline; S100 and 14-3-2, concentrations of these proteins in cell lysates.

* Exponentially growing cells.

† All assays were done in fused, multinucleate myotubes, except for the effects of dibutyryl cyclic AMP and O-serum medium on cell morphology. Myoblasts are of group 4 morphology.

grow loosely associated with the surface of the culture dish and have relatively spherical, phase-bright cell bodies. A fifth neuronal cell line, B104, has a group 1 morphology, with very long, thin and usually bipolar cells. The other groups consist of cells with short, highly branched processes (group 6), extremely large flat cells (group 5), cells which are large and flat during the exponential phase of growth, but which branch profusely in the stationary phase (group 3), and the fibroblast-like cell (group 4). Whenever more than one cellular morphology was observed during the growth cycle, stationary phase cells were the most complex. This resembles the situation with C1300 neuroblastoma, where under normal conditions cells with long neurites are rare except in stationary phase cultures[2,10].

The C1300 neuroblastoma characteristically responds to the removal of serum and the presence of dibutyryl adenosine 3′,5′-monophosphate (dibutyryl cyclic AMP) by rapidly extending neurites[12–14]. Although dibutyryl cyclic AMP induces morphological transformations in most cells, the effect of serum-free medium on the mouse neuroblastoma seems to be specific. If this response is restricted to cultured nerve cells, only nerve lines should respond to the removal of serum with similar morphological transformations. Table 1 shows that this was the case. Thus the morphological response to low serum concentrations seems to distinguish neuronal from non-neuronal cells in this system. As expected from work on the mouse neuroblastoma and primary cultures of nervous tissue[15], all the neuronal and most of the other cell lines were transformed morphologically in the presence of dibutyryl cyclic AMP (Table 1). A more detailed account of these changes will be presented elsewhere.

Neurotransmitter synthesis

Another distinction between the classes of cells in the nervous system is provided by the enzymes involved in neurotransmitter metabolism. The activities of choline acetyltransferase, tyrosine hydroxylase, glutamic acid decarboxylase, and both acetyl and butyrylcholine esterase were measured in stationary phase cultures as detailed elsewhere[16–19]. Table 1 shows that esterase activity was widely distributed among the cell lines, varying greatly with respect to both absolute amount and the ratio of activities on acetylthiocholine and butyrylthiocholine substrates. Al-

though B6, B19 and B104 have relatively high ratios, demonstrating true acetylcholine esterase, no cell lines approached the activities of C1300 neuroblastoma clones. Choline acetyltransferase is also widely distributed among the cell lines. It has been suggested, on the basis of indirect evidence, that glia synthesise acetylcholine[20]; the data in Table 1 indicate that some of the non-neuronal cell lines in our collection have the requisite enzyme. Amino-oxyacetic-acid-inhibitable glutamic acid decarboxylase activity was restricted to the neuronal cell lines, while γ-aminobutyric acid (GABA) was found in all of the putative nerve and glial lines. Although there was no strong correlation between glutamic acid decarboxylase activity and the ability of the cells to retain GABA, these data again suggest that glial cells can synthesise neurotransmitters. Tyrosine hydroxylase was found in B65 and at least two of the non-neuronal cell lines. With the exception of choline acetyltransferase activity in B65, the data indicate the enzyme specific activities for stationary phase cells only. These activities and the concentrations of the putative neurotransmitters vary as a function of the growth cycle (B. K., H. T., D. S., manuscript in preparation).

As Table 1 shows, several neuronal cell lines contain more than one neurotransmitter synthetic enzyme. For example, B65 contains choline acetyltransferase, tyrosine hydroxylase and glutamic acid decarboxylase activities; B35 and B103 both contain choline acetyltransferase and glutamic acid decarboxylase activities. Although it is generally assumed that an individual nerve cell makes but one transmitter, the evidence for this is not complete. In particular, it is not known if the newly formed nerve cell can synthesise a spectrum of neurotransmitters before it becomes functionally coupled with another cell. Possibly the shift toward predominantly one transmitter is a result of this type of cell-cell interaction, an event not documented in these clonal cultures. Finally, it should be pointed out that the specific activities of the enzymes involved in neurotransmitter metabolism in the cell lines are lower than those observed *in vivo* (Table 1). Since transmitter enzymes may be concentrated at functional nerve endings, and their levels regulated by trophic interactions between cells, the relatively low activities presented here may again reflect a lack of such interactions in these cultures.

Proteins unique to the nervous system

Of several proteins which seem to be localised in the nervous system, S100 and 14-3-2 are thought to be associated with glia and nerve, respectively[21,22], in spite of exceptions[23–26]. Since stationary phase cells exhibited the greatest morphological complexity seen during the growth cycle, they were used for the quantitation of these proteins. (It should be pointed out, however, that the intracellular concentrations of some enzymes and other proteins depend on the stage of the growth cycle both in these and in other cell lines[27,28].) To assay for S100 and 14-3-2, cells were homogenised and the soluble cell fraction was assayed by an indirect radioimmune procedure using iodinated antigen and antisera prepared against the pure antigen[29]. Assays were routinely linearly sensitive between 1 ng and 100 ng for S100 and between 10 ng and 1000 ng for 14-3-2. (Concentrations of 14-3-2 and S100 in whole brain determined by this procedure were similar to those reported using other procedures[30,31].) Table 1 indicates that these proteins are not uniquely associated with either neuronal or non-neuronal cells. Although all the neuronal lines contain detectable 14-3-2, both proteins seem to be distributed independently among the remaining lines. Cells with undetectable levels of both proteins may not be of nerve or glial origin, an observation compatible with the fibroblast-like morphology of most cell lines in this group. These cells, which represent approximately 25% of the induced tumours, have not (with the exceptions of B1 and B56) been included in Table 1. However, cells which contain S100 or 14-3-2 and are not electrically excitable are of probable glial origin[21–26], and the presence of either protein cannot distinguish nerve from glia. Finally, all the cell lines of Table 1 were screened for neurophysin by the methods described for S100 and 14-3-2; none was found.

Some of the cell lines were also screened for secreted hydroxyproline, a marker for collagen[32]. Cells were labelled with ^{14}C-proline and the secreted protein assayed for 4-hydroxyproline[33]. Table 1 shows that all the cell lines examined secreted hydroxyproline. Although there was no absolute correlation with cell groups, these data suggest that more nerve-like group 2 cell lines secrete relatively less hydroxyproline-containing protein than the other morphological groups. This may explain the characteristic loose adhesion of the group 2 cells to the culture dish.

Conclusions from these data

We have drawn the following conclusions from our data. (1) Neoplasms of both neuronal and glial origin can be induced by established procedures in BDIX rats, and the resultant cells can be adapted to clonal cell culture. (2) At least 4% of our neoplasms yielded permanent nerve cell lines. (3) Nerve and glial cells can apparently be distinguished in cell culture on the basis of the effect of serum-free medium on cell morphology. All nerve and most glial cells undergo morphological changes in the presence of dibutyryl cyclic AMP, but only the nerve cells extend processes in the absence of serum. (4) Detectable glutamic acid decarboxylase activity is restricted to nerve lines, while low levels of GABA are also found in glial lines. Choline acetyltransferase and tyrosine hydroxylase activities are found in both nerve and glial cells. These results suggest that glia can synthesise neurotransmitters. (5) Some clonal nerve cell lines contain the enzymatic activities required to synthesise more than one neurotransmitter. (6) The nervous system proteins S100 and 14-3-2 are found in various combinations in both nerve and glial cell lines. All nerve cells contain detectable 14-3-2 while not all putative glial cells contain detectable S100.

Neuronal and non-neuronal lines can be distinguished on the basis of membrane excitability and neurotransmitter synthesis, but the sorting of non-neuronal lines has been difficult. On the basis of S100 and 14-3-2 protein synthesis, it can be argued that most of the cell lines presented in Table 1 are of either nerve or glial origin. It has been impossible, however, to group the putative glial cells on any basis except morphology. More specific criteria are needed.

Because all the cell lines in Table 1 derive from independently arising CNS tumours, they have greater functional diversity than is possible using one neoplasm. For example, clones of C1300 neuroblastoma have many different properties[34] but probably the original tumour was derived from a single cell, and the various stem cells resulted from later mutation and chromosome rearrangement[10]. In contrast, the rat nerve cell lines are not far removed from the original neoplastic cell, their karyotype approaches normal, and the clonal lines seem stable. Functional diversity and genetic stability are required if clonal lines are to be used with maximum effectiveness in the study of the mechanisms underlying the function of the mammalian nervous system.

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[1] Augusti-Tocco, G., and Sato, G., *Proc. natn. Acad. Sci. U.S.A.*, **64**, 311 (1969).
[2] Schubert, D., Humphreys, S., Baroni, C., and Cohn, M., *Proc. natn. Acad. Sci. U.S.A.*, **64**, 316 (1969).
[3] Benda, P., Lightbody, J., Sato, G., Levine, L., and Sweet, W., *Science*, **161**, 370 (1968).
[4] Lightbody, J., Pfeiffer, S. E., Kornblith, P. L., and Herschman, H. R., *J. Neurobiol.*, **1**, 411 (1970).
[5] Pfeiffer, S. E., and Wechsler, W., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2885 (1972).
[6] Druckrey, H., Preussmann, R., Ivankovic, S., and Schmahl, D., *Z. Krebsforch.*, **69**, 103 (1967).
[7] Vogt, M., and Dulbecco, R., *Proc. natn. Acad. Sci. U.S.A.*, **49**, 171 (1963).
[8] Kuffler, S. W., and Nicholls, J. G., *Ergeb. Physiol.*, **57**, 1 (1966).
[9] Steinbach, J. H., Harris, A. J., Patrick, J., Schubert, D., and Heinemann, S., *J. gen. Physiol.*, **62**, 255 (1973).
[10] Schubert, D., Harris, A. J., Heinemann, S., Kidokoro, Y., Patrick, J., and Steinbach, J. H., in *Tissue culture of the nervous system* (edit. by Sato, G.) 55–86 (Plenum, New York, 1973).
[11] Patrick, J., Heinemann, S., Lindstrom, J., Schubert, D., and Steinbach, J. H., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2762 (1972).
[12] Seeds, N. W., Gilman, A. G., Amano, T., and Nirenberg, M. W., *Proc. natn. Acad. Sci. U.S.A.*, **66**, 160 (1970).
[13] Furmanski, P., Silverman, D. J., and Lubin, M., *Nature*, **233**, 413 (1971).
[14] Schubert, D., Humphreys, S., de Vitry, F., and Jacob, F., *Devl Biol.*, **25**, 514 (1971).
[15] Shapiro, D. L., *Nature*, **241**, 203 (1973).
[16] Waymire, J. C., Bjur, R., and Weiner, N., *Analyt. Biochem.*, **43**, 588 (1971).
[17] Wilson, S. H., Schrier, B. K., Farber, J. L., Thompson, E. J., Rosenberg, R. N., Blume, A. J., and Nirenberg, M. W., *J. biol. Chem.*, **247**, 3159 (1972).
[18] Coggeshall, R. E., Dewhurst, S. A., Weinreich, D., and McCaman, R. E., *J. Neurobiol.*, **3**, 259 (1972).
[19] Schubert, D., Tarikas, H., Harris, A. J., and Heinemann, S., *Nature new Biol.*, **233**, 79 (1971).
[20] Bevan, S., Miledi, R., and Grampp, W., *Nature new Biol.*, **241**, 85 (1973).
[21] Cicero, T. J., Cowan, W. M., Moore, B. W., and Suntzeff, V., *Brain Res.*, **18**, 25 (1970).
[22] Perez, V. J., Olney, J., Cicero, T. J., Moore, B. W., and Bahn, B. A., *J. Neurochem.*, **17**, 511 (1970).
[23] Packman, P. M., Blomstrand, C., and Hamberger, A., *J. Neurochem.*, **18**, 479 (1971).
[24] Haglid, K. G., Carlsson, G. A., and Stavron, D., *Acta Neuropath.* Berl. **24**, 187 (1973).
[25] Hyden, H., and McEwen, B., *Proc. natn. Acad. Sci. U.S.A.*, **55**, 354 (1966).
[26] Sviridov, S. M., Korochkin, L. I., Ivanov, V. N., Maletskaya, E. I., and Bakhtina, T. K., *J. Neurochem.*, **19**, 713 (1972).
[27] Pfeiffer, S. E., Herschman, H. R., Lightbody, J., and Sato, G., *J. cell. comp. Physiol.*, **75**, 329 (1970).
[28] Blume, A., Gilbert, F., Wilson, S., Farber, J., Rosenberg, R., and Nirenberg, M., *Proc. natn. Acad. Sci. U.S.A.*, **67**, 786 (1970).
[29] Odell, W. D., and Daughaday, W. H., *Principles of competitive protein-binding assays*, (Lippincott, Philadelphia and Toronto, 1971).
[30] Herschman, H. R., Levine, L., and DeVellis, J., *J. Neurochem.*, **18**, 629 (1971).
[31] Cicero, T. J., and Provine, R. R., *Brain Res.*, **44**, 294 (1972).
[32] Eastoe, J. E., in *Treatise on collagen* (edit. by Ramachandran, G. N.) **1**, 1–72 (Academic Press, New York, 1967).
[33] Schubert, D., Tarikas, H., Humphreys, S., Heinemann, S., and Patrick, J., *Devl Biol.*, **33**, 18 (1973).
[34] Amano, T., Richelson, E., and Nirenberg, M., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 258 (1972).

Lunar magnetism and an early cold Moon

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Models of lunar magnetism have involved dynamo action in a fluid core in an early hot Moon; an early cold Moon magnetised some time before 4.0×10^9 *yr ago, which has subsequently heated up; and local field sources which, in some models, are related to impact. Here we examine the second possibility and show that provided the Moon contained a few percent of metallic iron and was exposed to an extra-Lunar field of about* 10 *or* 20 *oersted while much of it was still below the Curie point of iron, a restricted class of thermal evolution models, which satisfy the known constraints, can be derived.*

One model of the thermal history of the Moon (see ref. 1–6) which has gained wide acceptance, involves a Moon which accreted cold but which was heated from the outside during the final stages of accretion. This would have given rise to a molten shell very early on in lunar history, and formed the crust which is indicated by seismic studies[7–9]. The interior of the Moon, however, would have been cool during its early history and would have warmed up because of radioactive heating only relatively recently, thus forming the molten or partially molten core which is indicated seismically [7–9]. Early papers accounting for the magnetic field of the Moon suggested an initially hot Moon in which an iron core formed. Although moment of inertia constraints require that the core is small, such a core would at least behave as a magnetohydrodynamic fluid even at the present slow rotation rates[10]. There are, however, many difficulties[11] and here we consider whether an early hot outside, cold inside Moon, which evolved into the present cold outside, hot inside Moon, could quantitatively account for the evidence of an ancient magnetic field.

Origin of early magnetisation

If the Moon accreted cold then any metallic iron which was present may have acquired a remanent magnetisation from any of several mechanisms, provided there was an ambient field[12]. We consider a mechanism in which the Moon was magnetised by an external steady field, thus acquiring isothermal remanent magnetisation (IRM). If the Moon were uniformly magnetised, it would need to have had a dipole moment of about 10^{23} gauss cm^3 to give an ancient surface field of 2,000 gamma (γ) which is typical of several palaeointensity studies, although a much higher value has been reported[13–16]. This is well above the present value of less than 10^{20} gauss cm^3 (ref. 17). We have measured the IRM of iron-bearing, igneous rocks from the Moon,

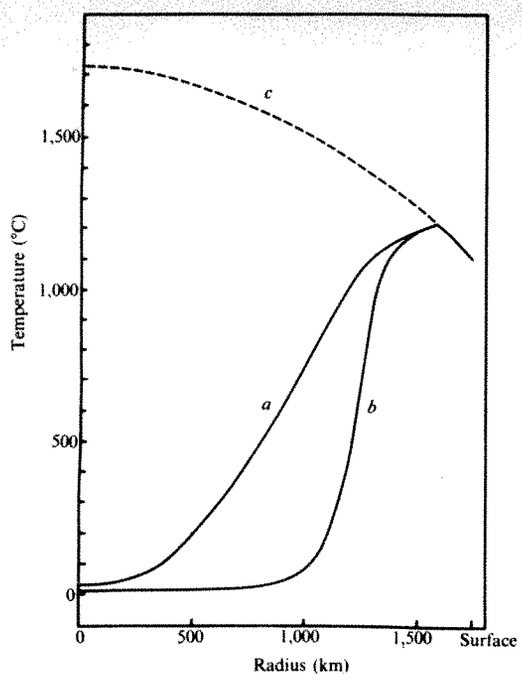


Fig. 1 Initial temperature distributions for an accreting Moon. The modified version permits the bulk of the Moon to remain below the Curie point of iron until 3.2×10^9 yr ago. *a*, Mizutani; *b*, modified; *c*, solidus.

which best represent the magnetic behaviour of rocks at depth These samples generally have a high proportion of multidomain (coarse) iron and readily acquire a soft IRM in a weak field. On the other hand, the soils are rich in superparamagnetic (~300Å) iron, and the breccias range from superparamagnetic, unwelded samples, to multidomain, highly recrystallised samples. Typically, surface igneous rocks, which contain about 0.05 weight % metallic iron, acquire an IRM of about 3×10^{-5} e.m.u. g^{-1} in a 10 oersted field[18]. By extrapolation, similar material with about 3% by weight of iron would acquire an IRM of about 2×10^{-3} e.m.u. g^{-1} in a field of 10 oersted, giving the required dipole moment. Large variations in the distribution of iron in the Moon, both laterally and vertically, could give rise to significant local variations in the field; perhaps accounting in part for the diversity of palaeointensity determinations.

Table 1 Parameter values used for lunar thermal models[1-6,28]

Model	Mass ratios Th/U	K/U	Source	Heat generation ($\times$ 317 erg g^{-1} s^{-1})	Decay constant ($\times$ 3.17 $\times$ 10^{-18} s^{-1})
Chondritic	4.15	80,000	U^{238}	2.97	1.54
Terrestrial	3.84	10,000	U^{235}	18.0	9.71
Lunar	4.00	2,000	Th^{232}	0.82	0.499
			K^{40}	0.94	5.5

Heat capacity, $cp = 10^7$ erg g^{-1} ° C^{-1}; heat of fusion, $hf = 4.0 \times 10^9$ erg g^{-1}; density, $\rho = 3.34$ g cm^{-3}; index of refraction, $n = 1.7$; opacity, $e = 100$ cm^{-1}; Stefan-Boltzmann constant, $s = 5.669 \times 10^{-5}$ erg cm^{-2} K^{-4} s^{-1}

Two questions need to be answered. First, what is the origin of the magnetising field? Second, once the Moon had become magnetised by an isothermal process could the memory have been retained until the termination of igneous activity about 3.2×10^9 yr ago. There is no definitive answer to the first question, because there are no clear records of planetary fields predating those frozen into the lunar rocks. It is, however, possible to speculate either that such fields existed early in the history of the Solar System in connection with local plasma instabilities, or that a close approach of the Moon to the Earth could have increased the turbulent motions in the Earth's core to the point at which the Earth's dynamo was much stronger than at present[13]. In either case a field of the order of 10 oersted needs to have existed, at least momentarily, some time after the bulk of the Moon had accreted.

The question of the time decay of IRM is hard to answer but extrapolation of the decay rate of IRM acquired in the laboratory in multidomain, iron-bearing rocks shows that the decay coefficient is very small[20].

Models of lunar evolution

A successful evolutionary model which will permit the Moon to retain an IRM long enough to provide a magnetic field in which the mare basalts would cool must also satisfy the following constraints.

(i) Early melting of the near surface regions, so that a crust may form by differentiation.

(ii) At least local melting in the near surface region between 3.2 and 4.0×10^9 yr ago, so as to provide a source for the mare basalts[21].

(iii) Cessation of this near surface melting no later than 3.2×10^9 yr ago because, first, there seems to have been no igneous activity after this time, and, second, the outer portion of the Moon must have cooled sufficiently to support the mascons which formed probably no later than this date.

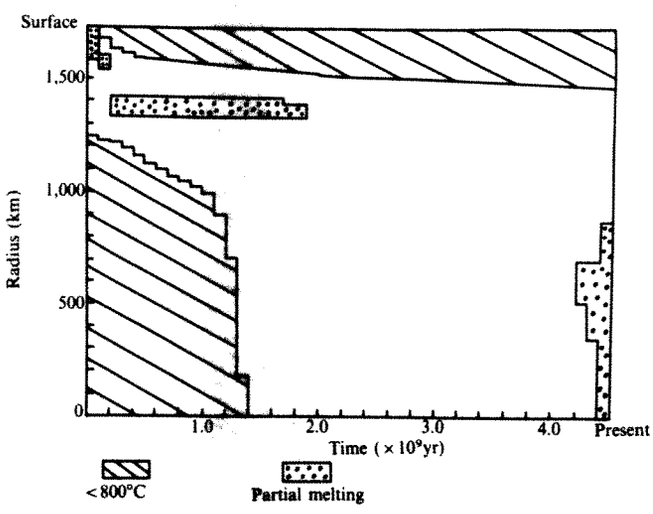


Fig. 2 Graph of radius against time showing the regions of partial melting and those regions which have temperatures below the Curie point of iron. For this model, the present-day surface heat flow is 23 erg cm^{-2} s^{-1}.

(iv) Much of the volume of the Moon must have been below 770° C (the Curie point) until 3.2×10^9 yr ago so as not to remove the magnetising field.

(v) A present day partially (or totally) molten core with a radius not exceeding 850 km, as suggested by seismic data[7-9] and predicted by Gast and Giuli[24].

(vi) The present day heat flow should lie in the range 20–30 erg cm^{-2} s^{-1} to be consistent with the Apollo 15 and 17 results[25].

In addition we have assumed that the Moon was of uniform composition at the time of accretion. We recognise this as an *ad hoc* assumption but chose to retain this simplicity until the model required otherwise. The basic initial temperature model (Fig. 1*a*) comes from Mizutani[23] and is close to his 'most likely' model, with $N_0 = 10^{-8}$ g cm^{-3} and $C = 3$ m s^{-1}, where N_0 is the

initial cloud concentration and C is the most probable particle speed for accretion. Using this particular model, the outer 150 km would be molten at the end of accretion and thus the Moon could have differentiated to give the present day crust.

The thermal history of the Moon was calculated using numerical methods but the analytical method was restricted to conduction solutions. The calculation is inherently nonlinear, because of the inclusion of a radiative term dependent upon temperature in the thermal conductivity. Details of the calculations will be published elsewhere.

The thermal conductivity used was $K + (16n^2s/3e)T^3$ where K is the lattice conductivity, T is the temperature and e the opacity (other terms are defined in Table 1). The parameters K and e are known from laboratory experiments to depend on temperature but in the range encountered in the models used here this is only a small effect[26]. The value of K was allowed to range from 0.1×10^5 to 10×10^5 erg cm^{-1} s^{-1} ° C^{-1}. The parameters used for n and e are given in Table 1.

Radioactive abundance ratios characteristic of lunar terrestrial and chondritic materials were used (Table 1). Our model does not account for any ratio changing upon fractionation. The values of uranium concentration were allowed to vary from 0.5×10^{-8} g g^{-1} to 10×10^{-8} g g^{-1}.

The calculations accounted for partial melting, total melting, differentiation of heat sources, resolidification and remelting, and convection of heat in fluid zones. The melting point criterion was that of Ringwood and Essene[27].

The Mizutani version (Fig. 1*a*) produced a Moon which was well above the Curie temperature for most of its history. We therefore arbitrarily reduced the interior temperature until suitable models could be found (Fig. 1*b*). This model implies that the outer part of the Moon accreted somewhat more rapidly, and/or the inner part more slowly than is implied by Mizutani's model. Alternatively it means that the initial temperature was less than 0° C.

The models which satisfied the constraints were almost unique in the choice of conductivity and uranium concentration. This is because of the conflicting requirements that the interior of the Moon remain cold enough not to destroy its IRM during approximately the first 10^9 yr, but that it should heat up rapidly enough during the next 3×10^9 yr so as to produce a partially molten interior out to the present radius of ~850 km. Only a very delicate balance between the thermal conductivity and uranium concentration could satisfy both constraints under the general assumption of an initially chemically and physically uniform Moon. If we relax the constraint of an early cold Moon, most of which is below the Curie point, until about 3.2×10^9 yr ago, models which satisfy the remaining five constraints can be produced. The uranium concentration is about 50 parts in 10^9, the thermal conductivity about 9×10^5 erg cm^{-1} s^{-1} ° C^{-1} and the heat flow value about 22 erg cm^{-2} s^{-1}.

The thermal conductivities tend to be slightly higher than typical experimental values for representative lunar analogues[28]. The uranium abundances are, however, very close to the average values commonly quoted for terrestrial, chondritic and lunar materials[1-6]. Finally, the indicated heat flow values all fall within the range of values determined by the lunar heat flow experiments[25]. Figure 2 illustrates that most of the Moon must remain below 770° C until about 3.2×10^9 yr ago.

We may now construct a sequence of events for the magnetic history of the Moon (Fig. 3). Initially the Moon was magnetised by a field of the order of 10 oersted when most of it was below the Curie point. The differentiation of the crust then occurred, concentrating a crust rich in anorthosite. Next, soils and breccia flows were formed primarily as a result of impacts and/or volcanic activity which enriched them in metallic iron. At or about the same time the mare basalts were formed. Finally, about 3.2×10^9 yr ago, the interior of the Moon heated up sufficiently to destroy the internal IRM, leaving only a magnetised crust. The crust could, in principle, still carry a dipole moment. The fact that none is observed could be because the Moon may have been magnetised after the crust was formed. It may also be because the crust is formed of material such as anorthosites or anorthositic gabbros which carry only a weak magnetic remanence[18]. Subsequent impacting may also have randomised directions in the crust.

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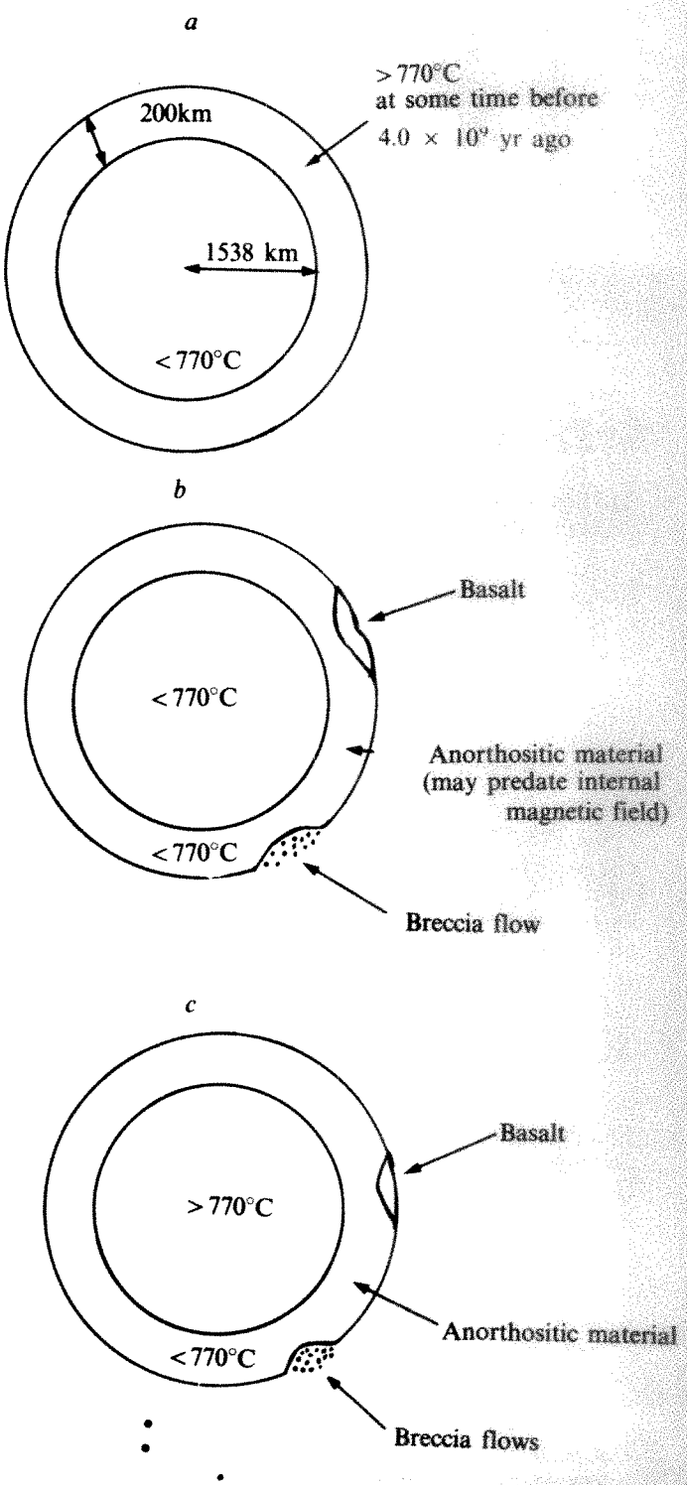


Fig 3. Schematic representation of the stages in the evolution of a cold Moon. The model presented here permits the magnetic, thermal, mean density, and moment of inertia constraints to be satisfied: *a*, before 4.0×10^9 yr ago; *b*, $4.0 - 3.2 \times 10^9$ yr ago; *c*, 3.2×10^9 yr ago.

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[1] Fricker, P. H., Reynolds, R. K., and Summers, A. L., *J. geophys. Res.*, **72**, 2649 (1967).
[2] Toksoz, M. N., Solomon, S. C., Minear, J. W., and Johnson, D. H., *The Moon*, **4**, 190 (1972).
[3] Anderson, D. L., and Phinney, R. A., *Mantles of the Earth and Terrestrial Planets*, 113 (Interscience, New York, 1967).
[4] Wood, J. A., *Icarus*, **16**, 229 (1972).
[5] Papanastassiou, D. A., and Wasserburg, G. J., *Earth planet. Sci. Lett.*, **11**, 37 (1971).
[6] Ringwood, A. E., and Essene, E., *Proc. Apollo* 11 *Lunar Sci. Conf.*, **1**, 769 (1970).
[7] Latham, G. V., *et al.*, *Apollo* 17 *preliminary Science Report*, (in the press)[8].
Nakamura, Y., Lammlein, D., Latham, G., Dorman, J., *Science*, **181**, 49 (1973).
[9] Toksoz, M. N., Press, F., Dainty, A. M., and Anderson, K., *The Moon* (in the press).
[10] Pearce, G. W., Strangway, D. W., and Gose, W. A., *Proc. third Lunar Sci. Conf.*, **3**, 2449 (1972).
[11] Levy, E. H., *Science*, **178**, 52 (1972).
[12] Runcorn, S. K., and Urey, H. C., *Science*, **180**, 636 (1973).
[13] Gose, W. A., Strangway, D. W., and Pearce, G. W., *The Moon*, **7**, 196 (1973).
[14] Helsley, C. E., *Proc. second Lunar Sci. Conf.*, **3**, 2485 (1972).
[15] Nagata, T., Fisher, R. M., Schwerer, F. C., Fuller, M. D., and Dunn, J. R., *Proc. second Lunar Sci. Conf.*, **3**, 2461 (1972).
[16] Nagata T., Fisher, R. M., Schwerer, F. C., Fuller, M. D., and Dunn, J. R., *Proc. third Lunar Sci. Conf.*, 2423 (1972).
[17] Mihalov, J. D., Sonett, C. P., Binsack, J. H., and Moutsoulas, M. D., *Science*, **171**, 892 (1971).
[18] Pearce, G. W., Gose, W. A., and Strangway, D. W., *Proc. fourth Lunar Sci. Conf.*, (in the press).
[19] Alfven, H., and Arrhenius, G., *The Moon*, (in the press). Alfven, H., *The Origin of the Solar System* (Oxford University Press, Oxford, 1954).
[20] Gose, W. A., Pearce, G. W., Strangway, D. W., and Larson, E. E., *The Moon*, **5**, 106 (1972).
[21] Mizutani, H., Matsui, T., and Takeuchi, H., *The Moon*, **4**, 476 (1972).
[22] McConnell, R. K., and Gast, P. W., *The Moon*, **5**, 41 (1972).
[23] Wood, J. A., *J. geophys. Res.*, **75**, 6497 (1971).
[24] Gast, P. W., and Giuli, R. T., *Earth planet. Sci. Lett.*, **16**, 299 (1972).
[25] Langseth, M. G., Keihm, S. J., and Chute, J. L., *Apollo* 17 *Preliminary Science Report*, (in the press).
[26] Schatz, J., and Simmons, G., *J. geophys. Res.*, **77**, (1972).
[27] Ringwood, A. E., Essene, E., *Proc. Apollo* 11 *Lunar Sci. Conf.*, **1**, 769 (1970).
[28] MacDonald, G. F., *Space Sci. Rev.*, **2**, 473 (1963).

Gravitational deflection of polarised radiation

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An upper limit is determined for the difference in the deflection of beams of orthogonally polarised radiation passing through the Sun's gravitational field. A null result is anticipated by present theories of gravitation but this prediction has never been tested.

PRESENT theories of gravitation tacitly assume that two orthogonally polarised beams of radiation will be deflected by equal amounts on passing near the Sun's limb. This hypothesis has never been tested, even though available techniques should permit measurement of a differential deflection up to 10^4 times smaller than the total deflection suffered by the radiation.

The equivalence principle predicts that orthogonally polarised radiation should show no differences in deflection by the Sun. An observer falling freely into the Sun should see all beams of radiation following straight trajectories at his own location. If radiation polarised in one sense were deflected at a rate different from oppositely polarised radiation, then initially coincident beams of mixed radiation would separate into two beams that curve away from each other. At least radiation polarised in one of the two senses would no longer appear to propagate along a straight line for a freely falling observer.

Most theories of gravitation incorporate the equivalence principle, at least in the limit of geometrical optics and weak fields, and we would expect gravitationally deflected radiation to follow paths unaffected by the state of polarisation. But we can think of situations in which polarisation must play an important role. One particularly limiting case, pointed out to us by Dr Stuart Shapiro, involves maximally rotating black holes.

Such a black hole is incapable of absorbing long wavelength radiation approaching along the hole's axis of rotation if the radiation is circularly polarised with spin angular momentum parallel to the angular momentum of the hole. This is because absorption of this radiation would endow the hole with excessive angular momentum and would add too little rest mass to the hole. As a result this radiation is not absorbed at all. In contrast, oppositely polarised radiation is readily absorbed since it merely tends to slow down the rotation of the black hole.

Although this picture is quite extreme, we would expect some measure of differentiation between left and right polarised radiation whenever long wavelength, circularly polarised radiation is scattered or absorbed by a rapidly rotating compact object (ref. 1 predicts a small Faraday-like rotation of the polarisation vector near a rapidly rotating mass). For shorter wavelengths, and larger, less rapidly rotating objects, these effects diminish, so that we again retrieve the result that gravitational effects should clearly distinguish different states of polarisation only in the diffraction limit, namely where the wavelength becomes comparable to the Schwarzschild or Kerr radius of the massive object.

We may eventually hope to make wavelength and polarisation-dependent observations that show such gravitational effects in eclipses of compact binary stars. But for the moment we have been attempting to establish, by observations, whether or not orthogonally polarised—linearly or circularly polarised—components of radiation are deflected by identical amounts in the Sun's gravitational field. Here we are clearly in the classical geometrical optics limit, and we are dealing with very low angular momentum in the deflecting mass. We would therefore expect no measurable difference in the deflection of orthogonally polarised radiation components.

Using very long baseline radio interferometry, this null result can, in principle, be determined with very high accuracy. The principal limitations are provided by the length of the available

baseline and Faraday rotation in the solar corona.

It is well known that the orthogonal polarisation components of a radio wave may be affected differently in propagation through a magnetised plasma. Faraday rotation of the plane of polarisation and plasma birefringence could obscure any differential gravitational deflection. For the present experiment the interplanetary plasma is expected to give the largest effect due to the Faraday rotation. We calculated, however, that even this Faraday rotation is negligible for solar elongations larger than $5R_\odot$. No significant plasma effects are expected from the ionosphere. Very long baseline interferometry, using a transcontinental baseline, offers a potential accuracy of almost 10^{-4} arc s at 8 GHz—of the order of one part in 10^4 of the total deflection.

If any effect is present, then the position of a small diameter radio source when viewed in one polarisation will be displaced from the position viewed with the orthogonal polarisation. This will show up as a slight change in the interferometer fringe rate between the two polarisation channels, an effect analogous to the beating of the separate components for a multicomponent source when viewed with a single polarisation.

As we are only searching for a relative displacement, the quantity to be measured is the relative phase difference between the orthogonal polarisations from the same radio source. This is independent of the local oscillator phase at each site provided that the same signal is applied to the two receivers at each radio telescope. In this way the full resolution of the interferometer can be realised.

Effectively, the observations search for an apparent splitting of the source, into orthogonally polarised components, as it approaches the Sun. Such a split can be detected far more readily than an absolute deflection relative to another radio source placed some degrees away in the sky. This second situation is the one that describes measurements of the normal deflection of radiation by the Sun. There the total change in angular separation of the sources 3C273 and 3C279 is measured to an accuracy of a few parts per million to arrive at a total deflection known to an accuracy of a few per cent.

Observations

The observations were carried out with the Green Bank-Owens Valley baseline on September 30 (day 274) and October 4 (day 278), 1972, when the radiation from these sources passes near the solar limb. The baseline is 3,900 km (10^8 wavelengths), and is nearly east–west. The Mark II VLBI tape recording system was used. The observations in Green Bank were with the Howard E. Tatel 85-foot (85-1) radiotelescope of the National Radio Astronomy Observatory (NRAO), and the frequency standard was obtained from the hydrogen maser installed at the 140-foot dish. At the Owens Valley Radio Observatory (OVRO) both A and B 90-foot radiotelescopes were used, and a Sulzer crystal oscillator, phase locked to a Hewlett Packard 5065A frequency standard, was used. Synchronisation was obtained from Loran C radio transmissions at NRAO and from the Goldstone Tracking Station at OVRO.

Different polarisations were sampled by switching 85-1 between left and right circular polarisation (LCP and RCP) every 2 s, while switching between antennae A and B at OVRO every 1 s. Antennae A and B had linearly polarised feeds, which were preset to be orthogonal. Hence in a period of 4s all four combinations of polarisation were sampled. As the coherence time of the rubidium-driven Sulzer oscillator is longer than the switching rate, relative phases between the different polarisations were easily obtained. The preliminary reduction was accomplished with the NRAO Mark II processor, and the fringes corresponding to the various polarisations were sorted by computer and combined into their respective groups. The VLBI interferometer was sensitive to dual sidebands of 2 MHz width centred at 8,075 and 8,095 MHz. Each sideband was processed separately.

In Fig. 1 the relative positions of 3C273, 3C279, and the Sun during the experiment are shown. Unfortunately, the signal-to-noise ratio for 3C279 was too small to allow us to make any convincing measurements of relative phase, although weak fringes were detected. This is due to the weak correlated flux[2] in 3C279 ($\leqslant 3$ f.u.). The correlated flux in 3C273 was about 12 f.u., and the relative phases between orthogonal circular polarisations were easily measured. On the two days of observations 3C273 was 5.0° and 7.8° from the Sun.

We have not been able to determine the phase difference in orthogonal linear polarisations due to a discrepancy, as yet not understood, in fringe rate between antennae A and B (although the change in baseline was properly taken into account). But two independent determinations of phase differences in the circular polarisations were obtained from separate analyses of the fringes produced by correlating antenna 85-1 with antenna A, and by correlating 85-1 with antenna B. For a source separated into two components, with angular separation, $\Delta\theta$, at position angle A, the fringe phase difference is $\Delta\Phi = \Delta\theta\,[(B_x\sin A - B_y\sin\delta\cos A)\sin H - (B_x\sin\delta\cos A + B_y\sin A)\cos H + B_z\cos\delta\cos A] + \text{constant}$

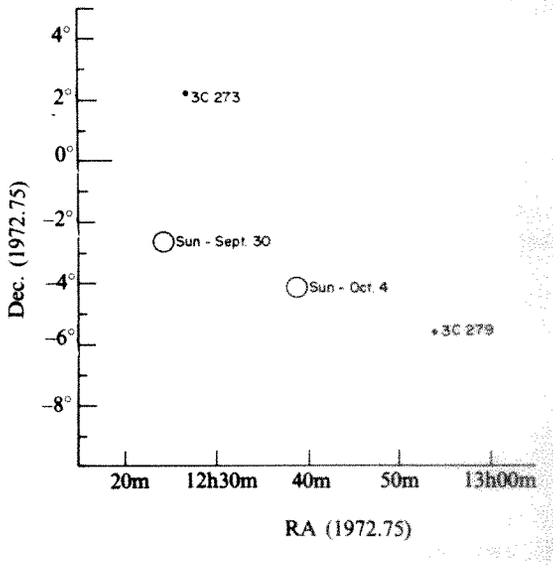


Fig. 1 Positions of 3C273, 3C279 and the Sun on the observing days.

where H is the hour angle, (α, δ) is the source position, and B_x, B_y, and B_z are the baseline components in wavelengths. Symmetry considerations lead us to expect that any separation of circular polarisation components will be perpendicular to the radial direction. Thus the position angle of the splitting, A, is orthogonal to the position angle from the Sun to the radio source. Phase differences between the orthogonal circular polarisations were calculated for scans of 15 min during the observing sessions. The data shown in Fig. 2 were fitted to the equation by the method of least squares. This was done for the B antenna experiment on October 4 and for the A antenna experiment on both days. The B antenna was not functioning properly on the first observing day. The phase differences for the upper and lower sidebands have been kept separate, since the exact times of relative phase determination, and the values of B_x, B_y and B_z differ slightly. Also the data from both days in the A antenna experiment were, in one case, taken together in the same least squares fit by arbitrarily assuming the splitting, $\Delta\theta$, to have a $1/r$ dependence on solar elongation. None of these least squares fits yielded significant splitting above the error level. Upper limits for all of these cases were obtained by calculating χ^2 for assumed values of $\Delta\theta$. The upper limits of the splitting, corresponding to the 95% confidence level are given in Table 1. It is noted that by assuming a yet stronger radial dependence

Table 1 Upper limits to the polarisation splitting

Polarisation	Observing days used	Data used	Solar elongation ($R_\odot$)	Splitting upper limits (95% confidence) Tangential $\Delta\theta$ clockwise (10^{-3} arc s)	Tangential $\Delta\theta$ counter-clockwise (10^{-3} arc s)	Radial $\Delta\theta$in (10^{-3} arc s)	Radial $\Delta\theta$ out (10^{-3} arc s)
Circular	278	Even	7.8	2.4	1.9	4.1	3.3
Circular	278	Odd	7.8	2.2	0.5	—	3.0
Circular	274	Even	5.0	27	25	336	467
Circular	274 and 278 Dependence on R neglected	Even	7.8	2.1	1.5	3.7	3.5
Circular	274 and 278 $1/R$ dependence assumed	Even	7.8	1.6	1.1	3.5	3.4
Linear	Change of polarisation technique		4 to 6			2.5×10^{-4}	2.5×10^{-4}
Circular	Faraday rotation technique		7			2.1×10^{-3}	2.1×10^{-3}

Clockwise tangential splitting is defined as that seen by an observer in which right circular polarisation appears rotated clockwise about the Sun away from left circular polarisation. In outward radial splitting right circular polarisation appears at greater solar elongation than left circular polarisation. The 'even' data are the result of correlating antennae A and 85-1, and the 'odd' data from correlating antennae B and 85-1.

than $1/r$, smaller upper limits are obtained since additional weight is effectively put on the data obtained at smaller solar elongations.

Results for circular polarisation

Symmetry considerations suggest that two circularly polarised radiation components could at best be split along a line perpendicular to the direction of the (non-rotating) deflecting mass.

As shown in Table 1, our observations set an upper limit of roughly 0.002 arc s for the difference in the deflection of the two circularly polarised components along a direction perpendicular to the solar radius vector. Along the direction of the radius vector, our upper limit is approximately 0.0035 arc s.

These upper limits are to be compared to a total gravitational deflection of ~0.2 arc s for radiation passing at these distances from the Sun.

Linearly polarised radiation

Radiation reaching us from a source that lies beyond the Sun can be decomposed into two orthogonally linearly polarised radiation components, with one polarised in a direction parallel to the radius vector from the Sun and the other polarised along a tangential direction. If these two components are not deflected identically by the Sun's gravitational field, it can be because one of the two components is slowed down more on approaching the Sun. This produces splitting along the radial direction. This situation is consistent with symmetry considerations. The component with the slower velocity is deflected more strongly, since its wavefront is retarded more strongly in the Sun's vicinity.

One way of measuring the time delay due to such a differential reduction in the velocity of propagation of the wave is to search for phase delays in the propagation of the two components. If the radially polarised component R and the tangentially polarised component T are equally strong, and the source is initially linearly polarised in a direction at 45° to the solar radius vector, then any difference in the propagation time past the Sun would make itself felt as a transition toward elliptical polarisation. If the difference in propagation time became as large as one quarter of a cycle, the initially linearly polarised wave would become purely circularly polarised. At a delay of half a cycle the wave would again be linearly polarised, but at an angle 90° removed from the initial direction (Fig. 3).

Thereafter there would be a second stage of elliptical polarisation, culminating in circular polarisation for a delay of 3/4 cycle. But the sense of circular polarisation between delays of 0 and 1/2 cycle is opposite to that between 1/2 and 1 cycle. A delay by a full cycle gives a wave linearly polarised in the initial sense, as emitted at the source. The intermediate state of elliptical or circularly polarised radiation would be right handed or left handed depending on which of the two components—the radial or the tangential—was propagated less rapidly.

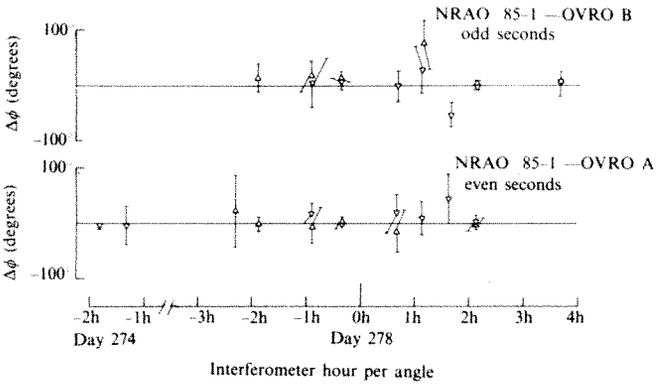


Fig. 2 Relative fringe phase between left and right circular polarisation for 3C273 is shown as a function of hour angle. Each point corresponds to approximately 15 min of observing. Upper (△) and lower (▽) sidebands are plotted separately. The arbitrary additive phase constant has been removed.

Observations carried out by Stelzried *et al.* in 1968 on the plane polarised carrier wave of 2.29 GHz (13 cm) transmitted by the solar orbiting spacecraft Pioneer 6 followed the source to within about $4R_\odot$ from the Sun. Although the polarisation emitted by the spacecraft was almost purely tangential to the solar radius vector[3], strong Faraday rotation was observed when the transmitted beam passed within about $6R_\odot$ from the Sun. By $4R_\odot$, where the signal became too difficult to follow, the polarisation angle had rotated from 90°—perpendicular to the ecliptic plane—through 0° and on to −45°.

It is virtually certain that at some time during this period Faraday rotation would have produced a polarisation angle of ~45° near the ray's closest point of approach to the Sun. Nevertheless, no ellipticity was detected, although the instrumental resolution of power axial ratio was ~0.1. This implies a maximum phase shift between the two linearly polarised components amounting to much less than 1 cm in separation. In contrast, a wave passing within $4–6R_\odot$ of the Sun is[4] delayed ~ 10^6 cm, indicating that the time delay and its associated bending should be identical for both linear polarisations to within one part in ~10^6.

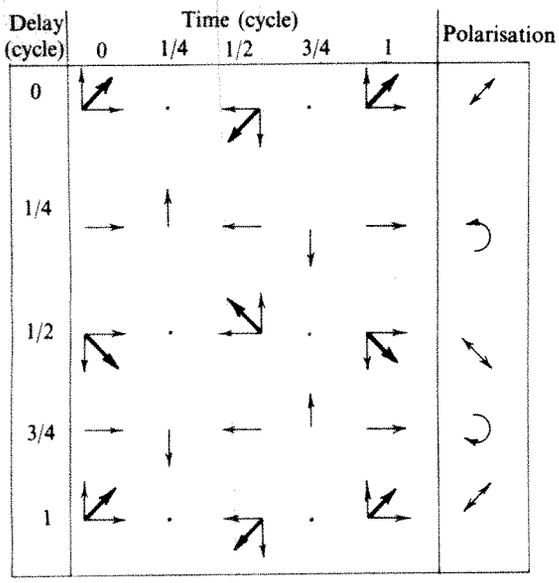


Fig. 3 The polarisation of a wave formed by the superposition of two orthogonal linearly polarised waves. The resulting polarisation depends on the phase relationship or delay between the two waves.

Beyond $7R_{\odot}$, where Faraday rotation effects seem to be negligible, the Pioneer 6 experiment also gives an upper limit on the time delay difference for orthogonally polarised circular components. This appears to be less than about one part in 10^5 of the total effect.

Space probe experiments of this type in the X band or at higher frequency could provide more stringent results.

The future

We have been able to place upper bounds on the possible difference in the deflection of orthogonally polarised radiation components passing through the Sun's gravitational field. Although these bounds are already quite stringent, order of magnitude improvements can be expected within the next few years by straightforward improvements in the techniques described here. Without extensive innovations, we may expect sensitivities to reach one part in 10^4 to 10^6 of the total gravitational deflection. Even a null result obtained in such observations should be a useful constraint for the construction of theories of gravitation.

We mentioned earlier that there should exist differential deflection effects that depend not only on polarisation but also on wavelength. These effects may be too small to measure in the deflection of radiation passing by the Sun, but we may hope to obtain significant results in astronomical observations involving eclipses of compact binary stars.

Very long baseline interferometer observations such as ours may also eventually have application to the polarisation structure of compact radio sources. Indeed one model of expanding doubles predicts that each component will have weak circular polarisation orthogonal to the other (unpublished work of R. H. Sanders). For our purposes polarisation structure can be separated from any gravitational effects by observing each source at different solar elongations. The observed lack of circular polarisation[5] in 3C273 implies that any structural features that could be detected should be much smaller than 0.001 arc s.

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Note added in proof:

After this paper had been submitted, our attention was drawn to a paper by Mashhoan[6], in which the questions discussed here are placed into a general relativistic framework. The author derives several important results that help in predicting the size of expected effects.

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[1] Skrotskii, G. B., *Soviet Phys. Dokl.*, **2**, 226 (1958).
[2] Kellermann, K. I., Clark, B. G., Shaffer, D. B., Cohen, M. H., Jauncey, D. L., Broderick, J. J. and Niell, A. E., *Astrophys. J.* (in the press).
[3] Stelzried, C. T., Levy, G. S., Sato, T., Rusch, W. V. T., Ohlson, J. E., Schatten, K. H. and Wilcox, J. M., *Solar Phys.*, **4**, 440 (1970).
[4] Shapiro, I. I., *Phys. Rev. Lett.* **13**, 789 (1964).
[5] Roberts, J. A., Ribes, J. C., Murray, J. D. and Cooke, D. J., *Nature phys. Sci.*, **236**, 3 (1972).
[6] Mashhoan, B., *Phys. Rev. D*, **7**, 2807 (1973).

LETTERS TO NATURE

PHYSICAL SCIENCES

Dust in the head of Comet Kohoutek

We have been making photographic observations of Comet Kohoutek (1973f) at the observatory of Izaña, Tenerife, with the aim of assaying the dust content of the tail. We have taken many photographs on various films and with various filters; having enlarged and printed some of the negatives we have noticed on many of them a straight dark streak running away from the head for a distance of about $2\frac{1}{2}$ arc deg. The streak is to be seen on exposures made on 1974 January 9, 10, 11 and 12 on Kodak Tri-X Pan, RAR 2495 and Eastman Kodak High Speed Infra-red (HIE-135) emulsions and with focal lengths of 85, 135 and 225 mm (Fig. 1).

We conjectured that the streak might be the shadow of the head of the comet cast on to the tail and so we computed the position angle of the Sun with respect to the comet head. We found that it agreed with the streak direction to within our limits of accuracy of measurement (about 10 arc min). We assumed that our objectives were free from distortion and that the photographs represented a stereographic projection of the celestial sphere centred on the comet's head.

If this explanation of the streak is accepted, several interesting conclusions follow:

(1) The opaque region casting the shadow has a diameter

of about 4×10^7 m (25,000 miles). This follows from the observed length of the streak and the known distance of the comet from the Earth at the time. It does not follow, of course, that there is a solid nucleus of this diameter since even sparse dust in sufficient depth can cause enough opacity.

(2) The opacity is caused by dust, not gas, and this dust has a low albedo, otherwise the comet would have been much brighter.

(3) If we assume as usual, that the particle size distribution obeys a power law $n(a) = ka^{-p}$ with $p = 3$ for small particles[1] and 4 for larger ones, and that the space density of particles varies inversely as the square of the distance from the centre of mass (or nucleus if there is a nucleus) as required by a simple continuity equation, then it is possible to calculate what we might call the 'opacity radius'—the distance from the centre of mass at which a light beam must pass in order to be attenuated by a factor 1/e. Alternatively if this radius is known then the mass enclosed within a sphere of this radius can be computed. A simple formula can be derived: $M = (2/3)a\rho D^2$, where D is the opacity radius, a is the upper limit of particle radius and ρ is the material density of the particles. Here we have apparently $D = 2 \times 10^7$ m and $a = 10^{-4}$ m (ref. 2) since the power law seems to break down for large particles; and if $\rho = 3$ then $M = 8 \times 10^{13}$ kg; $\rho = 8$ gives $M = 2 \times 10^{14}$ kg.



Fig. 1 Comet 1973f at 1945 on 1974 January 9. In white light, exposure 80 s at f/2 on Kodak Tri-X Pan film. The head had a magnitude of +3.5 at this time. The three bright stars at the top of the field are (from left to right): 46 Capricorni, ξ Aquarii, β Aquari.

These figures agree well with the estimates made by Whipple[3] for the total mass of a comet and suggest the possibility that the whole mass may be contained in the dust cloud. There would be ample surface area, 3×10^{15} m^2 if the particles are spherical, more if they are not, for the adsorption of gases and sufficient opacity to prevent them from volatilising too quickly.

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[1] Ingham, M. F., *Mon. Not. R. astr. Soc.*, **122**, 157 (1961).
[2] Finson, M. L., and Probstein, R. F., *Astrophys. J.*, **154**, 327 (1968).
[3] Whipple, F. L., in *The Moon, Meteorites and Comets*, (edit. by Middlehurst, B. M., and Kuiper, G. P), chapter 19 (Chicago University Press, 1963).

Atmosphere of Mercury

The recently released news concerning the possible atmosphere surrounding Mercury seems to have taken the astronomical community by surprise. It had generally been expected that because of the high temperatures (of the order of 450 K) existing there and also because of the low surface gravity, any atmosphere originally present, or any released from subsequent events on the surface would quickly evaporate since the random thermal speed of the atoms would be greater than the escape velocity. If V_E is the escape velocity from Mercury, ρ_A the density of its atmosphere and A the surface area, then a simple computation indicates[1,2] that the rate of mass loss from this atmosphere is

$$\frac{dM}{dt} = \frac{A\rho_A}{\sqrt{(\beta\pi)}} [1 + \beta V_E^2] \exp(\beta V_E^2)$$

where $\beta = m/2kT$, k being Boltzmann's constant and m the atomic mass. All this ignores the solar wind which carries mass into the atmosphere. In reality the problem becomes a boundary layer problem in which the magnetic field of Mercury plays a part, but we can see simply that the main effect is to generate an atmosphere. If the wind has a density ρ_W and a velocity V_W, then it adds matter to Mercury at the rate

$$\frac{dM}{dt} = AV_W \rho_W$$

and the stead state situation is given when

$$V_W \rho_W = \frac{\rho_A}{\sqrt{(\pi \beta)}} [1 + \beta V_E^2] \exp(-\beta V_E^2)$$

or

$$\frac{\rho_A}{\rho_W} = \left[\frac{\sqrt{(\pi\beta)} V_W \exp(\beta V_E^2)}{[1 + \beta V_E^2]}\right]$$

Inserting the appropriate numerical values, $\beta \approx 10^{-11}$, $V_E^2 \approx 2 \times 10^{10}$, and $V_W \approx 10^8$ (all in c.g.s. units gives)

$$\frac{\rho_A}{\rho_W} = 600$$

There should therefore be a very tenuous atmosphere, basically composed of hydrogen being maintained in this way. Any helium and argon released by radioactivity would become trapped in this atmosphere and should be detected.

If the atmosphere is signficantly denser than given above, it implies that volcanic activity or some such gas generating process must be active now.

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[1] Jeans, J., *Dynamical Theory of Gases* (Cambridge Univ. Press, 1921).
[2] Williams, I. P., and Crampir, D. J., *Mon. Not. R. astr. Soc.*, **152**, 261 (1971).

Existence of two groups in thermoluminescence of meteorites

The thermoluminescence (TL) properties of meteorites[1-4] may be applicable to the determination of exposure age, terrestrial age, thermal gradients, radiation history and preatmospheric shape. In an attempt to apply TL to the determination of terrestrial age we have discovered that meteorites can be divided into two groups, the existence of which is relevant to several of the applications.

Our apparatus consists of a molybdenum strip heated electrically at $5 \pm 0.05°$ C s^{-1} by an electronic control unit. The light emitted by 10 mg of 50 μm sieved powder placed on a defined area of the strip is measured by a cooled and shielded 9635B EMI photomultiplier tube. The amplified signal is plotted automatically against temperature. The resulting 'glow curve' has two peaks, one at about 200° C (LT) and one at about 350° C (HT).

Table 1 Details of meteorites of known terrestrial age

Meteorite	Terrestrial age (yr)	BM no. (if applicable)	Class	Thermoluminescence group
Barwell	8		L6	Low
Bruderheim	13		L6	Low
Khanpur	21		LL5	Low
Mangwendi	39		LL6	Low
Olivenza	49		LL5	Low
Saratov	55	BM1956, 169	L4	High
Crumlin	71	BM86115	L5	Low
Gambat	76	BM83864	L6	Low
Jelica	83		LL6	Low
Soko Banja	96		LL4	High
Tennasilm	101	BM1913, 218	L4	High
Butsura	112	BM34795	H6	High
Dhurmsala	113		LL6	Low
Aldsworth	138	BM61308	LL5	Low
Durala	158	BM32097	L6	High
Limerick	160		H5	Low
Wold Cottage	178	BM1073	L6	High
Mauerkirchen	205	BM19967	L6	High
Ogi	232	BM55256	H6	High

We recorded the glow curves of nineteen meteorites that were seen to fall and are therefore of accurately known terrestrial age (Table 1). Two groups were immediately apparent; one in which LT falls below HT in about 100 yr after fall, and one in which it takes several hundred years for this to occur. Although the two groups are evident by visual inspection of the glow curves they are more obvious in Fig. 1, a plot of $\log_e$ (peak height ratio, LT/HT) against the terrestrial age of the meteorite. The low retentivity group, in which LT is fading fastest, shows much scatter from the line but the high group shows surprisingly little. The cause of the scatter is probably small initial differences in the TL of the meteorites, different thermal histories since the decay is temperature sensitive and, in the case of the low group, varying degrees of shock.

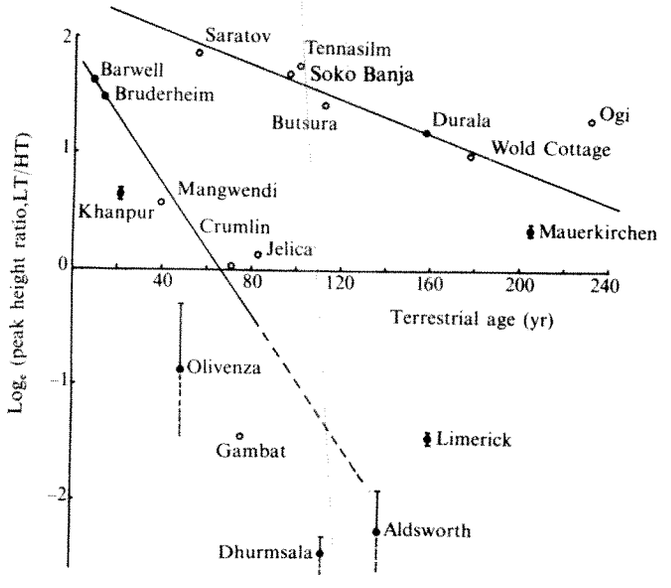


Fig. 1 Natural logarithm of the peak height ratio (LT/HT) as a function of terrestrial age, for meteorites of known terrestrial age.

To confirm that meteorites in the low retentivity group are fading faster than meteorites in the high retentivity group, the mean life (time to fall to 1/e) of LT in six meteorites was determined. We heated powder from three high and three low group meteorites in the dark at 120° C for various periods up to 70 h. Heating was done in an Abderhalden drying pistol surrounded by refluxing tetrachloroethylene. The results are shown in Fig. 2. Although the curves are not exponential, and the results must be treated as approximate, it is clear that the two groups are due to different mean lives of LT in the luminescent material. The mean life, τ, at room temperature can be calculated from

$$\tau(300\text{K}) = \tau(393\text{K}) \exp[(E/k300) - (E/k393)]$$

where E, the energy of the peak, can be found by the initial rise method and k is the Boltzman constant[5]. For the low retentivity group the mean life at room temperature is 6–8 yr and for the high group 45–55 yr.

We have not been able to determine with certainty the reason for the two groups but suspect that it is related to shock. Both metamorphism and shock change the nature of the luminescent feldspar, shock producing maskelynite and metamorphism causing recrystallisation. Chemical–petrological groups assigned on the extent of metamorphism[6] show no correlation

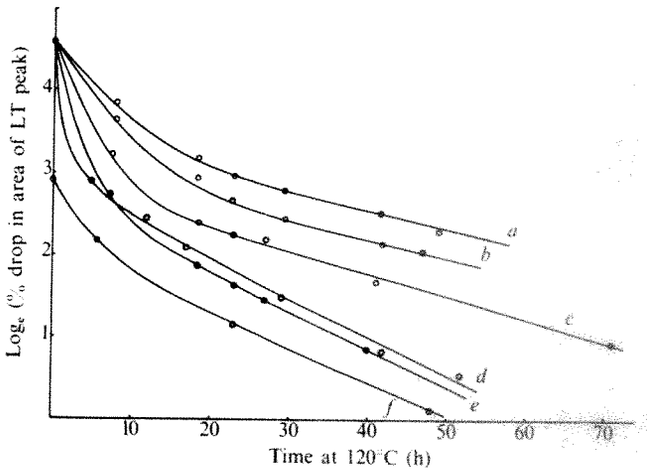


Fig. 2 Isothermal annealing curves for three high and three low retentivity meteorites. The ordinate is the natural logarithm of the percentage drop in the area under LT. Overlap has been allowed for where necessary and the curve for Jelica has been displaced vertically downwards for clarity. *a*, Wold Cottage (50–54 h); *b*, Saratov (50–55 h); *c*, Tennasilm (50–55 h); *d* Barwell (28–34 h); *e*, Bruderheim (26–34 h); *f*, Jelica (28–34 h). The numerical values are estimated for the mean lives at 120°C.

with the TL groups of the meteorites, inferring that metamorphism does not govern TL group membership. Shock is known to reduce TL intensity, however, and we have reason to believe that it also reduces the mean life of LT. Most of the members of the low group have been shocked or are breccias (R. Hutchison, personal communication). Olivenza and Barwell are anomalous in this respect, as is Soko Banja which is in the high group but is a breccia. Shock may also explain the scatter in the low group line in Fig. 1 since it can occur with various degrees of severity.

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[1] Houtermans, F. G., and Liener, A., *J. geophys. Res.*, **71**, 3387 (1966).
[2] Lalou, C., Nordemann, D., and Labeyrie, J., *C. r. hebd. Séanc. Acad. Sci., Paris*, Serie D, **270**, 2401 (1970).
[3] Chrostodoulides, C., Durrani, S.A., and Ettinger, K. V., *Modern Geology*, **1**, 247 (1970).
[4] Vaz, E., *Meteoritics*, **6**, 207 (1971).
[5] Garlick, G. F. J., *Luminescent Materials* (Clarendon Press, Oxford, 1949).
[6] Van Schmus, W. R., and Wood, J. A., *Geochim. cosmochim. Acta.*, **31**, 747 (1967).

Fission track dating of a vitric tuff from East Rudolf, North Kenya

ACCURATE dating of the Plio–Pleistocene sedimentary sequence in the East Rudolf basin of northern Kenya is urgently required to provide a time framework for the hominoid[1,2], archaeological[3,4] and other[5,6] important finds in the area. A sequence of water-lain tuff horizons are intercalated with lacustrine, deltaic and fluviatile sediments[7] and if a number of these tuffs can be dated with sufficient accuracy they could provide both the primary geochronological framework required and, by establishing correlations between East Rudolf sections and the world-wide geomagnetic reversal time scale, enable palaeomagnetic dating to be applied to those parts of the stratigraphy lying between the dated tuff horizons.

^{40}Argon/^{39}Argon age spectrum analysis by Fitch and Miller[8] has provided a firm date of 2.61±0.26 Myr for the prominent KBS Tuff horizon that occurs near the middle of the sedimentary sequence. This date is compatible with the probable age ranges of the sediments suggested by palaeontological correlation with dated sequences elsewhere in East Africa[5] and with the initial findings of the palaeomagnetic reversal study[9]. Further work by Fitch and Miller[10] indicates that straightforward K/Ar or ^{40}Argon/^{39}Argon dating of the East Rudolf tuff horizons is complicated by episodes of enhanced groundwater metasomatism which probably resulted from the periodic release of volcanic heat and/or hot fluids in the area. They suggest that although the East Rudolf succession contains clear evidence of a sequence of local, explosive ignimbritic eruptions over the period 5.0 to 0.5 Myr, in some parts of the sedimentary basin metasomatism has disturbed the argon and potassium isotope ratio of the component crystal and glass fractions of the tuffs produced by these eruptions in such a way as to cause partial and complete overprinting of the apparent ages obtained from them. The repeated occurrence of the same discrepantly low ages from different tuffs at different locations is explained as resulting from a series of distinct, short-lived overprinting events, rather than the postulation that the discrepancies result from the accumulative effect of prolonged, low energy geochemical activity in a diagenetic environment. One of the most widespread of the overprinting events recognised by Fitch and Miller occurred around 1.75 Myr (ref. 10).

We first investigated the feasibility and practical problems involved in applying fission track dating to East Rudolf tuffs and then tried to confirm or deny the presence of a widespread thermal annealing event around 1.75 Myr. The sample used was a vitric tuff from the upper part of the Kubi Algi Formation, inland from Allia Bay in the south-east of the East Rudolf basin (Kenya Grid Reference HBH 72001640). The tuff was collected by A. K. Behrensmeyer and V. J. Maglio in 1970 from section B5. A minimum age of 3.0 Myr was suggested by Maglio for the Kubi Algi formation; Fitch and Miller have proposed an age of around 3.9 Myr based on conventional K/Ar and ^{40}Argon/^{39}Argon age determinations. More precise dating was precluded by the very complex age spectrum obtained; analysis of the spectrum indicates both partial contamination by an older volcanic fraction included in the tuff and also minor discrepancies in argon loss caused by subsequent events.

Petrologically the sample used for this track study is an unconsolidated volcanic ash, largely composed of glass shards but also containing abundant calcite grains, small amounts of biotite and feldspar, and numerous minute grains of iron oxide. Only the glass shards can be regarded as definitely juvenile; microscopic examination shows the shards to be fresh, strongly vesicular and bearing no sign of devitrification.

The fission track dating method[11] and, more specifically, the use of volcanic glass in track dating[12–15] have been described previously. Uncontaminated glass shards were separated from the ash sample by hand picking beneath a binocular microscope; the shards were divided into two aliquots. One aliquot was heated to 250° C for 52 h, which was observed to anneal the spontaneous tracks present; it was then irradiated using a thermal neutron fluence of approximately 1×10^{14} neutrons cm^{-2}. The fluence was carefully monitored using NBS standard glass SRM614, with a uranium content of 0.82 p.p.m., allowance being made for the depleted ^{235}U. The natural and irradiated shards were simultaneously mounted in epoxy resin, polished and then etched in 2.75 M hydrofluoric acid for 25 min at 20° C. The two aliquots were consecutively counted using an eyepiece graticule in a Reichert Zetaplan microscope; incident illumination with a magnification of ×256 was used. The natural shards revealed tracks formed by the spontaneous fission of ^{238}U and the irradiated shards showed tracks resulting from the neutron-induced fission of ^{235}U.

Table 1 shows the resulting spontaneous and induced track densities; it presents an apparent fission track age of 1.8 Myr. No precise value for the error has been attached, since errors quoted in previous studies have been of questionable value. Considering the track counting, fluence dosimetry and the uncertainty of the value of the ^{238}U fission decay constant, a minimum value of 10% is given for the uncertainty of the date. The variation in track density between shards was about 20–30%, indicating a relatively homogeneous uranium distribution. Since fission tracks in glass are particularly susceptible to thermal annealing[16], the mean track diameters of both the induced and spontaneous tracks were measured. Comparison of these results revealed no significant difference in track diameter, indicating that the specimen has either suffered no thermal annealing or that it has been totally annealed at 1.8 Myr.

As this tuff is within the Kubi Algi Formation and is stratigraphically below the 2.6 Myr KBS Tuff, the second alternative is accepted as the correct interpretation. Thus this study has shown that fission track dating of Plio-Plistocene vitric tuffs from the Lake Rudolf area is practicable and that in appropriate circumstances this method can produce evidence of important events in the

Table 1 Spontaneous and induced track densities of vitric tuffs.

Sample and location	No. spontaneous tracks counted (1)	Spontaneous track density cm^{-2} ρ_s	No. induced tracks counted (2)	Induced track density cm^{-2} ρ_i	Fission track date (3)
AJH64 Kubi Algi SB5–R3	403	2.558×10^5	987	5.871×10^5	1.8×10^6 yr

(1) Total tracks in 100 natural shards; an area of 1,681 μm² counted in each shard.
(2) Total tracks in 100 annealed irradiated shards; an area of 1,681 μm² counted in each shard.
(3) Neutron fluence = 8.59×10^{13} neutrons cm^{-2}, induced track density in dosimeter glass 518 tracks cm^{-2}, 89 tracks counted in 10,000 fields; spontaneous fission decay constant for ^{238}U taken as 8.42×10^{-17} yr^{-1} (ref. 17).

history of the rocks, other than or in addition to the age of initial volcanism. The apparent age obtained from the sample studied indicates that it was subjected to thermal annealing around 1.8 Myr and this can be taken as additional confirmation of the geologic reality of the important overprinting event around 1.75 Myr, indicated by previous K/Ar and ^{40}Argon/^{39}Argon studies[10].

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1 Leakey, R. E. F., *Nature*, **242**, 170 (1973).
2 Leakey, R. E. F., *Nature*, **237**, 264 (1972).
3 Isaac, G., Leakey, R. E. F., and Behrensmeyer, A. K., *Science*, **173**, 1129 (1972).
4 Leakey, M. D., *Nature*, **226**, 228 (1970).
5 Maglio, V. J., *Nature*, **239**, 379 (1972).
6 Maglio, V. J., *Nature*, **231**, 248 (1971).
7 Bowen, B. E., and Vondra, C. F., *Nature*, **242**, 391 (1973).
8 Fitch, F. J., and Miller, J. A., *Nature*, **226**, 226 (1970).
9 Brock, A., and Isaac, G., in *Stratigraphy, Palaeoecology and Evolution in the Lake Rudolf Basin* (Wenner-Gren Foundation, New York, in the Press).
10 Fitch, F. J., and Miller, J. A., in *Stratigraphy, Palaeoecology and Evolution in the Lake Rudolf Basin* (Wenner-Gren Foundation, New York, in the Press).
11 Fleischer, R. L., and Hart, H. R., in *Calibration of Hominoid Evolution* (edit. by Bishop, W. W., and Miller, J. A.) (Scottish Academic Press for Wenner-Gren Foundation, New York, 1972).
12 Fleischer, R. L., Viertl, J., Price, P. B., and Aumento, F., *Science*, **161**, 1339 (1968).
13 Storzer, D., *Earth planet. Sci. Lett.*, **8**, 55 (1970).
14 Storzer, D., Horn, P., and Kleinmann, B., *Earth planet Sci. Lett.*, **12**, 238 (1971).
15 Macdougall, D., *Earth planet Sci. Lett.*, **10**, 403 (1971).
16 Storzer, D., and Wagner, G., *Earth planet Sci. Lett.*, **5**, 463 (1969).
17 Spadavecchia, A., and Hahn, B., *Helv. phys. Acta.*, **40**, 1063 (1967).

Oxidation effect on the analysis of iron in the interstitial water of recent anoxic sediments

RECENT studies on the changes in composition of interstitial water during its separation from sediments[1-3] prompted us to evaluate the effect of squeezing on the concentration of ferrous iron within the interstitial water. Sediment samples were taken in northern Chesapeake Bay (38°56′ N and 76°25′ W) with a Benthos gravity corer. Layers of sediment were immediately extruded from the plastic coring tube and loaded into nylon squeezer barrels. The interstitial water was squeezed from the sediment through a 0.22 μm Millipore filter by the pressure of nitrogen acting against a rubber diaphragm[4]. The water drained directly into a calibrated test tube containing a solution of *o*-phenanthroline, buffered at *p*H 3.6, and the iron content was determined colorimetrically[5].

During attempts to determine the precision of the analysis, we discovered that the dissolved Fe(II) concentration in the interstitial water increased during the squeezing process. Figure 1*a* shows the Fe(II) concentrations in consecutive aliquots of 3 ml as a function of volume of interstitial water emerging from the squeezer. The shape of the squeezing curve, a rapid initial rise in concentration to a constant value, was observed in every subsequent experiment.

We investigated five factors which could cause the increase of the interstitial Fe(II) concentration: filter brand, filter pore size, temperature and pressure of squeezing, and the presence of oxygen. In all of the experiments, excluding the one comparing oxygen-rich with oxygen-free conditions, all manipulations were carried out in a nitrogen atmosphere. During squeezing we found no significant differences in the concentration of dissolved Fe(II) in interstitial waters squeezed through Millipore, Gelman and Nucleopore filters, nor in waters squeezed through Millipore filters with pore sizes of 0.22, 0.10, 0.05 and 0.025 μm. Interstitial Fe(II) concentrations did not vary with squeezing temperatures of between 7 and 20° C nor with squeezing pressures of between 50 and 200 pound inch^{-2}. The only factor which appreciably affected the iron concentration was the presence of oxygen.

Although there have been several studies on the concentrations of trace constituents in the interstitial water of anoxic sediments[6-9], nobody to our knowledge has attempted to prevent the oxidation of these waters by any means other than rapid handling of the sediment. We have evaluated the importance of oxidation. Nine cores were taken in rapid succession; a layer of each core, 67–72 cm below the sediment–water interface, was extruded, homogenised and loaded into two 2.5 cm squeezers containing deoxygenated filters in a glove bag from which oxygen had been purged with nitrogen.[11,12] One of the pair of squeezers from each core was never exposed to

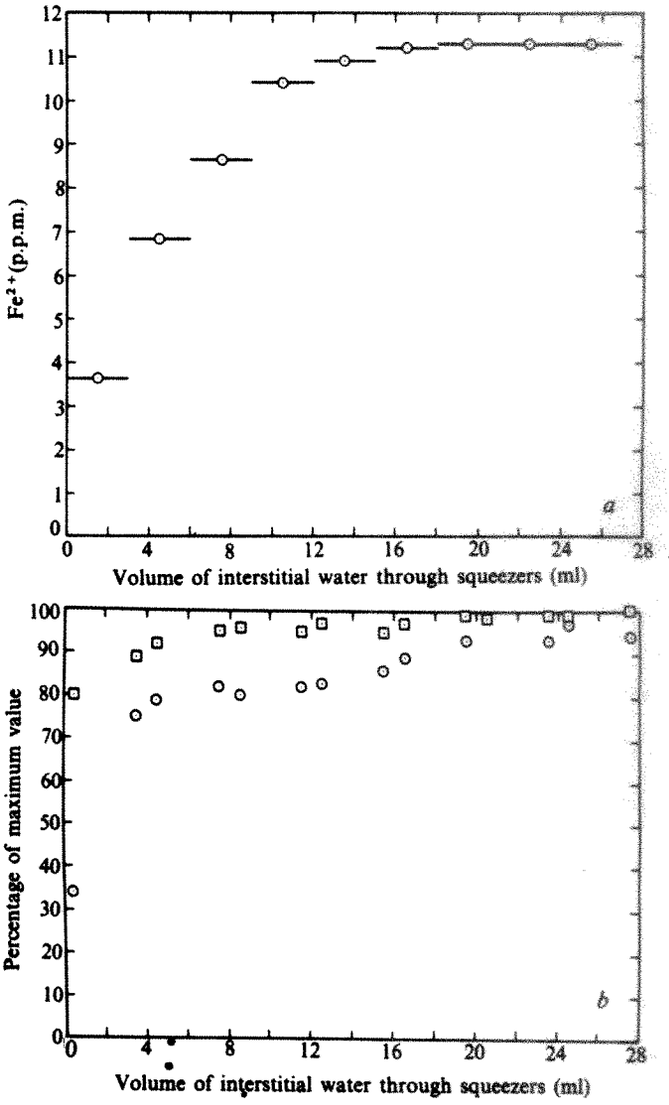


Fig. 1 *a*, Change in concentration of dissolved ferrous iron as interstitial water is squeezed. Horizontal lines indicate consecutive 3 ml aliquots analysed. *b*, Comparison of the changes in concentration during squeezing under nitrogen (□) and air (○).

the atmosphere and was manipulated under nitrogen. The second squeezer of each pair was removed from the nitrogen glove bag after loading and exposed to the atmosphere for 15 min with its top removed. Consequently, the surface of the sediment and the top of the drainage tube were exposed to oxygen for a period of time approximating rapid manipulation of a sediment core. These latter samples were then squeezed in air. Squeezing began at the same time for both the exposed and unexposed samples. An aliquot was taken simultaneously from each of the squeezers after 15 ml of interstitial water had been squeezed. In eight of the nine cores the Fe(II) concentrations in the interstitial waters squeezed under nitrogen were greater than those squeezed in air; and in five of these eight, the difference between the exposed and unexposed samples exceeded 50%. The mean of the nine samples squeezed in air was 10.0 p.p.m.; in nitrogen, 12.9 p.p.m.

We repeated this experiment using a sediment core stored at 4° C. Successive 1 ml aliquots of the interstitial water passing through duplicate squeezers were analysed for ferrous iron in order to determine the change in concentrations as a function of volume. Figure 1*b* shows the concentrations plotted as a percentage of the maximum value. The results indicate that the difference in ferrous iron concentration of the interstitial water squeezed in air and in nitrogen occurs primarily during the squeezing of the first 15–20 ml. We conclude that discrete sampling and analysis of the aliquot directly from the squeezer, under a nitrogen atmosphere, is the best method of ensuring analytical accuracy.

Inorganic oxidation of dissolved ferrous iron is rapid in the neutral *p*H range, 6.8–8.0, typical of interstitial waters from Chesapeake Bay[10]. We observed a noticeable change in colour, from black to grey, extending several millimetres into the top and bottom of sediment samples extruded and squeezed in air. This same change in colour occurred in sediment samples exposed to air and is a result of oxidation[11]. Sediment protected by a nitrogen atmosphere during the entire sampling operation remained black for the duration of the procedure.

The rapid change in Fe(II) concentration observed in the initial period of squeezing must result from the oxidation of ferrous ion to ferric hydroxide by the small amount of oxygen trapped in the squeezer during transfer of sediment from the core to the squeezer. As oxygen is consumed, the Fe(II) concentration levels off at its true value. In our samples oxidation significantly affected the concentration of Fe(II) up to values of 10–20 p.p.m. The effect on other trace metals whose concentrations fall in the parts per 10^9 range, either directly through reaction with oxygen or indirectly through interaction with amorphous ferric hydroxide[12] would be even more pronounced. Acidification of squeezed samples does not alleviate the problem because the dissolved Fe(II) is oxidised and lost from solution within the squeezer. Thus, trace metal analysis of anoxic interstitial waters rich in iron must be performed in an inert atmosphere.

We used methods of oxidation protection in an investigation of the composition of interstitial waters from a station in northern Chesapeake Bay (Fig. 2*a-f*). Phosphate was determined colorimetrically and carbonate alkalinity titrimetrically[14]. The distribution of sulphide, *E*h and *p*H were determined with electrodes. The core was sampled at intervals of 2 cm to a depth of 12 cm and then at intervals of 7.5 cm to a depth of approximately 100 cm.

Below a depth of 1 to 2 cm sediments from Chesapeake Bay are anoxic and the decomposition of organic matter enriches carbonate alkalinity and phosphate with depth in the interstitial water. The generation of sulphide from the concomitant bacterial reduction of sulphate is commonly assumed to immobilise iron as an iron sulphide. We observed, however, two layers of significant dissolved Fe(II) mobilisation into the interstitial waters throughout the sediments of the northern Chesapeake Bay: a narrow band between the surface and 10 cm, and a broad layer below 40 cm. The maximum concentrations of iron observed in the interstitial water, consistently between 500 and 1,000 μg atom l^{-1} (28–56 p.p.m.), are between one and three orders of magnitude greater than the concentrations observed in anoxic sediments by other investigators[6–9]. These

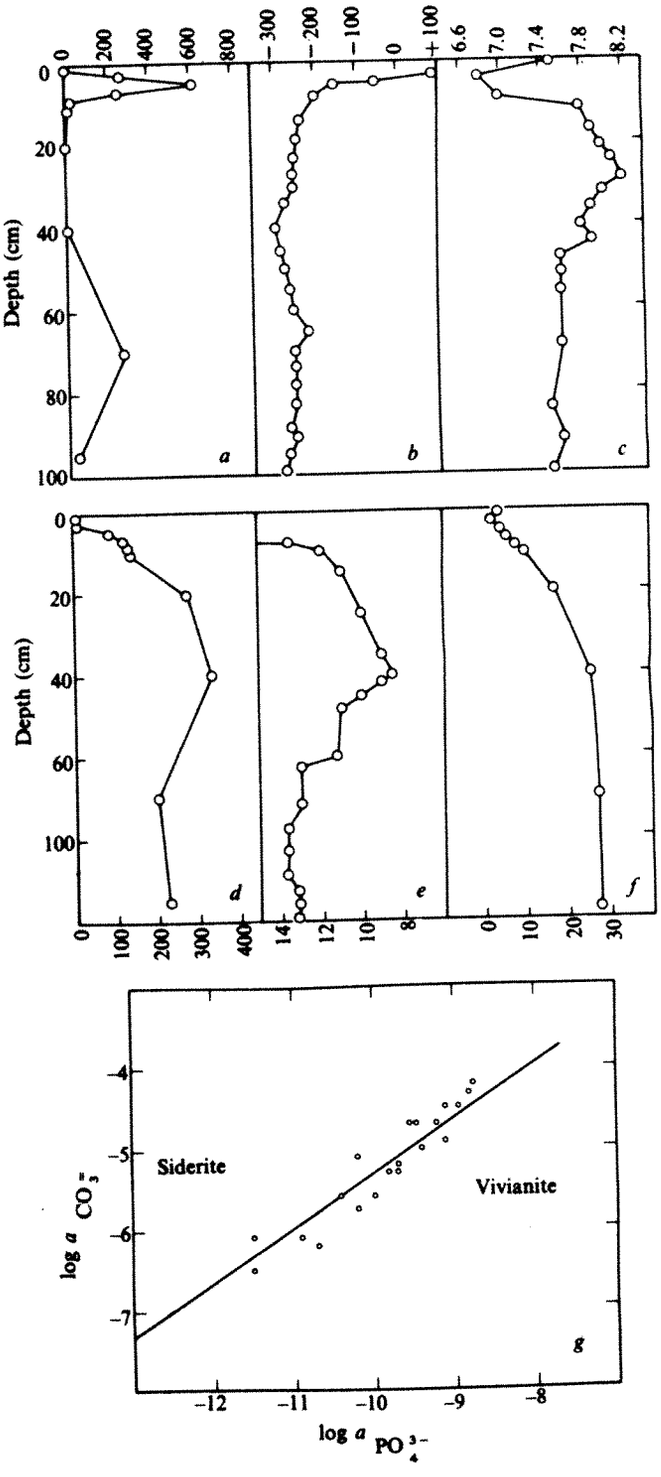


Fig. 2 Interstitial water chemistry at station 2, 38°56′08″N and 76°25′18″ W; water depth 12 m. Figures *a* to *f* are plotted as a function of depth below the sediment–water interface. *a*, Fe^{2+} (μg atom l^{-1}); *b*, *E*h (mV); *c*, *p*H; *d*, reactive phosphate (μg atom l^{-1}); *e*, PS^{2-}; *f*, carbonate alkalinity (meq l^{-1}); *g*, equilibrium among siderite, vivianite and interstitial water. The activities on the plot were calculated from the concentrations using an ion-pairing model and the Debye–Huckel equation. The speciation of all known inorganic ion pairs of importance was computed as a function of ionic strength from published stability constants; in this treatment, therefore, the activity of an ion is the product of the concentration of the free, uncomplexed ion and its individual activity coefficient.

unusually large concentrations may be due to the unique characteristics of the sediments of Chesapeake Bay or to the lack of sufficient oxidation protection in previous investigations. Between 2 and 5 cm below the sediment–water interface the strong positive iron gradient probably results from the equally strong negative *E*h gradient. Between depths of 5 and 10 cm we can pinpoint no single unambiguous cause for the rapid decrease in the ferrous iron concentration, since the concentrations of sulphide, phosphate, and carbonate, all increased rapidly.

The analytically determined concentration of a dissolved species consists of the free ion concentration plus the concentrations of that species present in ion pairs and soluble complexes[15]. The product of the free ion concentration and the appropriate activity coefficient yields the activity of the species. Our computed activities of carbonate and phosphate are plotted in Fig. 2*g*, in which the line represents the theoretical equilibrium between siderite, $FeCO_3$, and vivianite, $Fe_3(PO_4)\cdot 8H_2O$ (refs 16, 17). The close correspondence between the data points and the equilibrium line suggests that in these pore waters the activities of Fe(II), carbonate, and phosphate are co-dependent. Further, the close negative correlation between the Fe(II) and sulphide distributions suggests that sulphide may be controlled by iron in these iron-rich sediments.

In anoxic sediments, therefore, the distribution of Fe(II) in the interstitial water can be a sensitive indicator not only of general redox conditions but also of the chemical equilibria of certain nutrients. We conclude that the handling of anoxic sediments and the analysis of iron-rich pore waters must be carried out in an inert atmosphere if accurate results are to be obtained.

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[1] Mangelsdorf, P. C., Wilson, T. R. S., Daniell, E., *Science*, **165**, 171 (1969).
[2] Bischoff, J. L., Greer, R. E., and Luistro, A. D., *Science*, **167**, 1245 (1970).
[3] Fanning, K. A., and Pilson, M. E. Q., *Science*, **173**, 1228 (1971).
[4] Reeburgh, W. S., *Limnol. Oceanogr.*, **12**, 163 (1967).
[5] Sandell, E. B., *Colorimetric Determination of Traces of Metals*, 537 (Interscience, New York, 1959).
[6] Presley, B. J., Brooks, R. R., and Kaplan, I. R., *Science*, **158**, 906 (1967).
[7] Brooks, R. R., Presley, B. J., and Kaplan, I. R., *Geochim. cosmochim. Acta*, **32**, 397 (1968).
[8] Callendar, E., in *Proceedings of the 12th Conference on Great Lakes Research*, 124–160 (University of Michigan, Great Lakes Research Division, 1969).
[9] Presley, B. J., Kolodny, Y., Nissenbaum, A., and Kaplan, I. R., *Geochim. cosmochim. Acta*, **36**, 1073 (1972).
[10] Stumm, W., and Lee, G. F., *Ind. Engng. Chem.*, **53**, 143 (1961).
[11] Biggs, R. B., in *Estuaries* (edit. by Lauff, G. H.), 239 (American Association for the Advancement of Science, Publication No. 83, 1967).
[12] Jenne, E. A., in *Trace inorganics in water* (edit. by Gould, R. F.), 337 (American Chemical Society, 1968).
[13] Harwood, J. E., van Steenderen, R. A., and Kuhn, A. L., *Water Res.*, **3**, 417 (1969).
[14] Whaley, R. C., Carpenter, J. H., and Baker, R. L., *Chesapeake Bay Institute Special Report*, **12** (John Hopkins University, Baltimore, 1966).
[15] Garrels, R. M., and Thompson, M. E., *Am. J. Sci.*, **260**, 57 (1962).
[16] Garrels, R. M., and Christ, C. L., *Solutions, Minerals, and Equilibria*, 413 (Harper and Row, New York, 1965).
[17] Nriagu, J. O., *Geochim. cosmochim. Acta*, **36**, 459 (1972).

Spirorbid — algal stromatolites

Modern stromatolites may be formed in certain environmental settings by several different processes and organisms[1], although they are predominantly produced by blue-green algae. Algal stromatolites occur in the Lower Triassic Bunter formation (Buntsandstein) in the Krosno Odrzańskie region of western Poland. Spirorbid tubes often occur in the immediate vicinity of these stromatolites (Fig. 1), which are usually found unattached. Because spirorbid tubes are generally attached to

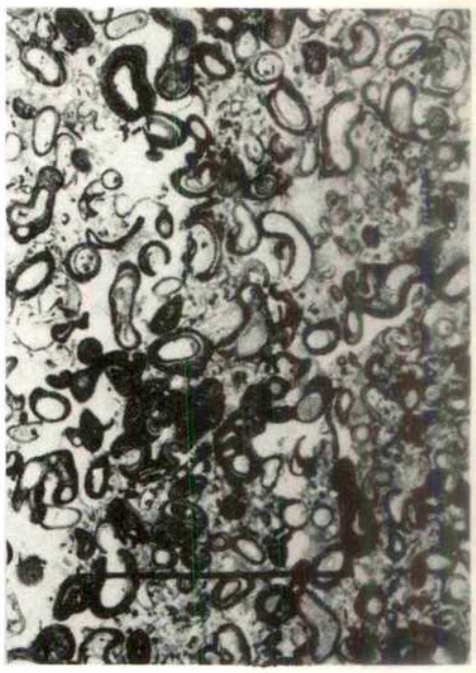


Fig. 1 Photomicrograph of organodetritic carbonate. Fossils are predominantly spirorbids. Some spirorbid tubes form oolitic nuclei. Scale bar, 1 cm.

submerged objects, mainly shells and pelagic and sedentary algae[2], such unattached occurrences seem to indicate that the spirorbids lived on algae.

Associations of stromatolites and spirorbids[3] or serpulids[4] have been found before. Garwood[3] mentions that in the Lower Carboniferous Main Algal Series of Tuedian Beds in Cumberland and Roxburghshire, the surfaces of small domelike

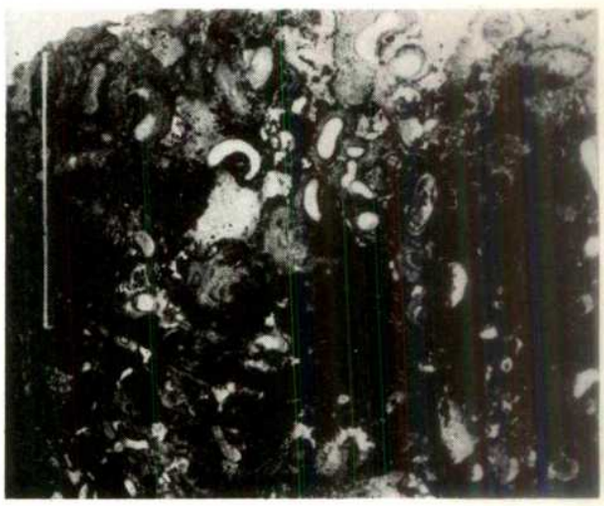
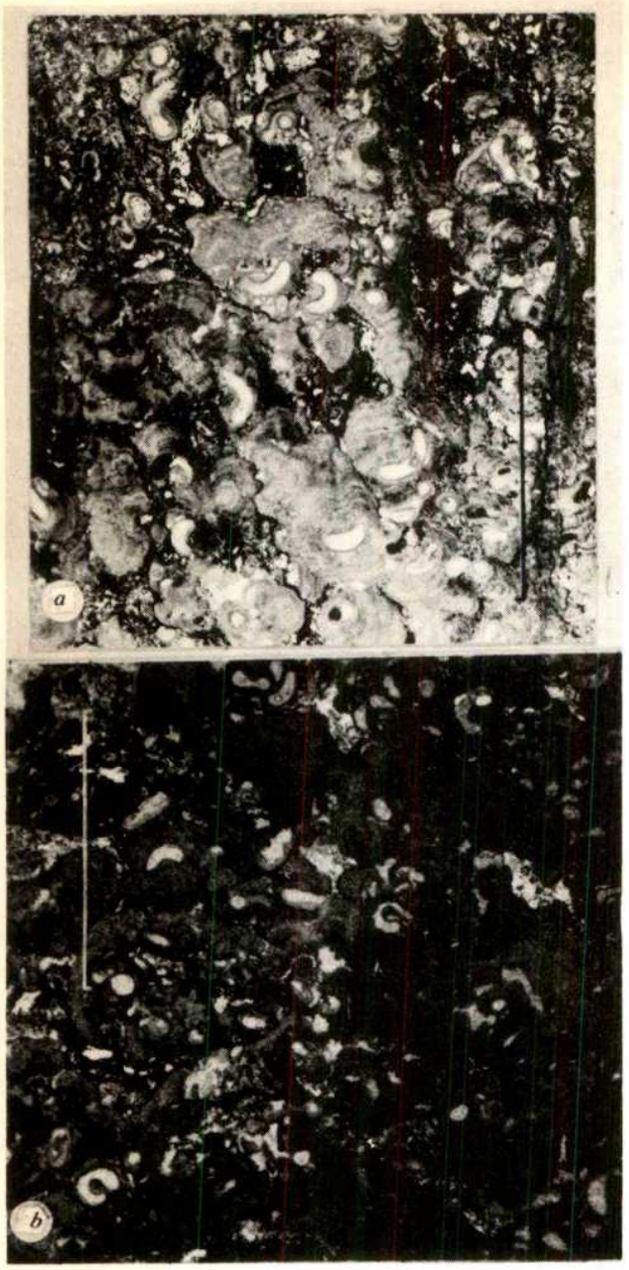


Fig. 2 Photomicrograph of spirorbid and algal-built stromatolites. Larger stromatolite fragments are found in any position. Subsequent algal laminae on the top of the debris. Scale bar, 1 cm.



Figs. 3 *a* and *b* Photomicrograph of spirorbid and algal-built stromatolites. Scale bar, 1 cm.

'protuberances' (stromatolites?) are frequently encrusted with adherent tubes of *Spirorbis*. Spirorbids also occur within the Bunter stromatolites (Figs 2 and 3). The stromatolitic structures developed on spirorbid tubes, and the detritus may contain small amounts of spirorbid fragments. As a rule, however, spirorbids occur in great number inside stromatolites (Figs 2 and 3) which are built not only by algae but also by spirorbids themselves. In contemporaneous worm–algal stromatolites from the Persian Gulf[5] burrowing worms build mounds which may be coated with algae but the worms do not enter into the framework of the stromatolitic structure.

The unique nature of spirorbid–algal association in the Krosno Odrzańskie region is best explained as resulting from the particular environmental conditions prevalent during the deposition of the Bunter stromatolite-bearing rocks. The Bunter algal stromatolites originated in upper subtidal and lower peritidal zones. Stromatolites only occur sporadically in the upper peritidal zone. Tidal influence on sedimentation is reflected in changes of stromatolitic structures. These can best be interpreted as resulting from repeated periodic events: influxes of coarse sediment, commonly evident in stromatolitic sequences, could have resulted from periodic catastrophic erosion and deposition. Crust-building communities lived in such tidal marine environments. Flakes and chips of stromatolites are common in the interareas of stromatolitic structures (Fig. 2). Larger stromatolite fragments also occur as clumps which have been rolled and are found in any position, commonly on their sides or upside down (Fig. 2). On top of the debris later colonies of algae may develop (Fig. 2). Stromatolitic intraclasts and characteristic sequences of stromatolitic structures indicate a high energy environment.

The spirorbid–algal community therefore seems to be an ecological adaptation to a rather high energy environment. The spirorbids probably reinforced the structure of spirorbid–algal stromatolites, which were therefore more resistant to the erosional effects of waves and currents than were ordinary algal stromatolites.

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[1] Logan, B. W., Rezak, R., and Ginsburg, R. N., *J. Geol.*, **72**, 69 (1964).
[2] Brönnimann, P., and Zaninetti, L., *Riv. ital. Paleont. Stratigr.*, **78**, 68 (1972).
[3] Garwood, E. J., *Q. J. geol. Soc. Lond.*, **87**, 111 (1931).
[4] Leeder, M. R., *Nature*, **242**, 41 (1973).
[5] Shinn, E. A., *J. sedim. petrol.*, **42**, 837 (1972).

Infrared Zimm plots for macromolecular characterisation

The light scattering method is an accepted procedure for the measurement of the molecular weight (M) and the size of macromolecules in solution. This information is most readily available for particles the major dimension (ξ) of which is of the order of the wavelength (λ) of the light incident on the system. Provided that they are not too optically dense, such particles satisfy the conditions

$$|m-1| \ll 1 \text{ and } \frac{2\pi\xi}{\lambda}|m-1| \ll 1$$

and are described in terms of the Rayleigh–Gans–Debye[1,2] (RGD) scattering theory. In this equation, m is the ratio of the refractive index (n) of the solute to that of the solvent (n_0).

If the particles are relatively small in comparison to λ (that is $\xi < \lambda/20$) then the simple Rayleigh[1,2] theory is applicable but no information is obtained about the particle size. Alternatively, if the particles are too large the scattered intensity becomes an oscillating function of θ and complicated interference effects occur. For this case, a general solution has been developed[3], but only for spheres.

With the improvement of sources and detectors which operate in the infrared, it is possible to increase the wavelength of scattering experiments so as to bring some particles which exhibit interference peaks in the visible region into the realm of the RGD theory, at least over a restricted angular range. Measurements can then be made on readily modified commercial apparatus and the data can be analysed according to the straight forward Zimm plot[1] method. The radius of gyration (S) and M can then be directly determined, even with polydisperse samples. An alternative procedure[4], that of measuring at extremely low angles θ to the forward direction of the incident beam, requires a purpose built apparatus.

We are at present engaged in a study of the scattering from aqueous suspensions of bacteria, with dimensions typically of the order 1 – 2 μm. Preliminary studies on *Serratia marcescens* provide an example. Figure 1 shows that this system exhibits

angular scattering peaks in the visible spectrum, even at the lowest values of θ accessible to a commercial scattering instrument. The source of our scattering photometer, a SOFICA 4200, was replaced by a continuous working Nd^{3+} YAG laser which operates at $\lambda = 1{\cdot}06$ μm. The detector was replaced by a combination of an infrared converter tube (type EMI 9606) and a 13-stage photomultiplier (type EMI 9635QB). Infrared-sensitive photomultipliers are a simpler alternative to this arrangement. The light trap was modified so that measurements could be made up to $\theta = 10°$ and an oscilloscope was used to record the photomultiplier response. These are all relatively trivial changes. The Zimm plot (Fig. 2) was then obtained from data for four different dilutions of the bacterial suspension. The experimental precision was commensurate with that of visible scattering experiments.

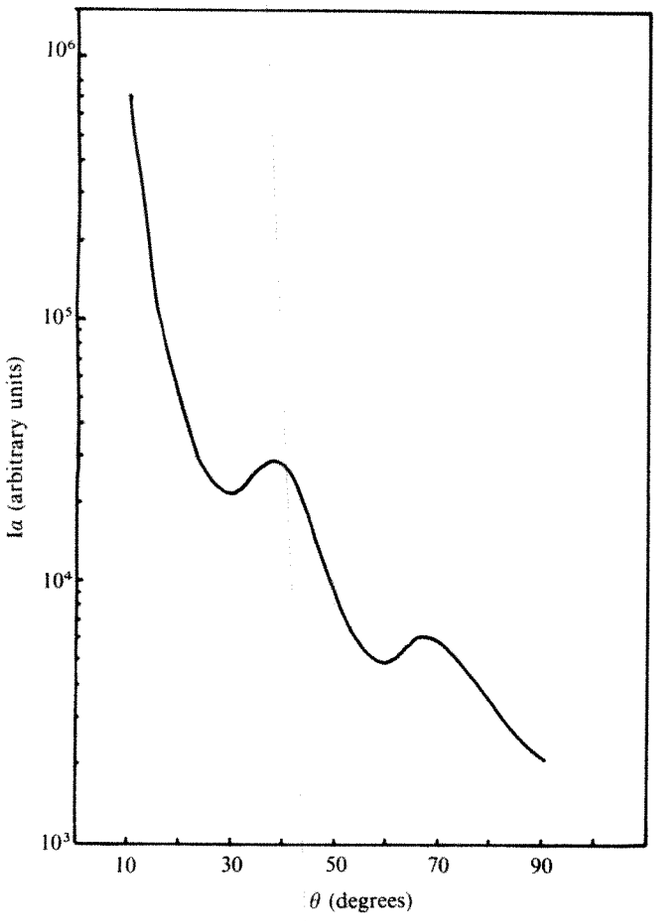


Fig. 1 Part of the angular scattering profile for *S. marcescens* in water. Data for $\lambda = 436$ nm and a suspension concentration of $2{\cdot}4 \times 10^{-6}$ g ml^{-1}. Oscillations are present even at low angles. The factor $\alpha = \sin\theta\,(1+\cos^2\theta)^{-1}$.

A value of 0·18 was assumed[5] for the refractive index increment ($^{dn}/_{dc}$), necessary for the interpretation of the plot intercept[1]. This value is strictly for the visible region of the spectrum and does not vary much with wavelength provided that no absorption band exists between visible wavelengths and 1·06 μm. This was verified experimentally. We are developing a method for the direct measurement of ($^{dn}/_{dc}$) in the infrared region. From Fig. 2, values of $M_W = 1{\cdot}0 \times 10^{11}$ and $(\overline{S^2})_z^{\frac{1}{2}} = 0{\cdot}34$ μm were determined. The latter is equivalent to a spherical diameter $\bar{d}_z$ of 0·88 μm. The subscripts refer to the relevant polydispersity averages. These values correspond to an average bacterial mass of $1{\cdot}7 \times 10^{-13}$ g. This mass and the particle size agree with other experimental data for this system[6].

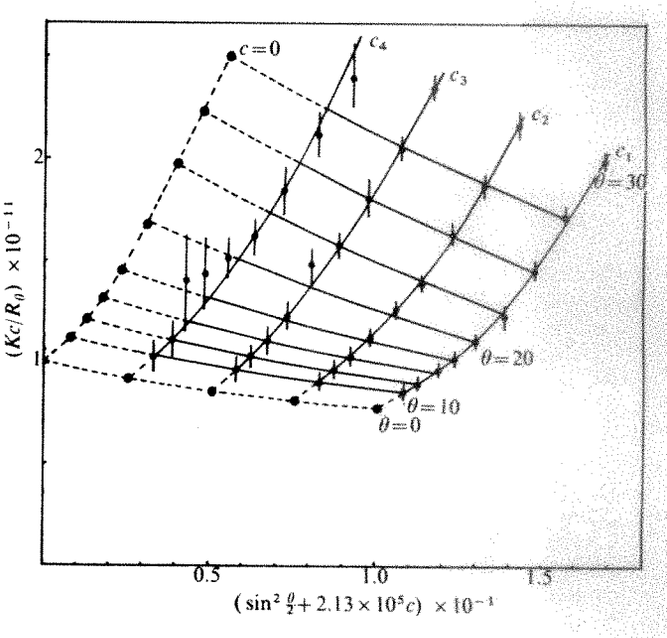


Fig. 2 Zimm plot for *S. marcescens* at $\lambda = 1{\cdot}06$ μm. C was the stock concentration of $4{\cdot}7 \times 10^{-7}$ g ml^{-1}. The dilutions were as follows; $c_1 = C$, $c_2 = 0{\cdot}75C$, $c_3 = 0{\cdot}50C$, $c_4 = 0{\cdot}25C$.

We therefore conclude that with the advance in infrared lasers and instrumentation it is possible to adapt conventional, commercial scattering photometers to work in the infrared. Provided that neither solute nor solvent are absorbing at the selected wavelength, particles which are too large to be studied through the RGD scattering methods in the visible region of the spectrum conform to the regular Zimm plot procedure when the incident wavelength is in the infrared. Their molecular weights and sizes may then be evaluated with relative ease.

Finally, the method can be extended well beyond the wavelength of the present preliminary experiment. For example, by modifying the optics, the present experiments could be extended to the 10·6 μm wavelength of the CO_2 laser. Airborne particles could then be studied. The combination of both increased wavelength and low angle measurement is doubly beneficial.

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Received January 8, 1974.

1 Stacey, K. A., *Light Scattering in Physical Chemistry* (Butterworths, London, 1956).
2 Van de Hulst, H. C., *Light Scattering by Small Particles* (Wiley, New York, 1957).
3 Mie, P., *Ann Phys.* **25**, 377 (1908).
4 Kaye, W., *J. Colloid Interface Sci.* **44**, 384 (1973).
5 Davies, H. G., Wilkins, H. F., Chayen, J., and La Cour, L. F., *Q. Jl microsc. Sci.* **95**, 271 (1954).
6 Morris, V. J., and Jennings, B. R., *Proc. R. Soc.* (in the press).

Fossils in mélanges

So-called 'tectonic' mélanges in many parts of the world seem to contain a disproportionately high number of fossils relative to the enclosing rocks. This is particularly true of mélanges in 'greywacke type' terrains, in particular the Esk Head Mélange[1] in the otherwise sparsely fossiliferous Torlesse Zone of the Alpine Assemblage[2] in the New Zealand Geosyncline, the mélange belts of the Franciscan Assemblage of California (M. C. Blake, jun., personal communication), and the Liptrap Formation of southern Victoria, Australia. In addition, the fossils contained in mélange belts commonly contain shallow water forms, and in extreme cases even terrestrial fossils (D. L. Jones, personal communication).

These facts alone seem to preclude postdepositional tectonic modification of part of a sedimentary sequence as the sole means of mélange formation. Rather, we suggest that the processes leading to the formation of at least some mélange belts resulted in the development of localised, specialised sedimentary environments during deposition, probably in shallow water and commonly accompanied by submarine volcanism. Continuation of the same tectonic regime, possibly in an accentuated or modified form, then led to the formation of mélange belts in parts of the deposited, but only partly consolidated, sedimentary sequence.

We fully support the suggestion[3] that the term mélange should be used only in a descriptive sense and that the prefixes 'sedimentary' and 'tectonic' should be used with the utmost caution.

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[1] Bradshaw, J. D., *J. R. Soc. N.Z.*, **3**, 161 (1973).

[2] Carter, R. M., Norris, R. J., Landis, C. A., and Bishop, D. G., *J. R. Soc. N.Z.*, **4**, 5 (1974).

[3] Berkland, J. O., Raymond, L. A., Kramer, J. C., Moores, E. M., and O'Day, M., *Bull. Am. Ass. Petrol. Geol.*, **56**, 2295 (1972).

Light scattering from soap films

If a strong light beam falls on the surface of a liquid, most of it will be reflected and refracted in certain directions, but a small part will be scattered in all other directions. This light scattering is caused by small surface ripples (corrugations) arising from thermal motion. The scattering intensity is proportional to kT/γ, where k is the Boltzmann constant, T the absolute temperature and γ the surface tension. In the case of a thin, free, liquid film (as found in soap bubbles), the scattered light also contains information about the interaction forces between the two film surfaces. According to the theory of Vrij[1], the second derivative, d^2F/dh^2, of the interaction free energy, F, between the two film surfaces, with respect to the film thickness, h, can be obtained in this way.

When electrical double layer and van der Waals forces are operative in the film one may write[1] $d^2F/dh^2 = B\kappa^3 \exp(-\kappa h) - A/(2\pi h^4)$, where B is a function of the surface potential, κ is the reciprocal Debye–Hückel thickness and A is the van der Waals–Hamaker constant.

Although a few results have been published[1,2], no measurements that would corroborate the theory in a completely satisfactory manner have ever been reported. Here we present some results for films drawn from a solution containing 8.2×10^{-4} M cetyltrimethylammoniumbromide (CTAB) and 8.4 (weight) % glycerol. Experiments were carried out on draining films (varying h) at constant angles. From plots of $\ln[d^2F/dh^2 + A/(2\pi h^4)]$ against h, the values of κ were calculated. In the (very small) van der Waals term we used $A = 6.28 \times 10^{-20}$J. From the values of κ, the electrolyte concentrations were calculated (Table 1) and compared with the concentration of the solution. The agreement between the two is good, in contrast with older experiments[1] where the calculated concentrations were up to 16 times too large.

Table 1 Results calculated from the thickness dependence of the light scattering (h between about 70 and 90 nm).

Angle of incidence (°)	Angle of observation (°)	κ (m^{-1})	Concentration of CTAB (mol l^{-1})
60	55.0	9.78×10^8	8.6×10^{-4}
60	54.0	8.70×10^8	6.8×10^{-4}
60	53.1	1.03×10^9	9.6×10^{-4}

After drainage of a film to constant thickness, we also studied the angular dependence of the light scattering. In Table 2 the observed scattering is compared with values predicted by calculation using the results of the dependence on thickness of the light scattering. It is clear that the experimental results fit the predictions very well.

We conclude that the measurements of the thickness- and angle-dependent light scattering show that the light scattering theory is internally consistent. Further, they show the validity of the exponential thickness dependence of the electrical part of d^2F/dh^2.

Table 2 Angular dependence of the light scattering

Angle of observation (°)	Predicted light scattering ratio ($\times 10^6$)	Measured light scattering ratio ($\times 10^6$)
54	6.56	6.69
53	5.86	5.37
52	4.41	4.42
51	3.68	3.78
50	3.10	3.30
49	2.64	2.66
48	2.26	2.34
46	1.69	1.78
44	1.30	1.37
41	0.91	0.97
37	0.60	0.63

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[1] Vrij, A., *J. Colloid Sci.*, **19**, 1-27 (1964). Vrij, A., *Adv. Colloid Interface Sci.*, **2**, 39-64 (1968).

[2] Mann, J. A., jun., *J. Colloid Interface Sci.*, **25**, 437-441 (1967).

Role of molecular aggregates in liquid drag reduction by polymers

SMALL quantities of certain polymers dissolved in a liquid can reduce considerably the frictional drag exerted by the liquid when it flows over a surface. This effect has been observed with various solvent/polymer combinations, but most investigations involve polyethyleneoxide (PEO), polyacrylamide or guar gum in water, where the concentrations required are of the order of a few parts per million.

Although the mechanism of this drag reduction is unknown, attention has generally focused on the role played by the individual polymer molecules. There might also be entanglements or aggregates of polymer molecules[1], especially in freshly prepared solutions[2], and the breakup of these under shear, like the degradation of polymer molecules themselves[3], may explain the frequently observed phenomenon of time-dependent drag reduction. But there has been little attempt to investigate these clusters, and most workers have been at pains to eliminate their effects. Recently, however, Hand and Williams[4] have shown that in drag-reducing solutions there is an 'adsorbed-entangled' layer of polymer molecules at the liquid/solid interface and have suggested that this is responsible for much, if not all, of the drag-reducing behaviour. We report here studies[5] of the time dependence of drag reduction in solutions of PEO and polyacrylamide which provide new evidence for the existence of such aggregates and indicate that they can be much more powerful drag reducers than the individual polymer molecules.

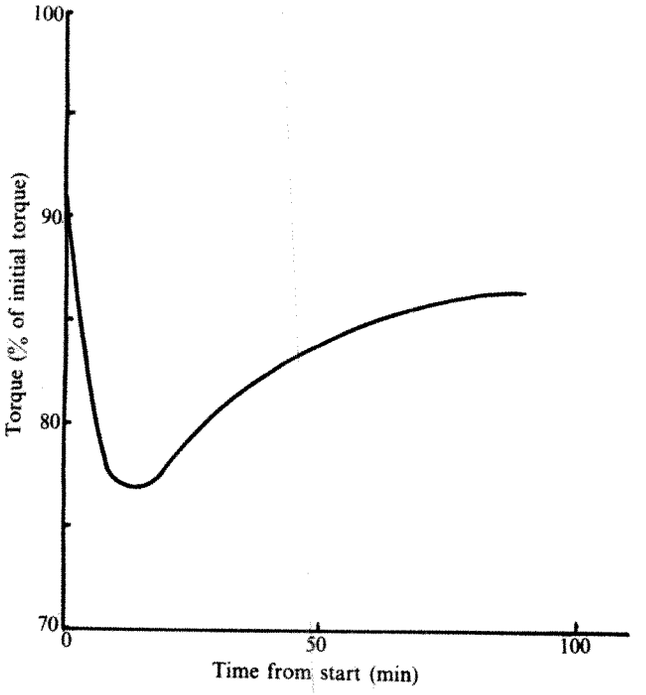


Fig. 1 Typical torque-time curve obtained with 'as received' (unsieved) material. Polyox Coagulant, 0.86 p.p.m., 840 r.p.m., 17° C.

The apparatus consisted of a vertical tapered stainless steel disk of diameter 0.23 m revolving, usually at 840 r.p.m. in a tank of water of volume 0.58 m^3. A dynamometer attached to the shaft of the disk enabled torque, and therefore drag reduction, to be measured as a function of time. The desired quantity of PEO powder was added below the water surface as a suspension in isopropanol. Most experiments were carried out using Polyox Coagulant with a molecular weight greather than 5×10^6.

The result of a typical experiment is shown in Fig. 1. The addition of the solid results in a rapid reduction of the required

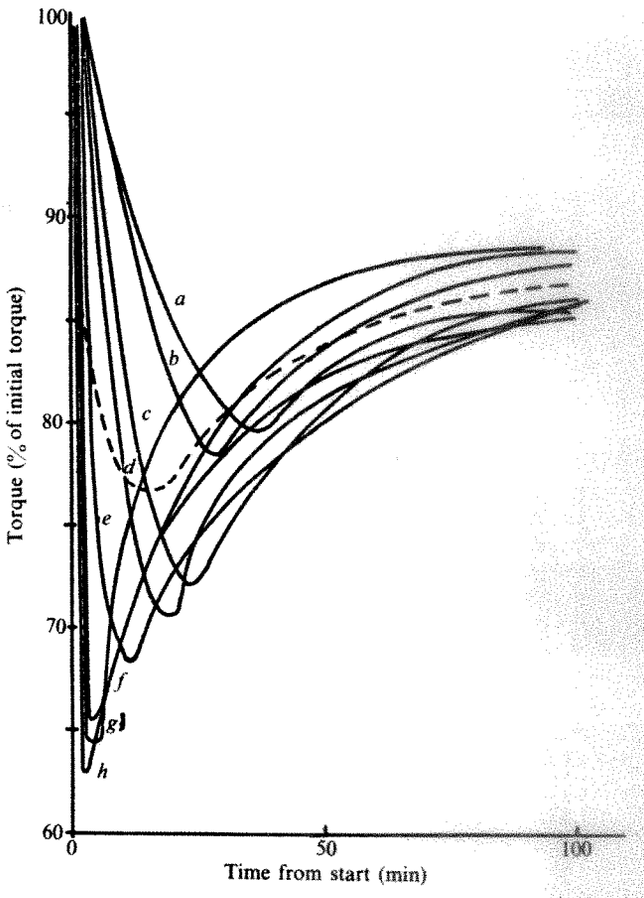


Fig. 2 Torque-time curves obtained with sieved material. The first figures refer to the BS sieve sizes, those in brackets to the mean aperture in millimetres. *a*, below 22 (over 0.71); *b*, 22–25 (0.655); *c*, 25–30 (0.55); *d*, 30–36 (0.46); *e*, 44–85 (0.26); *f*, 85–150 (0.141); *g*, 150–300 (0.079); *h*, over 300 (below 0.053). Polyox Coagulant, 0.86 p.p.m., 840 r.p.m., 17° C.

torque which, after passing through a minimum, gradually approaches an asymptotic value. This is significant because the torque might have been expected to decrease monotonously as the polymer dissolved. Experiments with sieved powder samples (Fig. 2) show that for a given final concentration, the finer the powder used the lower the minimum torque and the shorter the time taken to reach it; the 'steady-state' torque is,

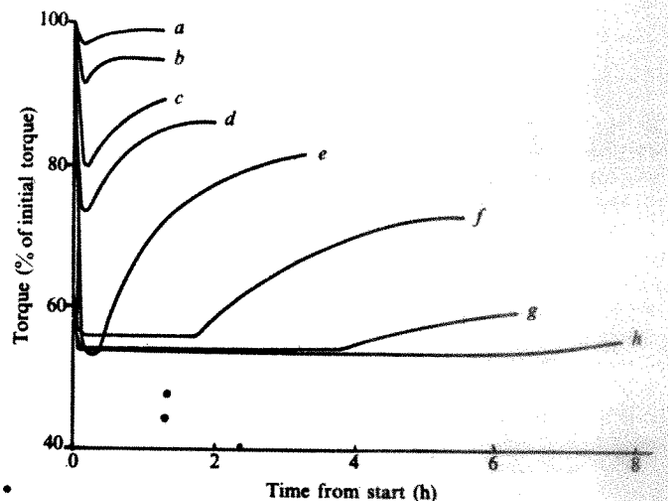


Fig. 3 Effect of polymer concentration (p.p.m.). *a*, 0.086; *b*, 0.171; *c*, 0.43; *d*, 0.86; *e*, 1.71; *f*, 4.30; *g*, 8.60; *h*, 17.1. Polyox Coagulant, 36–44 mesh (0.39 mm), 840 r.p.m., 17° C.

however, unaffected. The effect of polymer concentration using a powder of uniform particle size is shown in Fig. 3. In the particular conditions used the maximum attainable torque reduction was about 50%. This probably corresponds to complete flow laminarisation or some other hydrodynamic limitation, such as Virk's maximum drag reduction asymptote[6].

On the basis of these observations it is possible to advance the following hypothesis. When PEO powder is added to water, the particles rapidly swell to form a relatively small number of gel-like entities which circulate with the liquid and possibly attach themselves to the disk surface. By the action of shear forces at or near this surface they are broken down into a much larger number of aggregates of entangled molecules. At any one time there will be a particular distribution of aggregate sizes, which will tend to a steady state at long times.

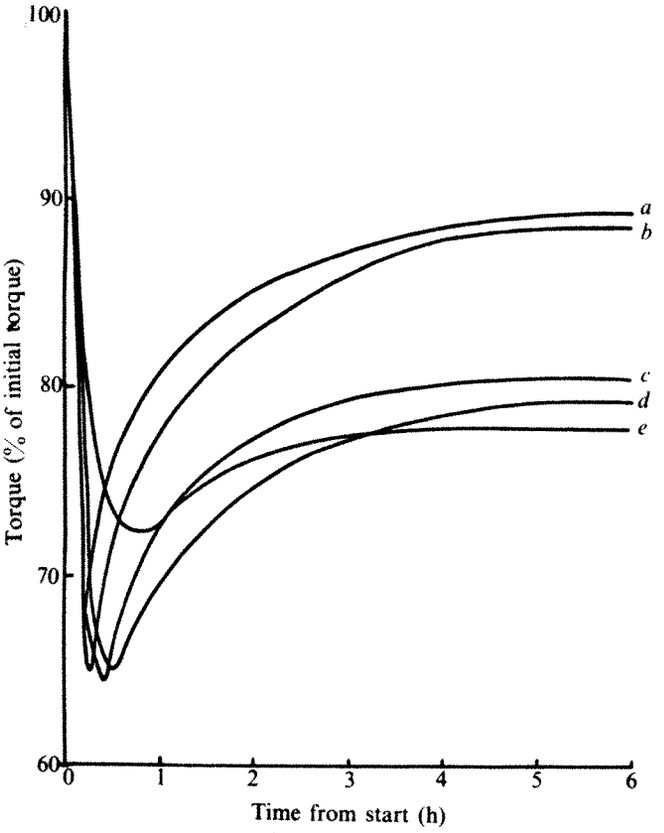


Fig. 4 Effect of disk speed (r.p.m.). *a*, 840; *b*, 720; *c*, 600; *d*, 420; *e*, 360. Polyox Coagulant, 30–36 mesh (0.46 mm), 0.86 p.p.m., 17° C.

Thus, the mean aggregate size will decrease monotonously with time while the number of aggregates or free molecules will increase. As the ability of polymer molecule aggregates to interact with eddies and thus bring about drag reduction is presumably a function of aggregate numbers and/or relaxation time, for a given set of hydrodynamic conditions the drag reduction could pass through a maximum as the optimum size distribution was reached and passed.

Strong evidence in favour of the aggregate hypothesis is provided by the effect of rotation rate on drag reduction (Fig. 4). It was found that the long-time value of the drag reduction decreased as the rotation rate increased. This observation runs contrary to the most firmly established fact in the field, that drag reduction in a stable system increases with shear rate, and suggests that the equilibrium (long-time) size distribution depends on the shear history of the solution. To test this idea, the apparatus described was used to shear a solution of PEO for 6 h at 420 r.p.m., after which the torque/speed characteristic of the disk was measured. This characteristic (Fig. 5) was quite different from that in an identical solution which had been sheared for the same length of time at 720 r.p.m. The solution subjected to the higher shear exhibited less drag reduction, presumably because of the reduced size of the active molecular aggregates.

Although these results could also be explained on the basis of molecular degradation, the effect of molecular weight (Fig. 6) supports the view that cluster formation and breakdown, rather than degradation, is the dominant effect. Thus, for a given polymer concentration, decreasing the molecular weight of the PEO from 4×10^6 to 6×10^5 more than halved both the maximum and steady-state drag reduction, while PEO of molecular weight 1×10^5 gave no drag reduction at all. Although the variation in chain length is only 40:1, the use of the 'entanglement coupling factor'[8] shows that the likelihood of chain entanglement in the highest molecular weight material is 7,000 times as great as in the lowest.

If the effects observed were a result of degradation, starting materials of different molecular weights would be expected to degrade, after a long time, to the same average molecular weight, that is, the asymptotic value of the drag reduction would be about the same in each case; in fact,

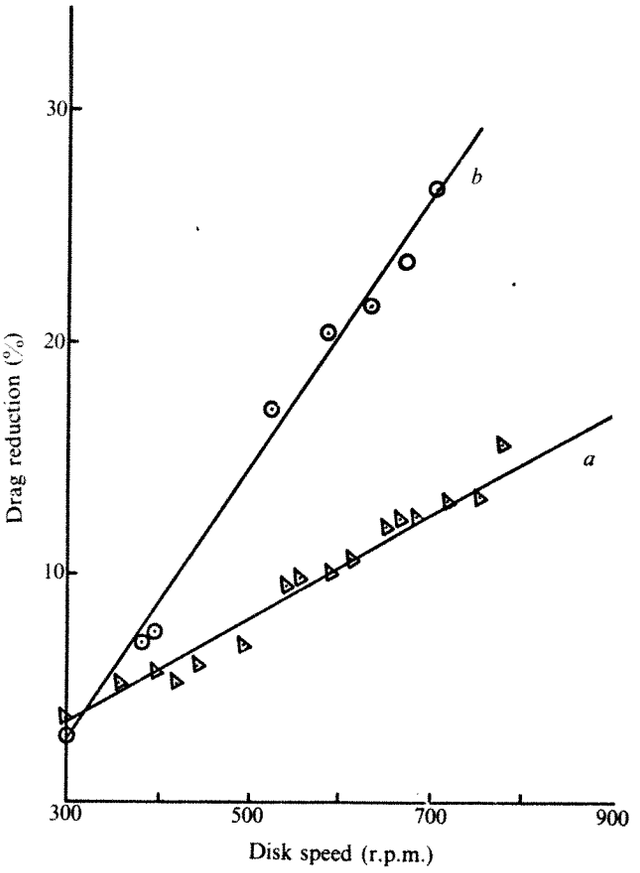


Fig. 5 Drag reduction as a function of speed for solutions sheared at 720 (*a*) and 420 (*b*) r.p.m. Polyox Coagulant, 0.86 p.p.m., 17° C.

Polyacrylamide of molecular weight about 5×10^6 exhibited similar drag-reduction characteristics to PEO, but the concentration required was somewhat greater. Guar and locust bean gums behaved differently, the drag reduction increasing with time up to an asymptotic value. The results obtained with PEO and polyacrylamide are consistent with the known tendency of many polymers to form molecular aggregates[8].

From the standpoint of drag reduction theory, the aggregate hypothesis provides an explanation of many of the anomalies reported in the literature for the PEO–water system. On the

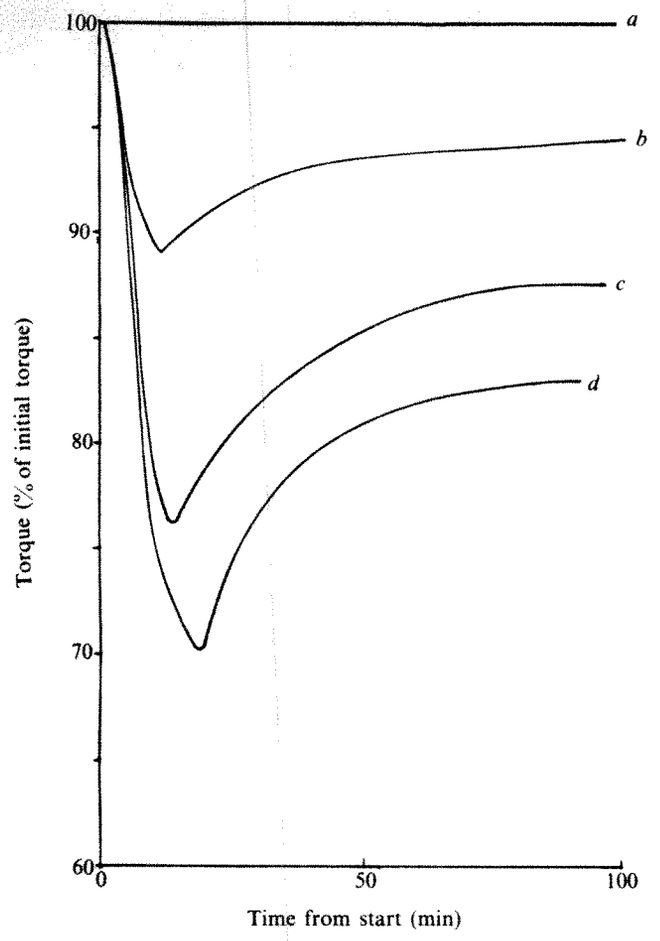


Fig. 6 Effect of molecular weight. Polyox, various grades, 30–36 mesh (0.46 mm), 0.86 p.p.m., 17° C. *a*, WSR N-10 (molecular weight 100,000); *b*, WSR 205 (molecular weight 600,000); *c*, WSR 301 (molecular weight 4,000,000); *d*, Coagulant 1 (molecular weight >5,000,000).

practical side, it suggests possible economies in the use of polymers, particularly PEO, in recirculating and non-recirculating systems; in the former the object should be to stabilise aggregates, in the latter to obtain the correct size aggregates at the right time.

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1 Fabula, A. G., *Sixth Symp. Naval Hydrodynamics, Washington DC*, (1966).
2 Brennan, C., and Gadd, G. E., *Nature*, **215**, 1368 (1967).
3 Gadd, G. E., *Nature*, **202**, 463 (1965).
4 Hand, J. H., and Williams, M. C., *Chem. Engng. Sci.*, **28**, 63 (1973).
5 Dunlop, E. H., *thesis*, (Univ. Strathclyde, Glasgow (1973)).
6 Virk, P. S., Merrill, E. W., Mickley, H. S., Smith, K. A., and Mollo-Christensen, E. L., *J. Fluid Mech.*, **30** (2), 305 (1967).
7 Ferry, J. D., *Viscoelastic Properties of Polymers*, second edition, 247 (Wiley, New York, 1970).
8 Shyluk, W. P., and Stow, F. S., *J. appl. Polym. Sci.*, **13**, 1023 (1969).

$NaAlO_2$ and $NaFeO_2$ Polymorphism

THE polymorphism of $NaAlO_2$ and $NaFeO_2$ is well known. Both oxides form β and γ polymorphs, and although their crystal structures are known, the relationship between the β and γ polymorphs has not been described. This relationship is described here and from it a simple topotactic mechanism for the $\beta \rightleftharpoons \gamma$ transformation is deduced. Movement of only half of the cations is necessary to complete the transformation in either direction and this movement is restricted to a single, filled–empty tetrahedron jump, for each cation. The polymorphisms may be summarised:

$NaFeO_2$: $\alpha \underset{760^\circ C}{\rightleftharpoons} \beta \underset{1010^\circ C}{\rightleftharpoons} \gamma$ (refs 1–3)

$NaAlO_2$: $\beta \underset{470^\circ C}{\rightleftharpoons} \gamma$ (ref. 4)

An α form may also be prepared at high pressure[5]. Both the β and γ polymorphs have 'tetrahedral structures', that is, the cations and oxygens are all tetrahedrally coordinated. (The α form has an ordered rock-salt structure.)

β-$NaFeO_2$ and β-$NaAlO_2$ are isostructural. They have the basic wurtzite structure but with ordering of cations on the tetrahedral sites[1,2]. This cation ordering causes a lowering of the symmetry from hexagonal $P6_3mc$ in wurtzite to orthorhombic $Pbn2_1$ in β-$NaFeO_2$. A schematic (001) projection of the structure (not drawn to scale) is shown in Fig. 1. The unit cell contains two layers of close-packed oxygen atoms at heights 0 and 1/2, and two layers of cations at heights 1/6 and 2/3 (ideally and ignoring differences in cation sizes). The cations for example those at 1/6 are coordinated to three oxygens at 0 and one oxygen at 1/2. Thus, the MO_4 tetrahedra all point upwards, along *z*; linkage of adjacent MO_4 tetrahedra is by corner sharing only (Fig. 2*a*).

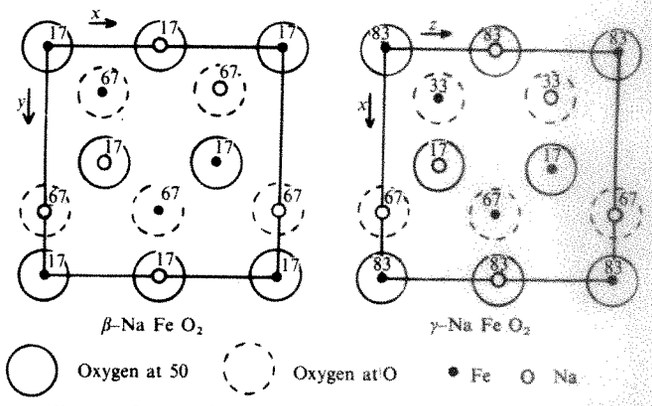


Fig. 1 Schematic projection of β-$NaFeO_2$ and γ-$NaFeO_2$ structures. The diagrams are not drawn to scale. Atomic heights, in units of *c*/100 and *b*/100 respectively, are idealised.

The structures of γ-$NaAlO_2$ and γ-$NaFeO_2$ have not been determined directly, but they are isostructural with γ-$LiAlO_2$, tetragonal $P4_12_12$ (ref. 8). The structure of γ-$LiAlO_2$ was determined independently by X-ray and neutron diffraction on single crystal and powdered specimens, respectively[7,8]. The structure has been described as a three-dimensional network of linked tetrahedra[7], its relation to cristobalite, SiO_2, has been noted[8], and it has been described as a stuffed, distorted cristobalite structure[8].

I made a model of γ-$LiAlO_2$ using cardboard tetrahedra and an alternative way of describing its structure became apparent (Figs 1 and 2*b*). Rather buckled layers of oxygen atoms occur, ideally at 0 and 1/2, which again approximate to hexagonal close packing. The cations are distributed over both sets of available tetrahedral sites; that is, at 1/6, 2/3 and

1/3, 5/6, but only half of the sites in each set are occupied (the set of tetrahedral sites at 1/3, 5/6 in β-$NaFeO_2$ are empty). The cations at for example 1/3 are coordinated to three oxygens at 1/2 and one oxygen at 0 and so these MO_4 tetrahedra point downwards. The manner of linkage of the tetrahedra is different from that in β-$NaFeO_2$ in that the tetrahedra occur in pairs. Each NaO_4 tetrahedron shares one edge with an adjacent FeO_4 tetrahedron, and *vice versa*. The remaining linkages are by corner sharing.

The β and γ structures may be classified using the nomenclature of Ho and Douglas[6]: . . . P_A T_+ P_B T_+ P_A . . . for β-$NaFeO_2$; . . . P_A $T_{+\frac{1}{2}}$ $T_{-\frac{1}{2}}$ P_B $T_{+\frac{1}{2}}$ $T_{-\frac{1}{2}}$ P_A . . . for γ-$NaFeO_2$. P_A P_B P_A . . . refers to anion layers in hexagonal close packing; T_+, T_-, indicate layers of tetrahedral sites which point up and down respectively, and $T_{\frac{1}{2}}$ indicates that only half of the sites in that layer are occupied.

The structural relationship between β-$NaFeO_2$ and γ-$NaFeO_2$ is shown in Fig. 1. The oxygen arrangement is the same in both cases and half of the cations, both Na and Fe, occupy similar positions in the two structures. The remaining Na and Fe cations, however, occupy different tetrahedral sites in the β and γ structures.

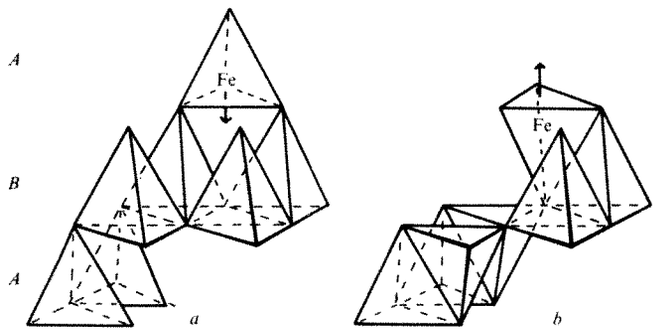


Fig. 2 Schematic structural relationship between β-$NaFeO_2$(*a*) and γ-$NaFeO_2$(*b*). *A*, *B* refer to a hexagonal close-packing sequence.

Closer examination of the structural differences between β and γ forms shows that the β⇌γ transformation may be effected by a simple topotactic mechanism. Cations at 1/3 in γ-$NaFeO_2$ can move to 2/3 (as in β-$NaFeO_2$) simply by jumping through their tetrahedron bases, that is, by passing between the three oxygens at height 1/2 to which they are coordinated. For each cation, the bond to the oxygen at 0 must therefore break and a new bond to the oxygen at 1 will form. Similarly, cations at 5/6 can transform to 1/6 and *vice versa*, by moving through the triangular window of oxygens at height 0. The relationship between the structures, and the mechanism of transformation, are shown in Fig. 2. The β-structure is shown schematically (Fig. 2*a*), with all of the tetrahedra pointing upwards, as is the corresponding γ structure (Fig. 2*b*), with half of the tetrahedra pointing upwards and half pointing downwards. The γ→ β transformation, written as . . . P_A $T_{+\frac{1}{2}}$ $T_{-\frac{1}{2}}$ P_B $T_{+\frac{1}{2}}$ $T_{-\frac{1}{2}}$ P_A . . . → . . . P_A T_+ P_B T_+ P_A . . . can be effected simply by inverting those tetrahedra which point downwards (Fig. 2*b*), and *vice versa* for the β→γ transformation.

Studies on related oxides have revealed several other sets of β, γ type systems. For example, Li_2BeSiO_4 and Li_3PO_4 have low temperature β polymorphs and high temperature γ polymorphs which are related to, but not isostructural with, β- and γ-$NaFeO_2$. Again a close structural relationship exists between the β and γ polymorphs of each pair and a simple topotactic mechanism can be postulated for the β⇌γ transformation. These results will be described soon.

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[1] Bertaut, F., and Blum, P., *C. r. hebd. Séanc. Acad. Sci., Paris*, **239**, 429 (1954).
[2] Bertaut, F., Delapalme, A., and Bassi, G., *C. r. hebd. Séanc. Acad. Sci., Paris* **257**, 421 (1963).
[3] Collongues, R., and Thery, J., *Bull. Soc. chim. Fr.*, 1141 (1959).
[4] Thery J., Lejus, A-M., Briançon, D., and Collongues, R., *Bull, Soc. chim. Fr.*, 973 (1961).
[5] Reid, A. F., and Ringwood, A. E., *Inorg. Chem.*, **7**, 443 (1965).
[6] Ho, S. M., and Douglas, B. E. *J. Chem. Educ.*, **46**, 207 (1969).
[7] Marezio, M., *Acta crystallogr.*, **19**, 396 (1965).
[8] Bertaut, E. F., Delapalme, A., Bassi, G., Durif-Varambon, A., and Joubert, J.-C., *Bull. Soc. Fr. Minér. Cristallagr.*, 88, 103 (1965).

Cr^{2+}-cobaloxime electron transfer reactions

Cobalt complexes of dimethylglyoxime (CH_3C:NOH.C:NOH.CH_3), commonly known as cobaloximes, form alkyl derivatives with unusually stable cobalt–carbon bonds[1,2]. These are regarded by some workers as 'model' compounds for vitamin B_{12}, a biological alkylating agent, the action of which depends on the reversible formation of a similar Co–C bond[3-5]. Various mechanisms have been postulated for this reaction[6]. The mechanisms of transalkylation reactions undergone by cobaloximes are thus of great interest; such reactions have been investigated with Hg^{2+} (refs 7 and 8) and with Cr^{2+} (ref. 9). For comparison we are studying the reaction kinetics of other cobaloximes with Cr^{2+}. These are apparently electron-transfer reactions[10-12], and should also be considered in the context of the large volume of work that has been done in that field.

Using DH^- to represent the mono-anion of dimethylglyoxime, the simple cobaloximes are of the form $[Co(DH)_2B_2]^+$, in the case where B is a neutral ligand such as NH_3 or H_2O. Neutral and anionic complexes are well known, with one and two anionic ligands respectively, and the alkyl complexes are usually neutral species of the type $[RCo(DH)_2B]$. In all cobaloximes the two dimethylglyoximato ions occupy a plane, and are linked by strong hydrogen bonds to form a pseudo-macrocyclic ligand with an N_4 donor set.

We have established that in cases in which both axial ligands are NH_3 molecules, the cobaloxime is reversibly reduced by Cr^{2+}:

$$[Co(DH)_2(NH_3)_2]^+ + Cr^{2+} \rightarrow [Co(DH)_2(NH_3)_2] + Cr^{3+}$$

$$\swarrow H^+ \qquad O_2 \downarrow$$

$$Co^{2+} + 2DH_2 + 2NH_4^+ \not\rightarrow [Co(DH)_2(NH_3)_2]^+$$
$$O_2$$

Cobaloxime(II), the reduced form, was indentified by its visible absorption spectrum, and the kinetics of the redox reactions were monitored at 464 nm, where the product absorbs strongly. Cobaloxime(III), regenerated by oxidation of the solution (in air), was isolated as the insoluble perchlorate salt, and microanalysis showed that no chromium was incorporated. In strongly acid solutions ($pH < 1$), aquation of the reduced species is rapid; this process is acid catalysed and has an equilibrium strongly dependent on $[H^+]$.

The initial redox reaction obeys the rate law

$$\frac{d[Co^{II}]}{dt} = k_{obs}[Cr^{2+}]\,[Co^{III}],$$

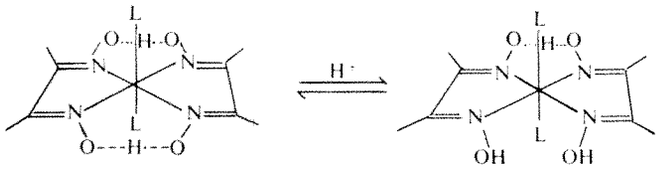
where $k_{obs} = 40 + 780\,[H^+]$ (at 25° C)

The precise mechanism of this reaction is not fully understood. Although there is no evidence for ligand transfer, nor for a binuclear complex intermediate, an inner-sphere pathway involving the binding of chromium to one or two oxime oxygen atoms cannot be entirely discounted.

The rate depends on $[H^+]$ because cobaloximes act as weak bases, thus

$$[Co(DH)_2B_2]^+ + H_3O \underset{}{\overset{K_B}{\rightleftharpoons}} [Co(DH)(DH_2)B_2]^{2+} + H_2O$$

The K_B values for many neutral alkylcobaloximes have been determined[1,7], and for these species are generally of the order of 3-4. For the cationic simple cobaloximes a lower value is expected because of the charge effect; our attempts to determine K_B values for the bisammino complex have so far been unsuccessful, but *p*H measurements in acid solution indicate an upper limit of about 0.5, and the linear dependence of the reaction rate on $[H^+]$, determined to $[H^+] = 0.7$ M, implies an upper limit of about 0.2. There is now considerable evidence [13, 14] that this protonation of cobaloximes leads to a significant change in structure:



Weakening of one of the strong hydrogen bonds of the pseudomacrocyclic equatorial ligands allows greater freedom of movement for at least two of the oxime nitrogen atoms relative to the cobalt ion. Thus the Frank–Condon energy barrier to the electron-transfer reaction, which results from the need for changes in coordination geometry on reduction of the metal ion, is greatly lowered. Endicott[15] has suggested that in a similar case, in which the ligand is a true macrocycle, this is the major barrier to redox processes. The reduction of electron density around the cobalt ion when the ligand is protonated should also increase the rate of electron transfer to that ion. The combination of these two effects clearly far outweighs the decrease in stability of the 'precursor' ion pair, or complex, formed by the two cationic reagents, when the charge on the cobalt complex is increased.

When alkylcobaloximes react with Cr^{2+}, the rate also depends on the acid. Both terms in the expression for the rate constant are lower than those that we have observed and the protonated and non-protonated species react at virtually the same rate.

If the cobaloxime is considered as a complex of Co^{III}, then the observed reaction

$$[RCo(DH)_2H_2O] + Cr^{2+} \rightarrow [RCr(H_2O)_5]^{2+} + Co^{II}$$

is an electron-transfer process. As an inner-sphere reaction it would be unique in that the bridging ligand (for example CH_3^-) has no lone pair available to coordinate to the reductant ion while bound to the oxidant. A process can, however, be considered, intermediate between inner and outer sphere, in which alkyl transfer accompanies electron transfer although no bridged precursor complex is formed. Alternatively the alkylcobaloxime can be regarded as a complex of Co^{II}, in which case the most plausible reaction mechanism is a bimolecular homolytic substitution at the carbon atom, an S_{H2} process. Both of these mechanisms are considered to be possible [9].

The absence of a rate increase on protonation of the alkylcobaloxime is however, inconsistent with the postulate of an electron-transfer process in this case, but it indicates a process in which the electron density at the Co ion is not greatly changed. (In this context is should be noted that only the non-protonated alkylcobaloximes react with Hg^{2+} (ref. 7). Consideration of the charge effect indicates that this is consistent with the formulation of that reaction as a net oxidative process on cobalt, although it may be expected that the diminution of the Frank–Condon barrier would be the dominant effect in such a reaction. It is generally agreed[7,8] that this is an electrophilic substitution reaction at carbon, an S_{E2} process.)

Further insight may be obtained from a consideration of the redox potentials of the reagents involved. These have been determined for both simple[16] and alkylcobaloximes[17,18]. Schrauzer and Windgassen[17] report one-electron reduction potentials for $[MeCo(DH)_2H_2O]$ of -1.7 and -2.42 V in acetonitrile against $Ag/AgNO_3$ (0.1 M), compared with -0.67 -1.44 and -2.42 for $[Co(DH)_2(Py)_2]^+$ under the same conditions. They conclude that the alkyl species are complexes of Co^{II}, and report[14] that the product of the first reduction is a Co^I species[19]. Honokabe and Yamazaki[18] seem to attribute the first cathodic wave to a Co^{III}–Co^I reduction, although they discuss it subsequently in terms of a one-electron process. Their results do however agree on the value of the first half-wave potential, when corrected for different reference electrodes. According to Maki[16], the first half-wave potential of the bisamminocobaloxime is only 0.43 V more negative than that of the bispyridine complex. The methylaquo species thus has a reduction potential 0.6 V more negative than the bisammine complex. In the latter case, the reduction is reversible[16], and can therefore be corrected for the reference electrode to give a value for the standard electrode potential[20] of -0.37 V. Because E^0 for the Cr^{2+}/Cr^{3+} couple is -0.41 V[21], the reduction by Cr^{2+} of any species with a reduction potential much more negative than that of the bisamminocobaloxime is, thermodynamically, an unfavourable process. The electron-transfer process by which that complex reacts with the chromous ion is therefore unavailable to the alkyl complexes.

We therefore conclude that it would be incorrect to regard the reaction of Cr^{2+} with alkylcobaloximes as a chromium–cobalt electron transfer reaction. The simplest mechanism consistent with all of the described observations is homolytic substitution at the carbon atom.

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[1] Dodd, D., and Johnson, M.D., *J. organometal. Chem.*, **52**, 1 (1973).
[2] Schrauzer, G. N., *Acc. Chem. Res.*, **1**, 97 (1968).
[3] Pratt, J. M., *The Inorganic Chemistry of Vitamin B_{12}* (Academic Press, New York, (1972).
[4] Prince, R. H., and Stotter, D. A., *J. inorg. nucl. Chem.*, **35**, 321 (1973).
[5] Brown, D. G., *Prog. inorg. Chem.*, **18**, 177 (1973).
[6] Agnes, G., *et al.*, *Biochim. biophys. Acta*, **252**, 207 (1971).
[7] Adin, A., and Espenson, J. H., *J. Chem. Soc., Chem. Commun.*, 653 (1971).
[8] Schrauzer, G. N., *Tetrahedron Lett.*, 275 (1971).
[9] Espenson, J. H., and Schveima, J. S., *J. Am. chem. Soc.*, **95**, 4468 (1973).
[10] Taube, H., and Gould, E. S., *Acc. chem. Res.*, **2**, 321 (1969).
[11] Sykes, A. G., *Adv. Chem. Radiochem.*, **10**, 153 (1967).
[12] Linck, R. G., *Reaction Mechanisms in Inorganic Chemistry*, **9**, 303 (1972).
[13] Gillard, R. D., and Wilkinson, G., *J. chem. Soc.*, 6041 (1963).
[14] Crumbliss, A. L., *et al.*, *J. chem. Soc., chem. Commun.*, 415 (1973).
[15] Endicott, J. F., *et al.*, *J. Am. chem. Soc.*, **95**, 5097 (1973).
[16] Maki, N., *Nature*, **188**, 227 (1960).
[17] Schrauzer, G. N., and Windgassen, R. J., *J. Am. chem. Soc.*, **88**, 3738 (1966).
[18] Hohokabe, Y., and Yamazaki, N., *Bull. chem. Soc. Japan*, **44**, 1563 (1971).
[19] Schrauzer, G. N., and Windgassen, R. J., *J. Am. chem. Soc.*, **89**, 1999 (1967).
[20] Kolthoff, I. M., and Lingane, J. J., *Polarography* (Interscience, New York, 1941).
[21] Latimer, W. M., *The Oxidation States of the Elements and their Potentials in Aqueous Solution* (Prentice-Hall, New York, 1938)

BIOLOGICAL SCIENCES

Exciton-like splitting in acridine dye–nucleic acid complexes

COMPLEXES between cationic acridine dyes and nucleic acids are of considerable interest because of the wide variety of biological effects in which the interaction between the dye and nucleic acids have been implicated[1–3].

Lerman's evidence based on the X-ray study and hydrodynamic measurements of proflavine-DNA complexes remains the most persuasive for the intercalation model, that is the mass per unit contour length of DNA diminishes when complexed with the dye[4–6]. Most recent information about the nature of complexes in solution derives from studies of equilibria between free and bound dye in the form of binding curves[1] (Scatchard plots).

In spite of several studies of complexes between the chromosomal stain quinacrine (QAC)[7–13], and nucleic acids, the molecular nature of the complex is not understood[14–17]. We have now used ultraviolet absorption spectroscopy to look for exciton-like interaction in complexes between QAC and DNA, poly(dAT), and poly(dG)·poly(dC). We also measured rate constants for quenching of the fluorescence of the complexed dye by iodide ion.

The interaction of excited states of weakly coupled composite systems such as molecular crystals, dimers, aggregates and polynucleotides can be treated by the molecular exciton model[18]. The interaction between dyes and nucleic acids when the binding is strong (at high phosphate:dye (P:D) ratios) can be treated similarly[19]. In the simple theory, when only the transition dipole-dipole potential term is considered, significant interaction occurs if (*a*) there is a small energy difference between interacting states, and (*b*) the interacting transition dipoles have large transition moments.

On the basis of the intensity and energy of the transition, the S_3 absorption band of QAC (*p*H 7) (35,710 cm^{-1} (280 nm) $f = 1.15$) is the only band which could possibly show significant exciton-like interaction (in addition to dispersion and other solvent shifts) with the polynucleotide transition at 38,460 cm^{-1} (260 nm, $f = 0.10$). The other two bands, S_2 at 22,470 cm^{-1} (445 nm, $f = 0.18$) and S_2 at 29,070 cm^{-1} (344 nm, $f = 0.08$) ought to show dispersion and possibly other shifts in the complexes but not exciton-like interaction.

To ensure complete, strong primary binding[1], we worked with P:D ratios of at least 6:1 and usually 20:1. Consequently the strong 260 nm absorption in the spectrum of the polynucleotide which was unperturbed by bound dye, masked small changes in the QAC S_3 transition. Therefore, we recorded the difference spectra of dye–polynucleotide solutions (sample beam) against identical polynucleotide samples (reference beam) on our Cary 14 spectrophotometer.

Figure 1 shows spectra of free QAC and difference spectra of QAC bound to DNA, poly(dAT) and poly(dG)·poly(dC). In the spectrum of QAC bound to DNA the 445 nm and 344 nm bands of free QAC appeared at 455 nm (21,980 cm^{-1}) and 350 nm (28,570 cm^{-1}) respectively. These transitions showed dispersion shifts only. In the 260–300 nm region, however, two overlapping bands were observed at 290 nm (34,480 cm^{-1}) and 270 nm (37,040 cm^{-1}). If these two bands are decomposed into their components, we obtain two bands with maxima at 34,480 cm^{-1} and 37,540 cm^{-1}. The energy difference between these peaks (Δ) is 3060 cm^{-1}. Thus Δ is larger than the energy difference between the unperturbed interacting states, ΔE_{31} (38,460–35,710 = 2,750 cm^{-1}) by 310 cm^{-1}.

Relative to an area of 1.15 for the oscillator strength of the $S_0 \rightarrow S_3$ transition of the free dye, we observed areas of 0.40 and 0.30 for the 290 nm and 270 nm components, respectively, for the spectrum of the complex. A similar band pattern is observed with the QAC/poly(dAT) complex (Fig. 1*b*). Decomposing the two overlapping bands as before we obtained two bands at 37,315 cm^{-1} and 34,015 cm^{-1}, giving $\Delta = 3{,}300$ cm^{-1}, again larger (by 550 cm^{-1}) than $\Delta E_{31} = 2{,}750$ cm^{-1}.

In contrast to the results with DNA and poly(dAT), the difference spectrum of QAC bound to poly(dG)·poly(dC) (Fig. 1*c*) contained only a single broad band in the region of the 280 nm absorption of the spectrum of free QAC.

No noticeable change in the difference spectra was observed when the P:D ratio was varied from 50:1 to 5:1. However, when the P:D ratio was reduced from 5:1 to 1:1, the doublet became a broadened maximum at 280 nm. Since the low P:D region corresponds to that in which weak binding occurs[20], the absorption at 280 nm can be attributed to weakly bound or free dye.

The dramatic difference in the 280 nm region of the spectra of free QAC bound to DNA and poly(dAT) is good evidence that the interaction between the S_3 state of QAC and its environment is not due to a simple dispersion shift.

Since the interaction energy (310 cm^{-1}) is smaller than the initial level separation (2,750 cm^{-1}) by almost an order of magnitude, the exciton model is not strictly applicable either. However, the transition moments are associated with broad bands rather than sharp lines and these bands overlap considerably (see Fig. 1). Therefore, resonance type interactions do occur as in Förster energy transfer and this may account for the exciton-like behaviour, that is the large intensity redistribution and pronounced band shifts and splitting. It therefore seems reasonable to attribute the two

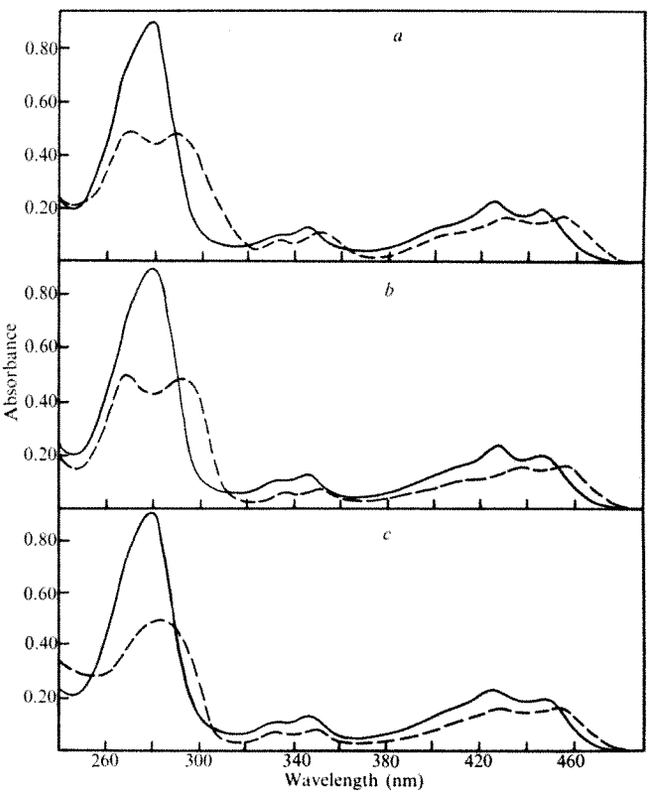


FIG. 1 Quinacrine-polynucleotide difference spectra. ——, Free quinacrine 1.55×10^{-5} M; – – –, quinacrine 1.55×10^{-5} M and polynucleotide 3.1×10^{-4} M. *a*, Calf thymus DNA; *b*, poly(dAT); *c*, poly(dG) · poly(dC). Difference spectra were run on a Cary 14 spectrophotometer (dispersion = 20 A° mm^{-1}) using 1 cm cells at 23° C. The reference cell contained a concentration of polynucleotide equal to that in the sample cell which contained the quinacrine–polynucleotide solution. Identical maxima were observed for reference optical densities of 0.6 (slits, 1 mm) and 1.85 (slits, 2.5 mm).

TABLE 1 Fluorescence quenching of quinacrine and quinacrine/nucleic acid complexes by iodide ion

	Φ_F^f/Φ_F^{f+b}	τ, ns	$kq \times 10^{-9}$ corrected	$M^{-1}\,s^{-1}$ uncorrected
QAC	1	4	7.8	—
QAC + poly(dAT)	0.4	20	0.19	0.20
QAC + poly(dG)·poly(dC)	7	6	2.3	3.0
QAC + DNA	2.1	10	1.1	1.2

[QAC] = 2×10^{-5} M; P:D = 20:1. The solution was buffered at *p*H 7 with 0.01 M cacodylate. The ionic strength was maintained constant at 0.1 M by the addition of appropriate amounts of NaCl such that $[Cl^-] + [I^-] = 0.1$ M. The polynucleotides were purified by dialysis for 24 h. Calf thymus DNA was purchased from Worthington Biochemicals; poly (dG)·poly (dC) and poly (dAT) were purchased from Miles Laboratories. Fluorescence intensities were measured using a Farrand spectrofluorimeter exciting at 365 nm (bandwidth 5 nm) and monitoring at 490 nm. Lifetimes were measured by the TRW model 75A fluorescence lifetime apparatus. Rate constants were obtained from Stern-Volmer plots[23] of fluorescence intensity compared with [iodide]. Φ_F^f/Φ_F^{f+b} (=A) is the ratio of the quantum yield of the free dye to that of free + bound dye. The corrected rate constants were derived from values of I_b^0/I_b obtained according to[22]:
$I_b^0/I_b = [1 - (1 - \alpha)A]/[(I_f^0{}_{+b}/I_{f+b})^{-1} - (1 - \alpha)A(I_f^0/I_f)^{-1}]$
where I is the fluorescence intensity, the superscript zero designates the fluorescence at zero iodide concentration; the subscripts f, b, $f + b$ designate free dye, bound dye, and free plus bound dye respectively; α is the fraction of bound dye (0.95). I_{f+b} are the observed values of the fluorescence intensity at various iodide concentrations.

maxima which we have observed from the difference spectra as being due to two states formed from the dipole-dipole interaction between the S_3 state of QAC and the S_1 state of DNA with intensity being stolen from the intense $S_0 \rightarrow S_2$ transition of QAC. In the case of dipole-dipole interactions the interaction energy varies as r^{-3}. We conclude therefore that the binding of QAC to poly(dG)·poly(dC) and to the other two polynucleotides is such that the distance of approach between QAC and the G–C base pairs is much larger than that between QAC and the A–T base pairs. In this sense, the observed splitting in DNA and poly(dAT) and the limiting P:D ratio of 5 at which the splitting is observed is fully consistent with the intercalation model.

Since the more tightly wound G–C helix is less likely to unwind than the other two polynucleotides in order to accommodate the intercalating dye molecules, the different difference spectra observed for poly(dAT) and DNA dye complexes on the one hand, and the poly(dG)·poly(dC)–dye complex on the other supports the intercalation model in the former two polynucleotides.

The constant shape of the Franck Condon envelope of the difference spectra when the P:D ratio was varied from 50:1 to 5:1 demonstrates that the observed splitting results from highly localised interactions between dye molecules and their nearest neighbour. There is no evidence for dye-dye interactions in QAC-nucleic acid complexes.

This conclusion suggests that the dye is more sterically hindered, and hence more shielded from collisions with molecules in solution, when bound to poly(dAT) than when bound to poly(dG)·poly(dC). A test for this was to examine the quenching of the fluorescence of the complexed QAC molecule by species such as iodide ions—a collisional process[21]. We would expect differences in the fluorescence quenching rate constant (k_q) for QAC–poly(dAT) and QAC–poly(dG)·poly(dC) complexes. Fluorescence intensity values for quenching[22] are less physically significant than k_q, for they do not account for the significant variation in fluorescence lifetime of the dye in the different environments (Table 1).

In the three cases studied, Stern-Volmer[23] plots were linear. Table 1 shows a significant variation in k_q depending on the complex. We applied a correction[22] to the observed k_q values in order to ensure that the differences observed were not due to a small amount of free dye (estimated to be less than 5% from absorbance changes at 440 nm, compared with P:D) which may still be present even at high P:D ratios. There was no significant difference between the corrected and uncorrected values.

The value of k_q for the free dye agrees with the calculated diffusion controlled value in water[24]. The poly(dAT)–QAC complex had the lowest k_q and poly(dG)·poly(dC)–QAC complex the highest. These observations confirm our interpretation of the spectroscopic experiments described above; that QAC is bound considerably closer (intercalated) to A–T than to G–C base pairs in the polynucleotides.

In summary, (1) we have observed exciton-like splitting between the S_3 state of QAC and the lowest excited state of DNA and poly(dAT) with concomitant intensity redistribution but have not observed this effect with QAC and poly(dG)·poly(dC); (2) we conclude that QAC is bound closer to A–T base pairs (possibly by intercalation) than to G–C base pairs in polynucleotides; (3) the most important interactions responsible for the splitting are nearest neighbour interactions; (4) dye-dye interactions are negligible in QAC-nucleic acid complexes.

Coupled with theoretical calculations we believe that the exciton-like interaction could be quite important in unravelling the structures of acridine dye-nucleic acid and similar complexes as they occur in solution.

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1 Peacocke, A. R., in *Acridines* (edit. by Acheson, R. M.), 723 (Wiley, New York, 1973).
2 Sankaran, L., and Pogell, B. M., *Nature new Biol.*, **245**, 257 (1973).
3 Le Pecq, J. B., Jeanteur, P., Emanoil-Ravicovitch, R., and Paoletti, C., *Biochim. biophys. Acta*, **119**, 442 (1966).
4 Lerman, L. S., *J. molec. Biol.*, **3**, 18 (1961).
5 Lerman, L. S., *J. molec. Biol.*, **10**, 367 (1964).
6 Lerman, L. S., *Proc. natn. Acad. Sci. U.S.A.*, **49**, 94 (1963).
7 Caspersson, T., Zech, L., Modest, E. J., Foley, G. E., Wagh, U., and Simonsson, E., *Expl Cell Res.*, **58**, 128 (1969).
8 Caspersson, T., Zech, L., Wagh, U., Modest, E. J., Foley, G. E., and Simonsson, E., *Expl Cell Res.*, **58**, 141 (1969).
9 Caspersson, T., *Expl Cell Res.*, **58**, 451 (1969).
10 Lee, C. L. Y., Welch, J. P., and Lee, S. H. S., *Nature new Biol.*, **241**, 142 (1973).
11 Bosman, F. T., and Schaberg, A., *Nature new Biol.*, **241**, 216 (1973).
12 Peterson, W. D., jun., Simpson, W. F., Ecklund, P. S., and Stulberg, C. S., *Nature new Biol.*, **242**, 22 (1973).
13 Williams, R. E., Lurquin, P. F., and Seligy, V. L., *Eur. J. Biochem.*, **29**, 426 (1972).
14 Weisblum, B., and de Haseth, P. L., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 629 (1972).
15 Pachmann, U., and Rigler, R., *Expl Cell Res.*, **72**, 602 (1972).
16 Selander, R. K., and De la Chapelle, A., *Nature new Biol.*, **245**, 240 (1973).
17 Weisblum, B., *Nature*, **246**, 150 (1973).
18 McRae, E. G., and Kasha, M., in *Physical processes in radiation biology* (edit. by Augenstein, L., Mason, R., and Rosenberg, B.), 23 (Academic Press, New York, 1964).
19 Philpott, M. R., *J. chem. Phys.*, **53**, 968 (1970).
20 Drummond, D. S., Simpson-Gildemeister, V. F. W., and Peacocke, A. R., *Biopolymers*, **3**, 135 (1965).
21 Lehrer, S. S., *Biochemistry*, **10**, 3254 (1971).

[22] Korsunskii, V. I., and Naberukhin, Y. I., *Molec. Biol. Moscow*, **6**, 594 (1972).
[23] Turro, N. J., in *Molecular photochemistry*, 94 (Benjamin, New York, 1965).
[24] Calvert, J. G., and Pitts, J. N., in *Photochemistry*, 627 (Wiley, New York, 1967).

α-Hydrazino-ornithine blocks net synthesis of putrescine but not of RNA and DNA

THE polyamines spermine, spermidine and their precursor putrescine occur ubiquitously and in high concentrations in animals and plants[1]. Increased polyamine synthesis in animal cells and bacteria precedes or coincides with enhanced RNA formation, especially during growth stimulation, suggesting a close relationship[2]. Ornithine decarboxylase (ODC), a rate-limiting enzyme in polyamine synthesis, has an extremely short half life[3]. The early, large increase in its activity in all cases of rapid tissue growth examined suggests that the formation of its product, putrescine, triggers early enhancement of RNA synthesis during cellular growth. A critical test of this concept would be to block net putrescine and(or) polyamine synthesis selectively and determine whether nucleic acid synthesis is altered. Such a test might assess the role of putrescine as a potential modulator of growth in normal and neoplastic tissue.

α-Hydrazino-ornithine (HO) is a specific and potent inhibitor of ODC with *Ki* values of 0.5 μM and 2 μM for bacterial and mammalian enzymes respectively[4,5]. HO inhibits competitively with both the substrate ornithine and the coenzyme pyridoxal phosphate. HO is 100 times more potent an inhibitor of ODC than are other hydrazines and inhibits the pyridoxal phosphate-dependent glutamic acid and dihydroxyphenylalanine decarboxylases only 1% as efficiently as it inhibits ODC[4,5], indicating considerable selectivity for ODC. HO is relatively nontoxic, producing no gross behavioural abnormalities in rats in doses up to 2 g kg^{-1} (ref. 5). We now report that HO effectively blocks net putrescine synthesis in rat hepatoma cells (HTC) in culture and in the liver of intact rats. Doses of the drug which markedly reduce putrescine do not appreciably affect the synthesis of either RNA or DNA.

The growth stimulus produced when high density HTC cells were diluted with fresh medium led to a marked increase in putrescine (Fig. 1). This increase followed soon

TABLE 1 Effect of α-hydrazino-ornithine on DNA and RNA synthesis in rat tissues

Addition	DNA (c.p.m.) in HTC cells	RNA (c.p.m.) Intact liver	RNA (c.p.m.) Regenerating liver
None	19,700 ± 1,500 (n = 3)	1,290 ± 140 (n = 4)	1,610 ± 70 (n = 4)
α-Hydrazino-ornithine	20,600 ± 1,200 (n = 3)	1.200 ± 90 (n = 5)	2,250 ± 140 (n = 5)

HTC cells were collected and stimulated by dilution (1:5) with fresh growth medium in the presence and absence of α-hydrazino-ornithine (5 × 10^{-4} M). At 17 h, ^{3}H-thymidine (1 μCi ml^{-1}; 6–7 Ci mmol^{-1}) was added and incorporation into 5% trichloroacetic acid (TCA)-precipitable material was measured in 1 ml aliquots of cells (25 μg protein) after a 1 h pulse at 37°C (methods as in legend to Fig. 1). DNA incorporation is expressed as c.p.m. ± s.e.m. per sample. The incorporation of ^{14}C-orotic acid (6.6 mCi mmol^{-1}) into rat liver was measured in 5% TCA-precipitable material 10 min after intravenous administration of 1 μCi of orotic-6-^{14}C-acid[13]. α-Hydrazino-ornithine (200 mg kg^{-1}) was given subcutaneously to sham operated and hepatectomised animals at operation and 4 h later; animals were killed 7 h after hepatectomy. Hepatic tissue was excised, quick frozen, weighed and homogenised in 9 volumes of 0.32 M sucrose. Aliquots (0.5 ml) were precipitated by adding 0.3 ml of 20% TCA, and the precipitate was washed with four successive 4-ml portions of 5% TCA and two 4-ml portions of absolute methanol. The resulting precipitate was dissolved in 1 ml of hydroxide of hyamine at 70°C for 30 min and transferred with 1 ml of absolute ethanol to 10 ml of PCS solubiliser for scintillation counting. RNA incorporation is expressed as c.p.m. ± s.e.m. per sample (0.057 g wet weight of tissue).

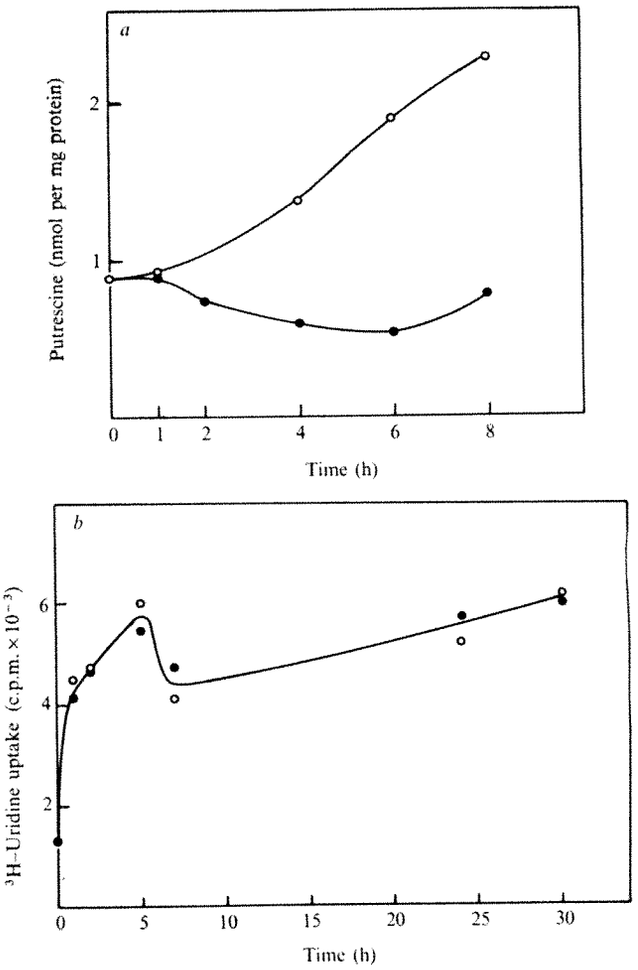


FIG. 1 Influence of α-hydrazino-ornithine on putrescine levels (*a*) and RNA synthesis (*b*) in HTC cells. *a*, HTC cells were grown to high density (about 2 × 10^7 per flask) in monolayer in 75 cm^2 Falcon flasks as described previously[8]. Cells were collected by scraping, and diluted (final concentration about 2 × 10^5 ml^{-1}) with fresh growth medium (10% foetal calf serum in Eagle's minimum essential medium with Hanks balanced salts, supplemented with 100 U each of penicillin and streptomycin, and equilibrated with 5% CO_2 in air). Putrescine was analysed by an enzymatic-isotopic microassay[14] in cell extracts. Aliquots (approximately 2.5 × 10^6 cells) were collected by centrifugation, washed twice at 4° with 10 ml phosphate-buffered saline, and disrupted by freezing and thawing four times in 0.25 ml distilled water. Protein was precipitated by heating at 95° C for 15 min and putrescine was assayed in the supernatant fluid. Results are expressed in nmol putrescine per mg cell protein, determined on aliquots of cell lysates by the method of Lowry *et al.*[15] *b*, High-density HTC cells collected by scraping were diluted 1:5 with fresh medium and incubated at 37° C in 1 ml aliquots (final concentration 2 × 10^5 cells per ml). ^{3}H-uridine (1 μCi ml^{-1}; 26 Ci mmol^{-1}) incorporation was measured by a 1 h pulse at timed intervals after dilution. Cells were washed by centrifugation at 4° C with three successive 5 ml aliquots of Hanks buffer, 5% TCA and absolute methanol. The final precipitate was solubilised in 0.5 ml of hyamine in methanol at 70° C for 15 min, and the solution was mixed with 3 ml of absolute ethanol for scintillation counting. Uridine uptake is expressed in c.p.m. incorporation per sample (average of triplicate samples). ○, Cells without α-hydrazino ornithine; ●, cells with 5 × 10^4 M α-hydrazino-ornithine.

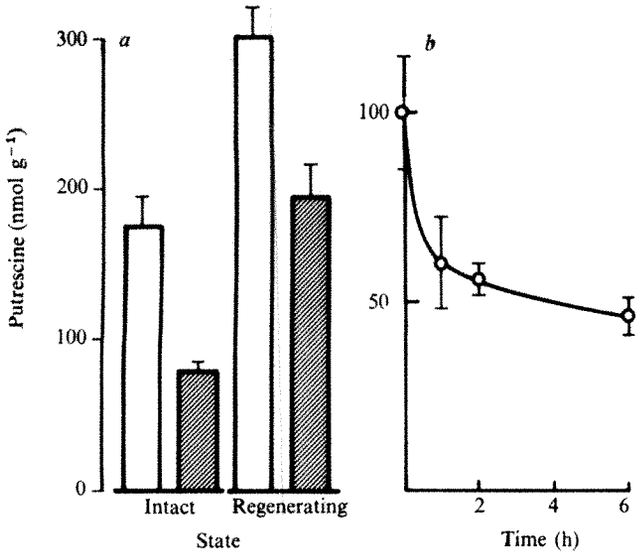


FIG. 2 Effects of α-hydrazino-ornithine on putrescine levels in intact and regenerating liver. *a*, Male rats (130 g, groups of three) under ether anaesthesia were nephrectomised bilaterally, with or without concomitant partial hepatectomy. Four hours after operation, animals received either saline (0.9% NaCl) or α-hydrazino-ornithine (75 mg kg^{-1}, subcutaneous), and were killed 2 h later. Livers were quick-frozen and tissue was homogenised in 9 volumes of distilled water. Protein was precipitated by heating for 15 min at 95° C and putrescine was measured in the supernatant[14]. Results are expressed in nmol of putrescine per g wet weight of tissue. Bars above the columns indicate s.e.m. *b*, Intact rats were killed at timed intervals after the subcutaneous administration of 200 mg kg^{-1} α-hydrazino-ornithine, and hepatic tissue was analysed for putrescine as outlined above. Results are expressed as nmol per g wet wt with vertical bars indicating 2 s.e.m. values. Open columns, saline; hatched columns, α-hydrazino-ornithine.

after that of ODC activity, which reached a maximum after 4–6 h and then declined[6–8]. In the presence of HO, putrescine levels not only stopped increasing, but they declined. In these cultures ODC activity is completely inhibited, although dialysis of tissue extracts to remove HO unmasks an enhancement of ODC levels[8]. The time course of incorporation of ^{3}H-uridine into high-density monolayer HTC cells diluted with fresh growth medium (Fig. 1) resembles that reported earlier for synchronised HTC cells in suspension[9]. It is interesting that the synthesis of RNA, as reflected by uridine incorporation, was virtually unaffected when putrescine levels were substantially lowered by HO. ^{3}H-uridine incorporation was unaltered in the presence of HO for periods up to 30 h. Because we did not measure specific activities of nucleotide triphosphate pools, changes in precursor uptake may have masked effects of HO on nucleic acid synthesis.

In agreement with previous studies on synchronised HTC cells in suspension[9], the high-density monolayer cells exhibited maximal ^{3}H-thymidine incorporation 18–20 h after stimulation by dilution with fresh growth medium. At this time there was no appreciable difference between cells stimulated in the absence or presence of HO (Table 1).

In intact animals HO depressed hepatic putrescine levels about 50%, an effect which persisted for at least 6 h (Fig. 2). In regenerating liver, HO prevented the 1.7-fold increase in putrescine usually elicited by partial hepatectomy[10,11] (Fig. 2). Hepatic putrescine levels were higher in controls, nephrectomised to impair HO excretion and permit administering lower doses, (Fig. 2, left) than in unoperated controls (Fig. 2, right), which is consistent with reports that stress increases hepatic ornithine decarboxylase activity[12]. In agreement with earlier data[13], partial hepatectomy caused a 25–50% increase in liver RNA synthesis, which is the same in nephrectomised and normal rats. In contrast to its influence on putrescine, HO (50 mg kg^{-1} to nephrectomised rats; 400 mg kg^{-1} to nonnephrectomised rats) failed to alter the apparent increase in RNA synthesis (Table 1).

Thus both in HTC cells in culture and in regenerating and intact rat liver, HO effectively blocks net putrescine formation. Neither RNA nor DNA synthesis is apparently altered in HTC cells, and RNA synthesis is unaffected in regenerating and intact liver. HO only partially lowered putrescine levels in our experiments. Conceivably, complete depletion of putrescine might affect nucleic acid formation. Moreover, more refined techniques may reveal influences of HO on the synthesis of certain classes of RNA or DNA. Although we did not measure spermidine and spermine, it is conceivable that their continued synthesis in the presence of HO could mediate enhanced nucleic acid synthesis. However, blockade of spermidine and spermine formation by methyl glyoxal *bis* (guanylhydrazone), which inhibits S-adenosylmethionine decarboxylase, fails to impair nucleic acid synthesis[16,17].

Our data indicate that enhanced ODC activity and putrescine formation are not an indispensable signal for new RNA and consequent DNA synthesis. Although increased levels of putrescine and presumably of polyamine formation may not be causally related to new RNA synthesis, the accumulated evidence[1,2] still suggests that the polyamines serve an important function in tissue growth.

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1 Tabor, H., and Tabor, C. W., *Pharmac. Rev.*, **16**, 245 (1964).
2 Cohen, S. S., *Introduction to the polyamines* (Prentice Hall, Englewood Cliffs, New Jersey, 1972).
3 Russell, D. H., and Snyder, S. H., *Molec. Pharmac.*, **5**, 253 (1969).
4 Harik, S. I., and Snyder, S. H., *Biochim. biophys. Acta*, **327**, 501 (1973).
5 Harik, S. I., Pasternak, G. W., and Snyder, S. H., in *Polyamines in normal and neoplastic growth* (edit by Russell, D. M.) 307 (Raven Press, New York, 1973).
6 Hogan, B. L. M., *Biochem. biophys. Res. Comm.*, **45**, 301 (1971).
7 Hogan, B. L. M., Murden, S., and Blackledge, A., in *Polyamines in normal and neoplastic growth* (edit by Russell, D. H.) 238 (Raven Press, New York, 1973).
8 Harik, S. I., Hollenberg, M. D., and Snyder, S. H., *Molec. Pharmac.*, **10**, 41 (1974).
9 Martin, jr., D., Tomkins, G. M., and Granner, D., *Proc. natn. Acad. Sci. U.S.A.*, **62**, 248 (1969)
10 Janne, J., *Acta physiol. scand.*, **300**, Suppl. 1, (1967).
11 Russell, D. H., Medina, V. J., and Snyder, S. H., *J. biol. Chem.*, **245**, 6732 (1970).
12 Schrock, T. R., Oakman, N. J., and Bucher, N. L. R., *Biochim. biophys. Acta*, **204**, 564 (1970).
13 Fujioka, M., Koga, M., and Lieberman, I., *J. biol. Chem.*, **238**, 3401 (1963).
14 Harik, S. I., Pasternak, G. W., and Snyder, S. H., *Biochim. biophys. Acta*, **304**, 753 (1973).
15 Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265 (1951).
16 Pegg, A. E., *Biochem. J.*, **132**, 537 (1973).
17 Fillingame, R. H., and Morris, D. R., *Biochemistry*, **12**, 4479 (1973).

Hormonal regulation of presumptive mRNA in the fungus *Achlya ambisexualis*

THE Oomycete fungus, *Achlya ambisexualis*, is one of the most primitive eukaryotes which differentiates in response to a steroid hormone[1]. Because it is fast growing, easy to manipulate and can be induced to undergo differentiation in a relatively synchronous manner (the entire thallus acts as target cells), *Achlya* lends itself to studies of the molecular control of gene activation by hormones.

Antheridiol, a sterol secreted by female strains of *Achlya* has been characterised from culture filtrates and synthesised from disnorcholenic acid[1]. The hormone is active at 6×10^{-12} g^{-1} when assayed with *A. ambisexualis* (strain E87)[1]. Addition of antheridiol to vegetative hyphae elicits the initiation of antheridial branches[2] which eventually differentiate into the male sex organs (antheridia). Thomas and Mullins[3] postulate that a prerequisite for hormonal induction of antheridial hyphae is a localised softening of the cell wall by cellulase. They suggest that this weakens areas of the walls, through which protoplasm is forced by the internal turgor pressure forming the sex organ initial. Their preliminary studies suggest that both transcription and translation are required for this process[4]. In animals steroid hormones can stimulate transcription of specific mRNAs which are then translated into proteins required for cellular differentiation[5–7].

Recent studies have shown that mRNAs of higher plants and animals contain a sequence of polyadenylic acid (poly(A)) which is added to the 3′ ends of the molecules[8–10]. Only histone mRNAs lack a poly(A) moiety[11]. We have demonstrated in *Achlya* the presence of a poly(A)-rich class of cellular RNA. This is the most primitive eukaryote to date in which poly(A) has been shown to be associated with presumptive mRNA. Furthermore, we have demonstrated that the sex hormone, antheridiol, has a considerable effect on the accumulation of this RNA during the early stages of male sex organ development. We have characterised the poly(A)-rich fraction and have demonstrated its similarity to the mRNAs of higher eukaryotes.

Poly(A)-rich RNA has been demonstrated in the cellular slime mould, *Dictyostelium discoideum*[12] and in the yeast, *Saccharomyces cerevisiae*[13,14]. The size of this poly(A) varies from 50 nucleotides in yeast[13] to more than 200 nucleotides in higher animals[15–17]. In contrast, *Escherichia coli* mRNA, so far as is known, does not contain any poly(A)[18]. Two interesting questions are (*i*) do all existing eukaryotes possess some poly(A) associated with their mRNA, and (*ii*) does the size of such poly(A) increase with phylogenetic complexity? We have found the size of the *Achlya* poly(A) moiety to be smaller than the poly(A) tracts found in animals and *D. discoideum* but similar to that of *S. cerevisiae*.

Stock cultures of *A. ambisexualis* were maintained on Emerson's YPSS agar (Difco). Asexual spores were prepared according to Griffin[19]. A suspension of 1×10^5 spore cysts was inoculated into PYG broth (300 ml)[19] and grown for 24 h at 23° C until a finely suspended mycelium had grown. This was stored at 5° C or used immediately as an inoculum. A 25 ml sample of this was transferred to flasks containing 275 ml of Barksdale's mating media[20] and was grown for 24–32 h at 23° C. Cultures were collected through four layers of cheesecloth followed by washing with four volumes of ice-cold deionised H_2O.

RNA was isolated at 5° C by suspending 10–20 g wet weight of mycelia in SSC (0.15 M NaCl, 15 mM Na-citrate, *p*H 8.3) 1% SLS (Na-lauryl sulphate) and an equal volume

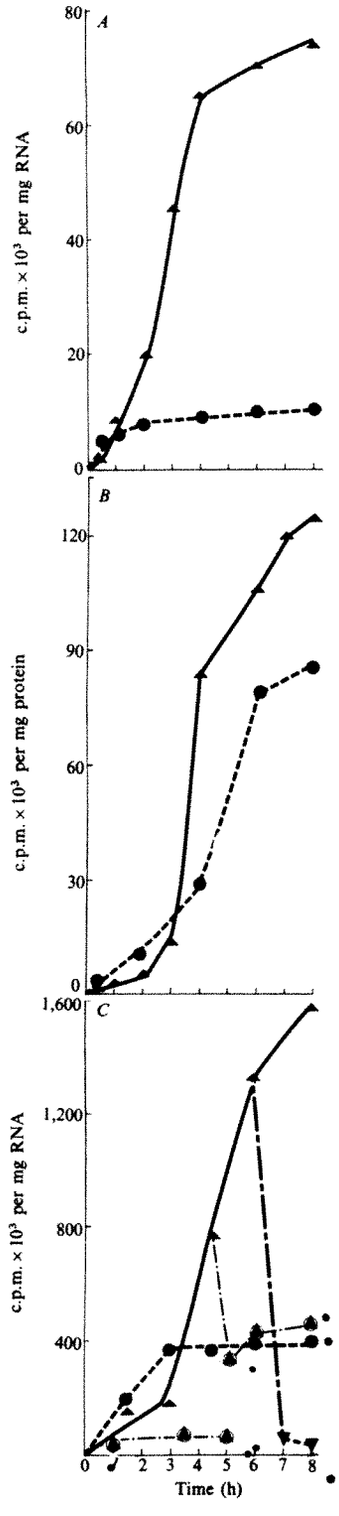


FIG. 1 The effect of antheridiol on the accumulation of RNA and protein during male organ initiation in *A. ambisexualis*. Total phenol-extractable RNA. Cells were exposed to continuous labelling conditions (10 μCi ^{3}H-uridine, specific activity 52 Ci mmol^{-1}) from time zero in the presence (▲) and absence (●) of 5×10^{-11} g ml^{-1} antheridiol. Concentration of RNA was determined by A_{260}/A_{280} ratios. Radioactivity was determined on a liquid scintillation counter. *B*, Total hot TCA-precipitated protein. Cells were labelled using steady state conditions (25 μCi ml^{-1} ^{3}H-leucine, specific activity 54 Ci mmol^{-1}) in the presence and absence of hormone. Concentration of protein determined by A_{280}/A_{260} ratios or by the Lowry procedure. *C*, Poly(A)-rich RNA accumulation. Cells were labelled using steady state conditions (10 μCi ml^{-1}) in the presence (▲) and absence (●) of hormone. Total RNA was extracted as described in the text and passed through a Sigma 38 cellulose column (Fig. 2). RNA concentration and radioactivity were determined as described above. When indicated 10 μCi ml^{-1} actinomycin D (– – –) (ref. 19) 25 μg ml^{-1} cordycepin (—•—) (ref. 9) was added to the hormone-treated cultures.

TABLE 1 Base composition analysis of *A. ambisexualis* RNA from unmodified cellulose columns

RNA fraction	Base composition (%) C	G	A	U
+ Hormone (bound)	17.3	21.5	33.5	27.5
Vegetative control (bound)	16.6	20.5	34.5	27.8
Unbound (both hormone and control)	17.4	23.1	29.4	29.9

Phosphorus-32 (15 μCi ml^{-1}, carrier free)-labelled RNA fractions were collected from Sigma 38 cellulose columns (Fig. 2) made up to SSC, 1% SLS, 0.3 M LiCl and precipitated in 2.5 volumes of ethanol. The precipitate was taken up in 0.3 N KOH and hydrolysed at 37°C for 24 h. Base composition was determined using descending paper chromatography following the procedure of Lane[25].

of phenol, equilibrated with 50 mM Tris-HCl, *p*H 8.3. The mixture was then homogenised in a Willems polytron homogeniser at the highest setting for 10 s. The aqueous phase was extracted three times with the Tris-washed phenol. This phase was made 0.3 M with respect to LiCl and the precipitated RNA was washed twice in ethanol, dried and dissolved in deionised H_2O.

To prepare partially purified nuclei, 30–50 g wet weight of tissue was suspended in SSC buffer containing 1% SLS, and 0.25 M sucrose according to the procedure of Jaworski and Horgen[21]. The nuclear pellet (containing some mitochondria) was resuspended in SSC, 1% SLS and extracted with phenol, precipitated with ethanol and taken up in deionised H_2O as described above. Polyribosomes were isolated according to the method of Lin and Key[22], except that buffers were at *p*H 9.0. Polysome fractions were pooled and the RNA was extracted as described.

In the presence of 5×10^{-11} g ml^{-1} antheridiol at 23° C, antheridial branch initials are visible between 3 and 4 h after the addition of hormone. The incorporation of ^{3}H-uridine into total phenol-extractable RNA is shown in Fig. 1*A*. The specific activity of the total RNA extracted from hormone-treated cells increased (after a lag of 30 min) markedly for 4 h, whereas control (vegetative) cell RNA reached a steady state 2-3 h after addition of the ^{3}H-uridine.

Figure 1*B* shows the effect of antheridiol on total protein accumulation. In the hormone-treated tissue a lag of 2-3 h was observed before the rate of incorporation of ^{3}H-leucine increased considerably. Conversely, incorporation of ^{3}H-leucine into protein in vegetative mycelia showed no detectable lag.

Total phenol-extractable RNA, from either the whole cell homogenate, nuclei or preparations of polysomes was passed through an unmodified Sigma 38 cellulose column[23], and the poly(A)-rich fraction was eluted with deionised H_2O (Fig. 2). The fraction eluted with H_2O (cellulose-bound) was found to be rich in adenylic acid (Table 1). The cellulose-bound fraction from both the hormone-treated and vegetative mycelia had greater than 33.5% AMP residues (Table 1). This was in contrast to the adenylic acid content found in the remainder of the cellular RNA. These values are similar to those reported for other enkaryotes[8,17].

The effect of antheridiol on the incorporation of ^{3}H-adenosine into poly(A)-rich RNA is shown in Fig. 1*C*. Antheridiol stimulated a three to four fold increase in the specific activity of the poly(A)-rich fraction after an initial lag period (2–3 h), whereas incorporation of label into the poly(A)-rich fraction of vegetative cells showed no lag, increased linearly, and reached a steady state after 3 h (Fig. 1*C*). The enhancement of incorporation between 3 and 4 h after addition of the hormone corresponds to the observation of the appearance of male sex organ initials. Addition of actinomycin D causes a rapid decline in the specific activity of the poly(A)-rich fraction within 1 h (Fig. 1*C*), suggesting that total poly(A)-rich RNA accumulation is affected by actinomycin D and that the life span of a given poly(A)-rich molecule is very short (less than 1 h). Cordycepin, a drug which is known to affect the post-transcriptional addition of poly(A)[9], rapidly inhibited the incorporation of ^{3}H-adenosine into *Achlya* poly(A)-rich RNA (Fig. 1*C*). This suggests that the mechanism for the addition of the poly(A) fraction to *Achlya* presumptive mRNA is similar to the mechanism operative in mammalian cells.

In order partially to insure that our incorporation studies were in fact measuring mRNA accumulation and not rapid changes in nucleotide pool sizes, we compared incorporation of label into poly(A)-rich RNA with the effect of antheridiol on leucine incorporation (Fig. 1*B* and 1*C*). Again, an enhancement of the total hot trichloroacetic acid (TCA) precipitable counts was observed when compared with the control. The rapid increase in specific activity of the total protein between 3 and 4 h corresponded to the rapid increase observed in total incorporation into poly(A)-rich RNA. These data indicate that antheridiol stimulates the accumulation and possibly the synthesis of an adenylate-rich fraction of cellular RNA. This enhancement of the total specific activity of the RNA fraction was observed whether the RNA was isolated from the total cell homogenate, from purified nuclei or from polyribosomes.

During male sex organ differentiation in *A. ambisexualis* (E87), the external morphogenetic stimulus, antheridiol, activates the accumulation of a class of RNA, presumably messenger, which is required for the initiation of the antheridial branches. The addition of actinomycin D and of cordycepin inhibits the accumulation of the messenger-like fraction and also prevents normal morphological differentiation. Similar stimulation of mRNA synthesis by oestrogen and progesterone has been reported during chick oviduct development[24].

If the poly(A)-rich RNA of *Achlya* is in fact mRNA, then it would be expected that differing size transcripts would be synthesised for differing size gene products. In an attempt to determine the relative size of the poly(A)-rich RNA from *Achlya*, polyacrylamide electrophoresis of the

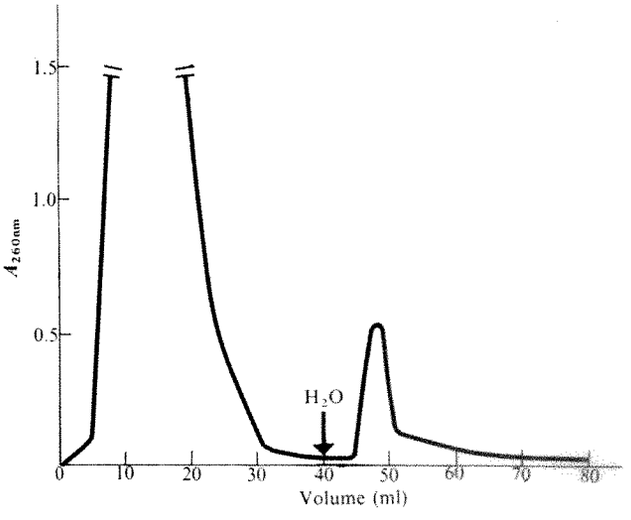


FIG. 2 Chromatography of *A. ambisexualis* RNA on Sigma 38 cellulose. RNA was extracted in SSC, 1% SLS precipitated in the presence of 0.3 M LiCl and taken up in deionised H_2O (1 ml). RNA concentration and radioactivity determined as in Fig. 1. The RNA was diluted to 10 ml with 10 mM Tris-HCl (*p*H 7.6), 500 mM KCl, 0.2 mM $MgCl_2$, and 400 A_{260} units were applied to a 3 ml cellulose column[23]. The column was washed with the above buffer until the A_{260} reading dropped below 0.02. Deionised water was then passed through the column to elute the poly(A)-rich RNA. This RNA was either used immediately or made up to SSC, 1% SLS and reprecipitated in ethanol.

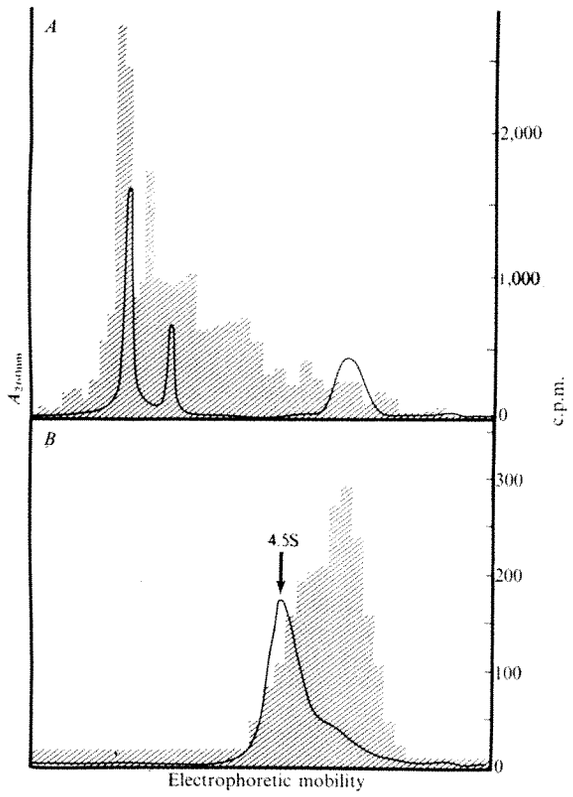


FIG. 3 Gel electrophoresis of *A. ambisexualis* poly(A)-rich RNA and RNAase digestion product. *A*, ^{3}H-adenosine-labelled poly(A)-rich RNA was isolated as described in Fig. 2 and reprecipitated with nonlabelled total *Achlya* RNA. Disc electrophoresis was performed using the procedure of Jaworski and Horgen[21] in 2.4% gels. The gels were scanned with a Gilford linear transporter, frozen in dry ice and sliced with a Mickle gel slicer. The slices were solubilised with NCS (Nuclear Chicago) and counted in a Beckman LS-150 scintillation spectrometer. *B*, ^{3}H-adenosine-labelled poly(A)-rich RNA was digested for 30 min at 37°C in 10 mM Tris-HCl (*p*H 7.6), 50 mM KCl, 1 mM $MgCl_2$ with pancreatic RNase (Worthington 56.8 μg ml^{-1}). The hydrolysed solution was reprecipitated in ethanol with 100 μg *E. coli* tRNA (4.5S). The precipitated polynucleotides were washed once in ethanol and taken up in electrophoresis buffer[21]. The mixture of tRNA and *Achlya* poly(A) was layered onto a 5% gel and electrophoresed for 2 h at 6 MA per gel. Gels were scanned, sliced and counted as above. Similar results were obtained when cells were pulse labelled for 2 h with 15 μCi ml^{-1} ^{32}P. RNA was extracted from the 5 h hormone-treated cells or from vegetative control cells that had been labelled for 5 h. Both gave identical gel profiles.

Sigma 38 cellulose-bound fraction with *Achlya* 26S, 18S, 5S and 4S RNA present as markers was performed. Figure 3*A* indicates that the poly(A)-rich fraction ranged from approximately 30S to almost 4S. These results are similar to what has been reported in other eukaryotic cells[16,17].

To determine the size of the poly(A) tract we took advantage of the fact that polyadenylic acid is insensitive to hydrolysis by T_1 and pancreatic RNase A[16,17]. After nuclease digestion and reprecipitation in ethanol, the *Achlya* poly(A) moiety was either subjected to alkaline hydrolysis or electrophoresis with *E. coli* 4.5S RNA as a marker.

In establishing if the RNase-resistant material of *Achlya* consisted of polyadenylic acid, it was subjected to base composition analysis after alkaline hydrolysis. The value for AMP was greater than 90%.

Coelectrophoresis with *E. coli* RNA showed that the poly(A) segment in *Achlya* migrated more rapidly than the 4.5S (25,000 daltons) marker suggesting a molecular weight of approximately 16–18,000 (Fig. 3*B*). This means that the poly(A) segment of *Achlya* presumptive mRNA is about 60 nucleotides long. This is significantly smaller than the poly(A) tract in animal mRNAs[9,15,16], somewhat smaller than that found in cellular slime moulds[12], but very similar to that reported for yeast[13,14]. It seems that evolution has resulted in progressively longer poly(A) pieces on the mRNA molecules in organisms with greater phylogenetic complexity.

The genus *Achlya* is a member of the most primitive group of eukaryotes that shows a developmental response to steroid hormones. We have demonstrated that in the male strain of *A. ambisexualis* (E87), associated with antheridiol stimulation of the development of sex organ initials, there is a corresponding effect of the hormone on the accumulation of a messenger-like fraction of RNA and on the accumulation of protein. These results suggest that the sterol affects morphogenesis by turning on the synthesis of mRNA which is then translated into protein required for the differentiation of the male sex organ initial.

We feel that *Achlya* provides a unique and valuable model system for the study of hormonal effects on development, both at the morphological and biochemical levels.

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1 Barksdale, A. W., *Science N.Y.*, **166**, 831–837 (1969).
2 Barksdale, A. W., *Mycologia*, **62**, 411–421 (1970).
3 Thomas, D., and Mullins, J. T., *Science N.Y.*, **154**, 84–85 (1965).
4 Kane, B. E., Reiskind, J. B., and Mullins, J. T., *Science N.Y.*, **180**, 1192–1193 (1973).
5 Means, A. R., Comstock, J. P., Rosenfeld, G. C., and O'Malley, B. W., *Proc. natn. Acad. Sci. U.S.A.*, **60**, 1146–1150 (1972).
6 Segal, S. J., Ige, R. O., Tuchimaa, P., and Burgos, M. H., *Science N.Y.*, **181**, 569–570 (1973).
7 Brawerman, G., Mendecki, J., and Lee, S. Y., *Biochemistry*, **11**, 637–641 (1972).
8 Manaham, C. O., App, A. A., and Still, C. C., *Biochem. Biophys. Res. Commun.*, **53**, 588–595 (1973).
9 Darnell, J. E., Philipson, L., Wall, R., and Adesnik, M., *Science N.Y.*, **174**, 507–510 (1971).
10 Singer, R. H., and Penman, S., *J. molec. Biol.*, **78**, 321–334 (1973).
11 Adesnik, M., Salditt, M., Thomas, W., and Darnell, J. E., *J. molec. Biol.*, **71**, 21–30 (1972).
12 Firtel, R. A., Jacobson, A., and Lodish, H. F., *Nature new Biol.*, **239**, 225–228 (1972).
13 Reed, J., and Wintersberger, E., *Febs. Lett.*, **32**, 213–217 (1973).
14 McLaughlin, C. S., Warner, J. R., Edmonds, M., Nakazato, H., and Vaughan, M. H., *J. biol. Chem.*, **248**, 1466–1471 (1973).
15 Edmonds, M., Vaughan, M. H., and Nakazato, H., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1336–1340 (1971).
16 Lee, S. Y., Mendecki, J., and Brawerman, G., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1331–1335 (1971).
17 Darnell, J. E., Wall R., and Tushinski, R. J., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1321–1325 (1971).
18 Perry, R. P., Kelly, E., and LaTorre, J., *Biochem. Biophys. Res. Commun.*, **48**, 1593–1600 (1972).
19 Griffin, D. H., and Breuker, C., *J. Bact.*, **98**, 689–696 (1969).
20 Barksdale, A. W., and Lasure, L., *Bull. Torrey Bot. Club.*, **100**, 199–202 (1973).
21 Jaworski, A. J., and Horgen, P. A., *Arch. Biochem. Biophys.*, **157**, 260–267 (1973).
22 Lin, C. Y., and Key, J. L., *J. molec. Biol.*, **26**, 237–247 (1966).
23 Schutz, G., Beato, M., and Fiegelson, P., *Biochem. Biophys. Res. Commun.*, **49**, 690–699 (1972).
24 O'Malley, B. W., McGuire, W. L., Kohler, P. O., and Karenman, S. G., *Rec. Prog. Hormone Res.*, **25**, 105–160 (1969).
25 Crane, B. G., *Biochim. biophys. Acta*, **72**, 110–113 (1963).

Serological evidence of calicivirus transmission between marine and terrestrial mammals

THE recent isolation of two serotypes of San Miguel sea lion virus (SMSV), a calicivirus indistinguishable from vesicular exanthema of swine virus (VESV), from California sea lions (*Zalophus c. californianus*) and Northern fur seals (*Callorhinus ursinus*) points to: (1) the widespread distribution of SMSV, and (2) the role of marine mammals as possible reservoirs of viral diseases of terrestrial animals[1,2].

The latter hypothesis has now been assessed further by a serological survey for SMSV neutralising antibodies in sea lion and fur seal sera, collected in 1972, and in feral swine sera obtained in 1973. Individual blood specimens were collected from twenty mature female California sea lions on San Miguel Island, one of the Channel Islands off the coast of southern California. Further specimens were obtained from 120 immature (3–4-yr-old) bachelor bull fur seals from four hauling grounds, Reef, Tolstoi, Lukanin and Zapadni during the annual seal harvest on St Paul Island, a member of the Pribilof Group of Alaska. Blood samples were collected from eighteen feral swine during the annual hunt on Santa Cruz Island, another of the Channel Islands. After processing, serum specimens were stored at −20° C until tested.

Vero cell monolayers, used for either neutralisation tests or production of SMSV viruses, were grown in Eagle's minimum essential medium (MEM) supplemented with 5% foetal calf serum and antibiotics (penicillin 200 U ml^{-1}; streptomycin 100μg ml^{-1}). Pools of the two serotypes designated SMSV-1MR and SMSV-2MR were produced by collecting inoculated Vero cell monolayers 18 h after infection and then titrating the supernatant fluids for 50% tissue infective doses ($TCID_{50}$ per 0.1 ml). The neutralising antibody test procedure was as follows. Serially diluted serum (inactivated by subjection to 56° C for 30 min) was mixed with equal volumes of virus suspension containing 100 $TCID_{50}$ per 0.1 ml, incubated at room temperature for 1 h, and 0.2 ml aliquots inoculated into each of four tubes of Vero cell monolayers per dilution tested. Inoculated tubes were then incubated stationary at 37° C and examined for cytopathic effect after 24, 48 and 72 h.

All twenty of the female sea lions had neutralising antibody titres to SMSV-2MR and eighteen had antibodies to SMSV-1MR (Table 1). The two distinct SMSV serotypes were isolated originally from different sea lions sampled on the same day. This together with serological evidence of SMSV antibodies against both isolates, confirms the simultaneous presence of two SMSV serotypes within the herd. This is not regarded as unusual for VESV; outbreaks have been reported where different VESV serotypes, attributed to mutation[3], occurred in the same domestic swine herd within a short time.

In contrast to the findings in sea lions, no serological evidence of SMSV-1MR antibodies was found in northern fur seals on St Paul Island. This is puzzling because a virus serologically identical to SMSV-1MR was isolated from an emaciated pup from the Reef rookery on this island[2]. One possible explanation is that the serum tested in our study was collected from 3–4-yr-old bachelor bulls who, due to their social behaviour, remained isolated from breeding adults and newborn pups. This would suggest that SMSV-1MR has been introduced recently into the fur seal herd on the Pribilof Islands.

One-third (40/120) of the fur seal sera tested had neutralising activity against SMSV-2MR (Table 1). Animals with antibody titres were from all four of the hauling grounds sampled. Although SMSV-2MR has not yet been isolated from any fur seals, serological evidence points to its past occurrence in the herd. It is important, however, that the same serotype of SMSV infected herds of two pinniped species as far apart as the Santa Barbara Channel and the Pribilof Islands. The annual fur seal migration from islands in the Bering Sea to waters off the coast of southern California and the presence of a small fur seal rookery on San Miguel Island, may account for the interspecies spread of SMSV.

TABLE 1 SMSV neutralising antibodies in sea lion, fur seal and feral swine sera

Antigen	Antibody titres	California sea lion (20)*	Northern fur seal (120)*	Santa Cruz feral swine (18)*
SMSV-1MR				
	<10	2	120	17
	10	7	0	1
	20	5	0	0
	40	5	0	0
	80	1	0	0
SMSV-2MR				
	<10	0	80	13
	10	1	20	0
	20	2	8	2
	40	5	10	1
	80	7	2	1
	160	4	0	1
	320	1	0	0

* No. of animals tested.

Feral swine on Santa Cruz Island have also had experience with the same SMSV serotypes; five out of eighteen serum specimens tested had SMSV-2MR and one out of eighteen had SMSV-1MR neutralising antibodies. Wild swine have existed on the Channel Island for many years, where they could have made contact with pinnipeds by foraging on beaches or scavenging on carcasses. The presence of antibodies in swine sera indicates that viral transmission has occurred. The age of the swine sampled was unknown, but their weight showed that only heavier, presumably older, animals had neutralising antibodies.

The finding of SMSV antibodies in feral swine and marine mammals may throw light on the successive outbreaks of VES in 1932, 1933 and 1934 in domestic swine[4]. The isolation of SMSV and its endemicity in pinniped herds suggests that VES outbreaks in domestic swine herds originated from a marine source. This coupled with the observation that SMSV produces a vesicular disease characteristic of VES in swine[5], and the finding of similar vesicles on fur seal flippers further substantiates inter-species transmission from a marine to a domestic animal[5,6]. Thus an exotic disease of swine thought to have disappeared 17 yr ago is now believed to have been rediscovered in pinnipeds and certain feral swine.

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1 Smith, A. W., Akers, T. G., Madin, S. H., and Vedros, N. A., *Nature*, **244**, 108–110 (1973).
2 Smith, A. W., Prato, C. M., Gilmartin, W. G., Brown, R. J., and Keyes, M. C., *J. Wildlife Diseases*, **10**, 54–59 (1974).

[3] Bankowski, R. A., in *Adv. Vet. Sci.*, **10**, 23–64 (edit. by Brandly, C. A., and Cornelius, C.) (Academic Press, New York, 1965).
[4] Madin, S. H., in *Diseases of swine* (edit. by Dunne, H. W.), 270–291 (Iowa State University Press, Ames, 1970).
[5] Bachrach, H. L., and Hess, W. R., *Biochem. biophys. Res. Comm.*, **55**, 141–149 (1973).
[6] Smith, A. W., Madin, S. H., and Akers, T. G., *Proc. Tenth Conf. Biological Sonar and Diving Mammals* (Stanford Research Institute, Menlo Park, California, 1973).

Distribution of adenosine triphosphatase during anaphase in cricket primary spermatocytes

THE idea that an adenosine triphosphase (ATPase) may be involved in the generation of the force that moves chromosomes comes by analogy with other motile systems[1]. In striated muscle, the ATPase is a part of heavy meromyosin[2]. It forms cross bridges with actin filaments, and is an irreducible component in the mechanochemical process[3]. ATPase is also present in nonmuscular systems, such as slime moulds[4], myonemes[5], cilia and flagella[6–8], where it is essential for motility[9–10].

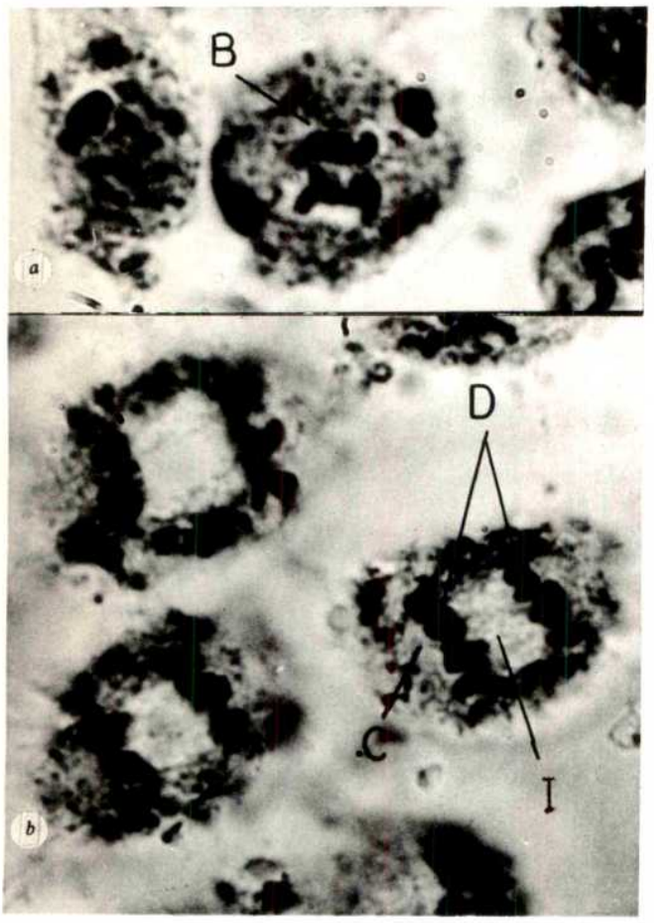


FIG. 1 The cytochemical distribution of ATPase in metaphase and anaphase primary spermatocytes from *Acheta domesticus*. ATPase is present in the stained areas (× 2,000). *a*, Metaphase: the stain is equally distributed in the cytoplasm and spindle, but heavier deposits occur in the bivalents (B). *b*, Anaphase: stain is absent in the interzone (I) between the separating dyads (D) but present in the chromosomal fibre regions, (C).

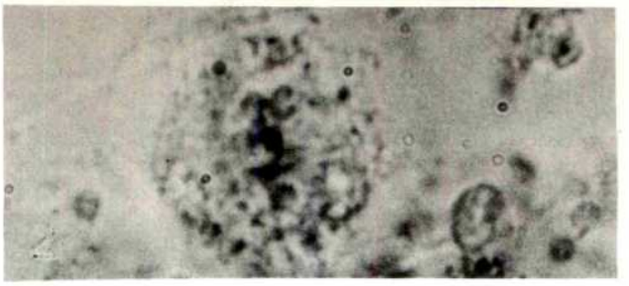


FIG. 2 Primary spermatocyte, metaphase, control: no stain is deposited in the spindle or cytoplasm (× 2,000).

An ATPase has been found in spindles isolated from sea urchins[1,10–11], but it is difficult to decide whether the enzyme has a functional role or is a contaminant, for the very low specific activity present in the spindle is the same as that in the cytoplasm[11]. Other problems, such as the lability of the enzyme[12–13], or the possibility that the spindle's force-producing mechanism is solubilised during isolation[14] add to the difficulties. Furthermore, information necessary for the interpretation of spindle ATPase function cannot be detected in bulk spindle isolates. Such information would concern possible changes in the distribution of the same amount of enzyme within the spindle accompanying chromosome motion. A cytochemical approach to this problem, using spermatocytes of the domestic cricket *Acheta domesticus*, L., now indicates that the distribution of ATPase at anaphase differs from that at metaphase.

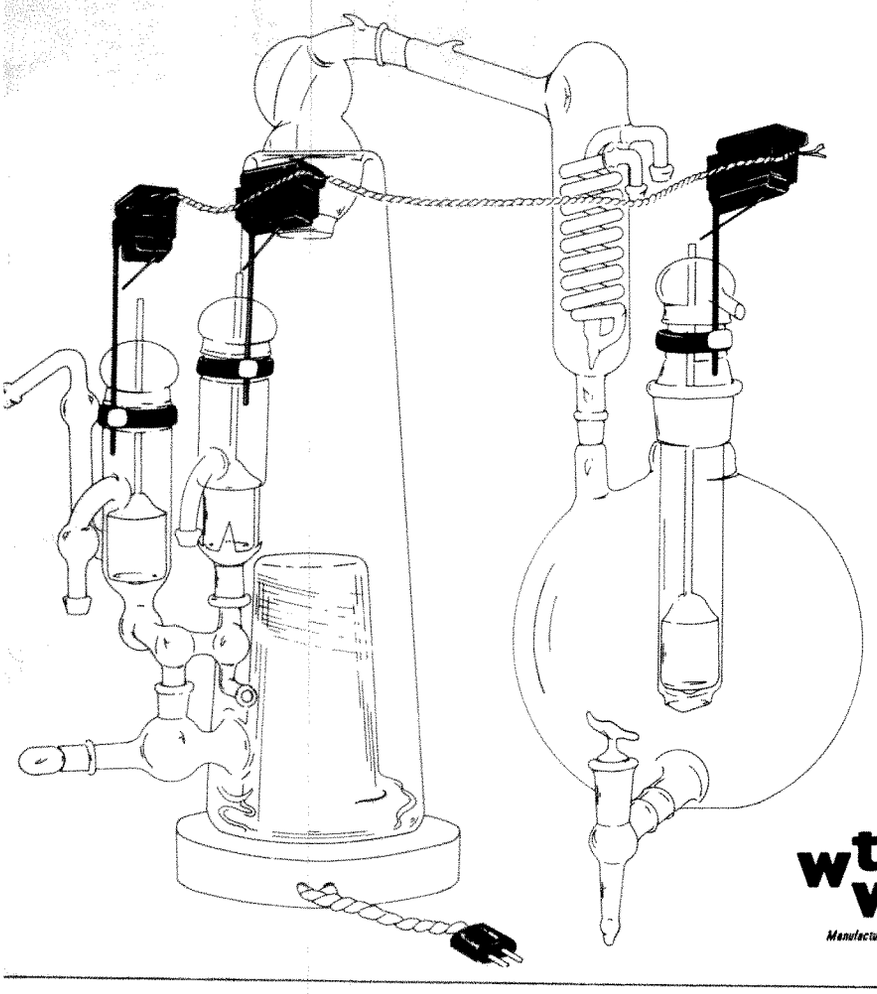
We cultured spermatocytes as described before[15], except that gelatin was incorporated into the medium to make the cells adhere to the coverslip to prevent their loss during cytochemical procedures. (A detailed account of the use of gelatin will be given elsewhere.)

When the cultures were established, they were observed until the cells reached the desired stage of division. Then the coverslips bearing the cultures were immersed in a fixative containing: 1 part 2.5% glutaraldehyde and 0.002 M $CaCl_2$ in 0.03 M cacodylate buffer (*p*H 7.2); 9 parts 2.5% hydroxyadipaldehyde in 0.55 M sucrose buffered with 0.03 M cacodylate (*p*H 7.5). After 12 min coverslips were washed once in buffered sucrose (0.03 M cacodylate and 8.5% sucrose) at 0° C and *p*H 7.4 and then given two 10-min rinses in buffered sucrose at *p*H 4.0 (to remove soluble phosphate). They were stored overnight at 6° C in the buffered sucrose, *p*H 7.4.

The fixed spermatocytes were treated by a modified Padykula–Herman method[17]. Incubation in substrate medium was at 37° C for 1 h. To test substrate specificity, ATP was replaced by 1.4×10^{-3} M ADP, GTP or ITP.

After the cytochemical procedure cultures were mounted in glycerol and ringed with clear nail polish. Controls were parallel cultures incubated without substrate. Sulphydryl dependence was tested by incubation in cysteine-free medium containing 0.01 M *p*-hydroxymethyl benzoate (POHMB). Enzyme localisation was considered positive when a black stain was present after incubation in medium containing substrate but was not present in control medium (Fig. 2).

Figure 1 shows the ATPase distribution. In the metaphase primary spermatocyte (Fig. 1*a*) both the cytoplasm and the spindle area stained with the same intensity. The chromosomes and the Golgi, however, stained more heavily. When spermatocytes were in anaphase (Fig. 1*b*), the stain was not equally distributed throughout the spindle area—there was a clear area in the interzonal spindle region, between the separating dyads. The stain, however, remained



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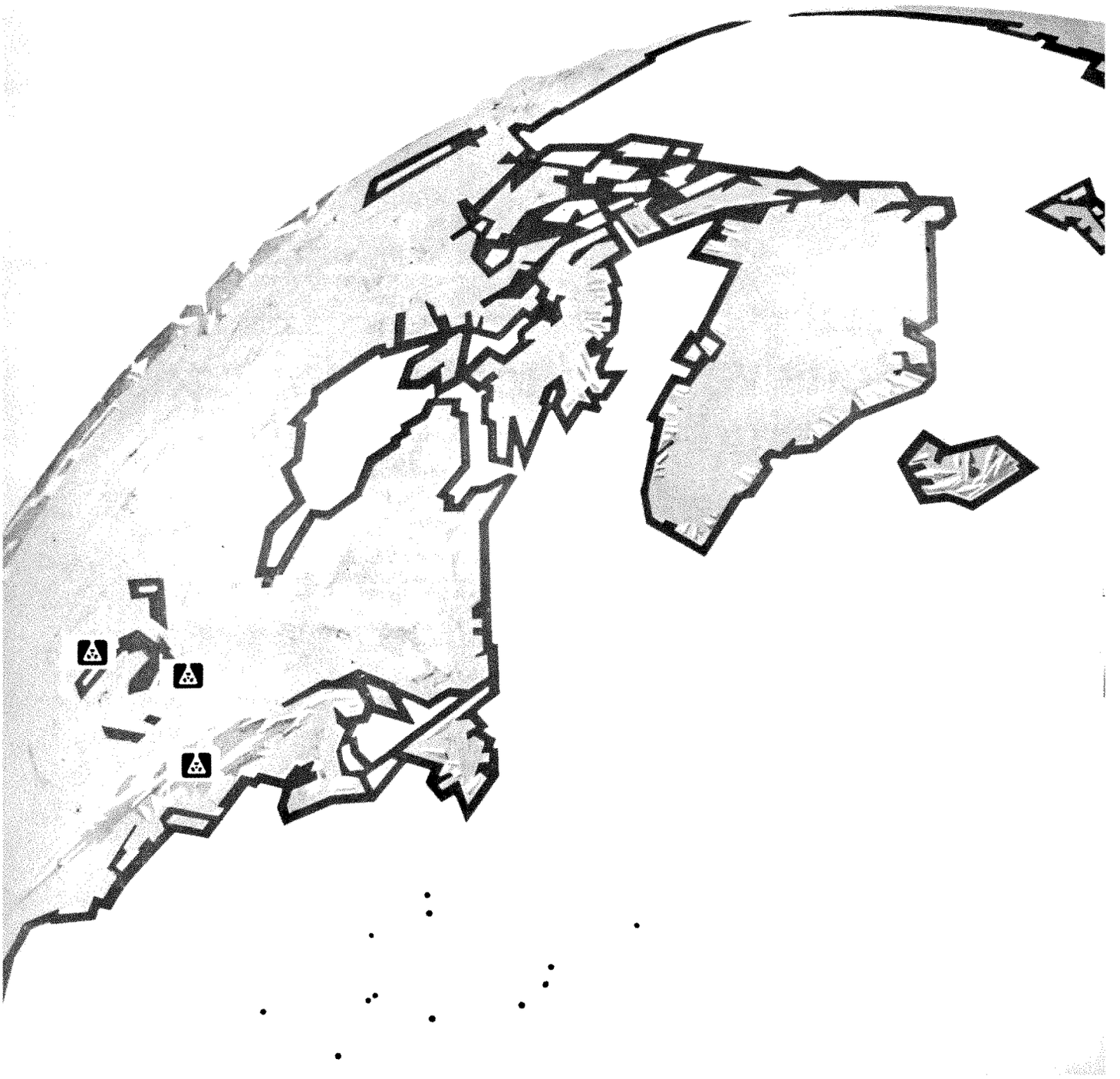
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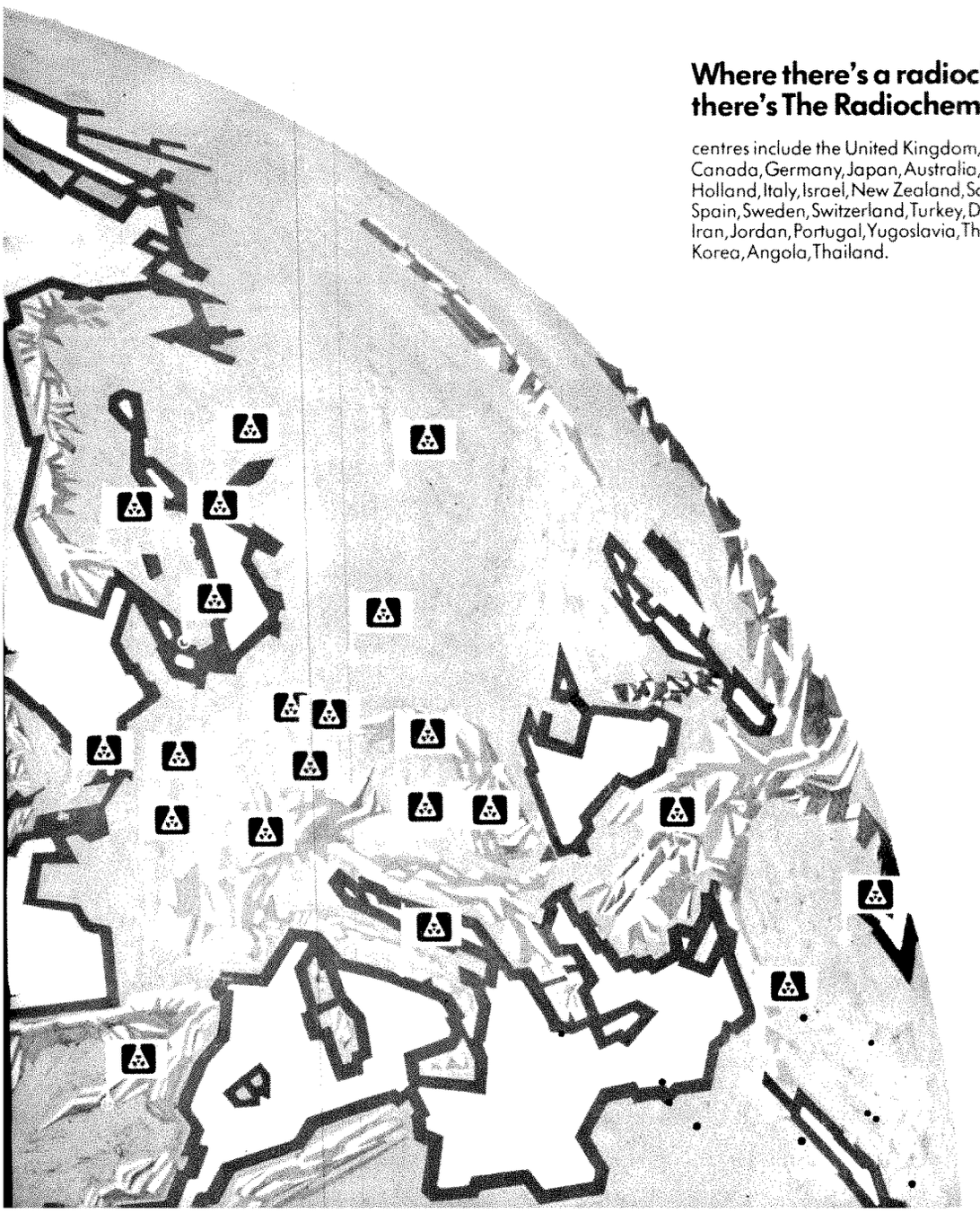
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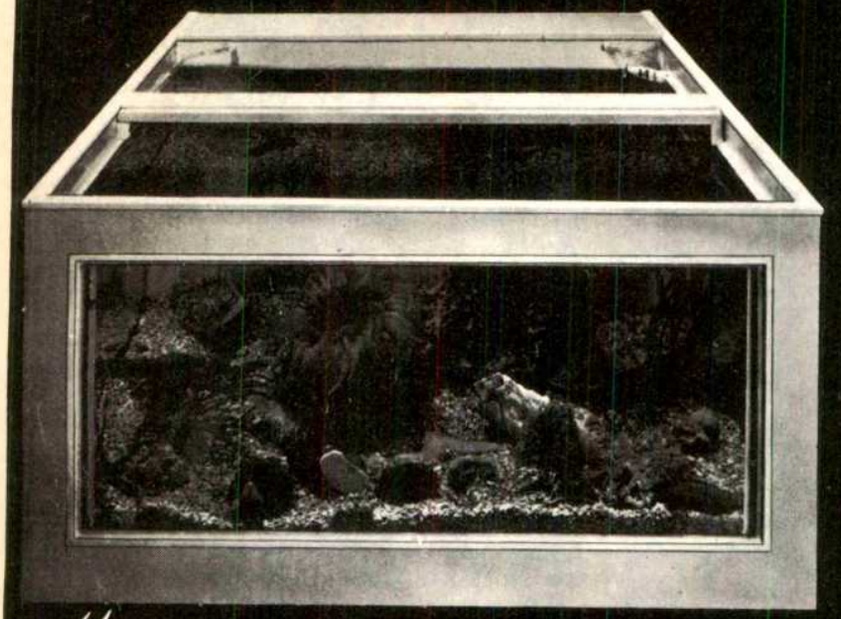
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in the chromosomal fibre region between the kinetochores and poles, and in the cytoplasm adjacent to the interzone. This difference in enzyme activity between the interzone and chromosomal fibre region persisted until the end of anaphase. The same pattern was found in secondary spermatocytes—equal distribution of enzyme activity between spindle and cytoplasm in metaphase and absence of activity in the interzone at anaphase. The stain remained undiminished in the chromosomes and other organelles. In control preparations, there were no differences between the interzones and half spindles under either bright field or phase contrast microscopy. With ADP (Fig. 3*a*), GTP (Fig. 3*b*) or ITP, both the interzone and the chromosomal fibre regions were stained in anaphase. In the presence of POHMB, the whole cell was more lightly stained and the degree of contrast between chromosomal fibre regions and interzones was reduced markedly (Fig. 4*a* and *b*). The Golgi, however, seemed to be unaffected by the presence of this sulphydryl inhibitor.

These observations on metaphase spermatocytes are consistent with those from the sea urchin[11], in that the ATPase was distributed equally between the spindle and the surrounding cytoplasm. They are not consistent with our failure to detect activity in spindles of root tip meristems[17]. This may reflect a difference in the two kinds of spindles (polar compared with apolar). But we used frozen sections of root tips, and have now determined that freezing denatures ATPase in spermatocytes. Thus our findings with root tips may have been artefacts.

The differences we observed in the anaphase distribution of ATPase were probably not a direct result of poor fixation of the interzone. Activity was evident in the interzone when nucleotide triphosphates other than ATP were used, and the interzone seemed to be morphologically intact when controls were viewed under phase contrast microscopy. We cannot, however, rule out the possibility that the ATP solubilises the interzone.

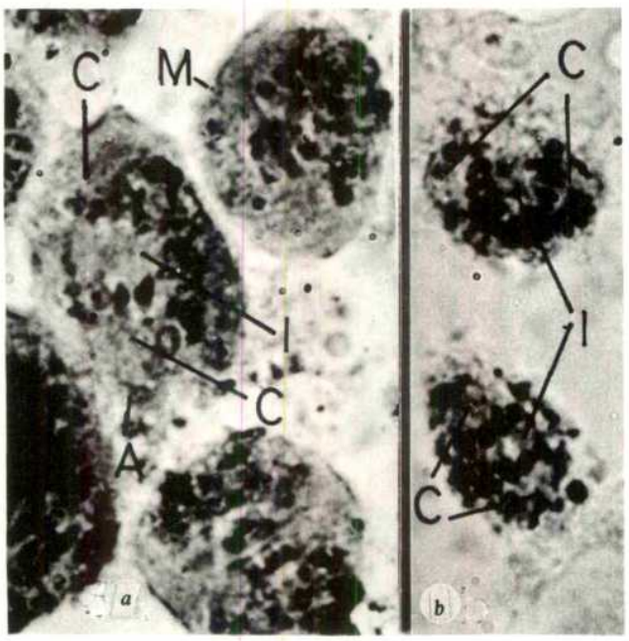


FIG. 3 The distribution of stain during anaphase when ADP and GTP replace ATP as substrate (× 2,000). *a*, ADP: primary spermatocytes, metaphase and anaphase. The stain is deposited in the interzone (I) and in the chromosomal fibre (C) regions of the anaphase cell (A). In the metaphase cell (M) the stain is evenly distributed in the cytoplasm and spindle area. *b*, GTP: secondary spermatocytes. The stain is present in the interzone (I) and chromosomal fibre (C) regions during anaphase.

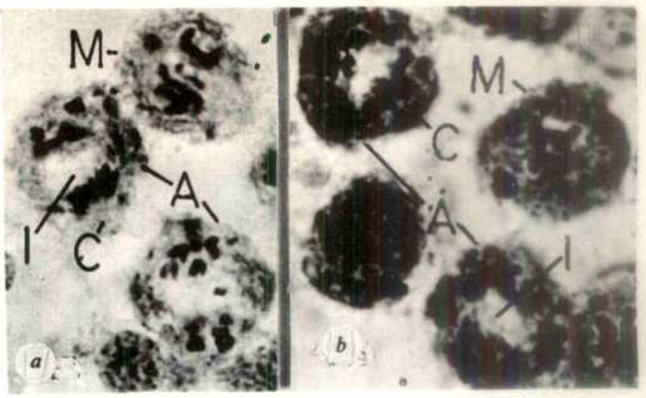


FIG. 4 Inhibition of ATPase by POHMB in metaphase and anaphase primary spermatocytes (×1,000). *a*, An ATPase determination in the presence of POHMB. Compare with (*b*). *b*, The control for (*a*). Cytochemical ATPase determined at the same time as (*a*) but without the inhibitor. The stain deposits are darker.

On the other hand, several facts suggest that the observed distribution of ATPase has functional significance. Thus, ATPase is present chiefly in the chromosomal fibre regions which are thought to generate the polarised forces to move the chromosomes or nonkinetochoric materials in anaphase[18,19]. Furthermore, ATPase is absent from the interzonal spindle, where significant polarised forces are not detectable at anaphase[18,19], and its distribution in metaphase and anaphase follows the changes in birefringence believed to coincide with the production of spindle force[20].

The spermatocyte ATPase is sulphydryl dependent, as is heavy meromyosin[21]. Actin-like filaments have been demonstrated in the spindles of grasshopper spermatocytes[22], and in the spindles and cortex of crane fly spermatocytes[23,24]. During anaphase these filaments are aligned parallel to the chromosomal fibres, but in the interzone they have a random orientation. Such orientation of actin filaments coincides with the reported distribution of ATPase and may explain the organisation of the ATPase in the chromosomal fibre region. If this were the case, then the interaction of actin and ATPase may be sufficient to generate forces that impart anaphase motion to chromosomes, and polarized motion to nonkinetochoric materials.

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1 Mazia, D., Chaffee, R. R., and Iverson, R. H., *Proc. natn. Acad. Sci. U.S.A.*, **47**, 788 (1961).
2 Mihalyi, E., and Szent-Gyorgyi, A. G., *J. biol. Chem.*, **201** (1953).
3 Huxley, H. E., *Science*, **164**, 1356 (1969).
4 Nakajima, H., in *Primitive motile systems in cell biology* (edit. by Allen, R. D., and Kamiya, N.) (Academic Press, New York, 1964).
5 Levine, L., *Science*, **131**, 1377 (1959).
6 Gibbons, I. R., and Rowe, A. J., *Science*, **149**, 424 (1965).
7 Gibbons, I. R., *Ann. Rev. Biochem.*, **37**, 521 (1968).
8 Warner, F. D., in *Adv. Cell molec. Biol.*, **2** (edit. by DuPraw, E. J.) (Academic Press, New York, 1972).
9 Brokaw, C. J., and Bendict, B., *Archs. biochem. Biophys.*, **125**, 770 (1968).
10 Miki, T., *Expl Cell Res.*, **29**, 92 (1963).
11 Weisenberg, R., and Taylor, E. W., *Expl Cell Res.*, **53**, 372 (1968).
12 Mazia, D., *Symp. Soc. exp. Biol.*, **9**, 335 (1955).
13 Stephens, R. E., *J. Cell Biol.*, **32**, 255 (1967).

[14] Forer, A., in *Handbook of molecular cytology* (edit. by Lima-de-Faria, A.) (North-Holland, Amsterdam, 1969).
[15] Levine, L., *Biol. Reprod.*, **7**, 211 (1972).
[16] Padykula, H. A., and Herman, E., *J. Histochem. Cytochem.*, **3**, 161 (1955).
[17] Shifrin, N., and Levine, L., *J. Cell Sci.*, **3**, 423 (1968).
[18] Bajer, A. J., and Molè-Bajer, J., *Intern. Rev. Cytol.*, Suppl. 3 (1972).
[19] Niklas, B. R. and Koch, C. A., *Chromosoma*, **39**, 1 (1972).
[20] Inoué, S., in *Primitive motile systems in cell biology* (edit. by Allen, R. D., and Kamiya, N.), (Academic Press, New York, 1964).
[21] Bendall, J. R., *Muscles, molecules and movement* (Heinemann Educational, London, 1969).
[22] Gawadi, N., *Nature*, **234**, 410 (1971).
[23] Forer, A., and Behnke, O., *Chromosoma*, **39**, 145 (1972).
[24] Forer, A., and Behnke, O., *Chromosoma*, **39**, 175 (1972).

Temperature immutability of adenyl cyclase-coupled β adrenergic recptors

OBSERVATIONS from several laboratories have suggested that the well established classification of adrenergic receptors into α and β subtypes is not immutable. Several groups have reported that in isolated perfused frog hearts, stimulation of cardiac rate and contractility by catecholamines has the properties of a classical β adrenergic response when experiments are performed at warm temperatures (25°–37° C) but of an α adrenergic response when experiments are performed at cold temperatures (5°–15° C)[1–3]. At warm temperatures, the order of potency of agonists in stimulating these preparations—isoproterenol>adrenaline>noradrenaline—is classical for a β adrenergic receptor. Similarly, effects of the catecholamines at warm temperatures are blocked by propranolol but not by the α adrenergic antagonist phentolamine. When the same experiments are performed at temperatures below 25° C, the order of potency of agonists is reversed to that characteristic of α adrenergic receptors. Also at lower temperatures α adrenergic antagonists such as phenoxybenzamine and phentolamine block the effects of adrenaline, whereas β adrenergic antagonists such as propranolol are ineffective. Gradations of response can be achieved by varying the temperature between 37° C and 10° C. Similar observations have been reported for the rat heart[1]. Also in a dog heart–lung bypass preparation the β receptors seem to become ineffective at 15° C, whereas α receptors retain their effectiveness[4]. On the basis of such observations, Kunos *et al.*[3] proposed that α and β adrenergic receptors may represent allosteric configurations of the same receptor macromolecule which could be modulated by among other factors, temperature.

During the past decade, many observations have supported the concept that the adenyl cyclase system in various tissues provides an excellent biochemical model for study of the molecular properties of β adrenergic receptors[5]. In almost all situations where adenyl cyclase is stimulated by catecholamines, the properties of this stimulation are those of β adrenergic receptors. Of course, virtually all experiments which have been reported have been performed at temperatures within the range of 30°–37° C. If, in fact, α and β adrenergic receptors merely represent temperature sensitive transitions in the state of a single macromolecule, then the ability of catecholamines to stimulate and of adrenergic antagonists to block stimulation of adenyl cyclase should vary with temperature in a fashion analogous to that reported above. Accordingly, we have tested the ability of α and β adrenergic agonists and antagonists to stimulate and respectively block stimulation of adenyl cyclase in several tissues from cold-adapted frogs, as well as from the hearts of several mammalian species, using a wide range of temperatures. Our findings do not support the contention that the adenyl cyclase-coupled β adrenergic receptors are easily convertible to α adrenergic receptors when the temperature varies.

Table 1 summarises results from several experiments performed with membranes prepared from dog and rat heart. In each case, stimulation of the adenyl cyclase activity by catecholamines had the characteristics of a β adrenergic response at high (37° C) and low (15° C) temperatures. The β antagonist propranolol was an effective inhibitor of catecholamine-induced stimulation of the enzyme and the α adrenergic antagonist phentolamine was ineffective under all the conditions examined.

We next examined preparations from frog heart, since most of the evidence supporting the hypothesis of temperature conversion of β adrenergic receptors was obtained in this tissue. We had limited success however; using various conditions, we could demonstrate only marginal stimulation of the adenyl cyclase activity by catecholamines in preparations of frog heart membranes.

But because the frog, which is cold blooded, seemed the most appropriate animal in which to test the possibility that the two forms of receptors are interconvertible, we have examined the properties of the adenyl cyclase-coupled adrenergic receptors present in the frog erythrocyte. These are known to be typical β adrenergic receptors[8].

As Figs 1 and 2 show, whether incubation was at 37° C or 10° C the stimulation of the frog erythrocyte cyclase was mediated by classical β adrenergic receptors. The order of potency of agonists was isoproterenol>adrenaline>noradrenaline>phenylephrine. Moreover, the stimulation of the enzyme by catecholamines could be blocked effectively by propranolol, a β adrenergic antagonist, whereas the α adrenergic antagonist phentolamine was virtually without effect. Similar findings were obtained with both summer and winter frogs, either warm or cold adapted.

TABLE 1 Effects of α and β adrenergic agonists and antagonists on adenyl cyclase activity of canine and rat heart membranes at different temperatures

Additions		Adenyl cyclase activity (pmol cyclic AMP generated)			
		Dog heart membranes		Rat heart membranes	
Agonist	Antagonist	37°C	15°C	37°C	15°C
None	None	63.0	51.8	23.1	15.2
Isoproterenol	None	141.0	76.7	39.7	24.8
Isoproterenol	Propranolol	63.1	49.3	21.8	15.9
Isoproterenol	Phentolamine	131.7	84.3	40.4	21.8
Adrenaline	None	113.8	86.9	36.6	24.8
Adrenaline	Propranolol	66.6	43.9	17.4	14.9
Adrenaline	Phentolamine	123.6	88.2	35.2	24.9
Noradrenaline	None	102.3	82.2	35.7	23.8
Noradrenaline	Propranolol	51.6	39.8	21.2	15.2
Noradrenaline	Phentolamine	110.1	77.7	35.1	23.9
Phenylephrine	None	—	—	20.5	15.7
Phenylephrine	Propranolol	—	—	18.7	14.3
Phenylephrine	Phentolamine	—	—	24.6	19.5
None	Propranolol	56.5	39.1	22.1	18.4
None	Phentolamine	66.6	46.3	22.2	18.5

Ventricular myocardium (0.8 – 1.0 g) was homogenised in 20 ml of 0.25 M sucrose, 0.05 M Tris-HCl, *p*H 7.4 by ten strokes of a motor driven Potter–Elvehjem glass Teflon homogeniser. After filtration through cheese cloth, the homogenates were centrifuged in a Sorvall RC2B centrifuge for 10 min at 10,000*g*. Pellets were washed twice with the sucrose buffer and once with 75 mM Tris-HCl 25 mM $MgCl_2$, *p*H 7.4, before being assayed for adenyl cyclase activity. Adenyl cyclase was determined using a modification[6] of Krishna's method[7]. Incubations were for 10 min at 37°C and for 30 min (canine heart membranes) or 60 min (rat heart membranes) at 15°C. Enzyme activity was linear for these periods of incubation at both temperatures. Reaction mixtures contained the following reagents in a total volume of 50 μl: 25 mM Tris-HCl, *p*H 7.4; 10 mM $MgCl_2$; 1.5 mM ATP; 49–59 μg of membrane protein; 0.1 mM cyclic AMP and an ATP regenerating system consisting of 5 mM phosphoenolpyruvate, 40 μg ml^{-1} pyruvate kinase and 20 μg ml^{-1} myokinase. Agonists and antagonists were added to a final concentration of 4×10^{-6} M. The values shown are the mean of duplicate determinations.

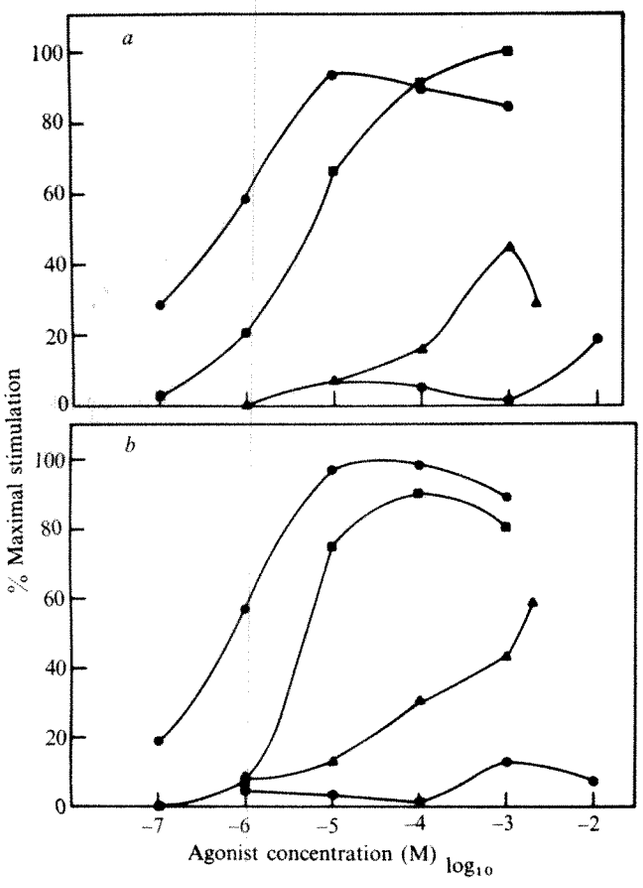


FIG. 1 Response of frog erythrocyte adenyl cyclase to adrenergic agonists at (*a*) 37°C and (*b*) 10°C. Isoproterenol (●), adrenaline (■), noradrenaline (▲) and phenylephrine (◆). Blood from cold-adapted southern jumbo grass frogs (Carolina Biological, Burlington, North Carolina) was collected and the red cells were washed three times with saline. Cells were lysed in 5 mM Tris-HCl, *p*H 8.1, buffer and the membranes were centrifuged at 18,000*g* for 15 min. The lysis process was repeated three times. Membranes were finally suspended in 75 mM Tris-HCl, 25 mM $MgCl_2$, *p*H 8.1 by homogenisation. Adenyl cyclase was assayed as described in Table 1, except that assays were carried out at *p*H 8.1 for 15 min at 37°C and for 60–90 min at 10°C. Cyclase activity was linear during these periods of incubation at both temperatures. Each sample contained 240–560 μg of membrane protein. In these experiments the mean basal enzyme activity was 285 pmol per mg protein. Maximal stimulation refers to activity observed in the presence of a maximally effective concentration of catecholamine which was generally about five times the basal activity. Each value is the mean of four determinations.

These findings suggest that the adrenergic receptors coupled to adenyl cyclase in mammalian heart as well as in frog erythrocytes are typical β adrenergic receptors, the characteristics of which are not altered at a wide range of temperatures. These observations do not seem to support the previous physiological observations reported and the hypothesis that β adrenergic receptors are convertible to α adrenergic receptors when temperatures are lowered, especially in the tissues of the cold-adapted frog. It should be noted that we were unable to test specifically the validity of the hypothesis for the frog heart, the tissue for which the most data has previously been reported. We cannot explain why only a marginal stimulation of frog myocardial cyclase activity was observed with catecholamines. As noted above, however, comparable changes in the specificity of the adrenergic receptors with temperature variation have been reported for rat heart[1]. In rat heart membranes, we saw no alteration in the adenyl cyclase-coupled β adrenergic receptors when the temperature was lowered.

Our observations raise objections to the hypothesis[2] that α and β adrenergic receptors are, in general, easily convertible allosteric forms of the same macromolecule. Although our data do not specifically disprove the hypothesis for the limited case of the frog heart, they do negate the hypothesis for the adenyl cyclase-coupled β adrenergic receptors of another frog tissue, the erythrocytes, as well as the mammalian heart in several species. It should be noted that whereas previously reported studies involved intact tissue preparations, our results were obtained exclusively with subcellular membrane fractions. Thus, it is possible that the apparent discrepancies in the results are due to a requirement for an intact cellular structure to mediate the changes in receptor specificity. The mechanisms involved in such an effect, however, are not clear.

It has not been shown definitively that β adrenergic effects on the myocardium are mediated by adenyl cyclase and cyclic AMP[9]. Moreover, the function of the frog erythrocyte β adrenergic receptors is not known. Nonetheless, as demonstrated here, the receptors which mediate effects of catecholamines on adenyl cyclase in each of these tissues are clearly β adrenergic receptors and hence represent a valid biochemical model of β receptors for studies such as these.

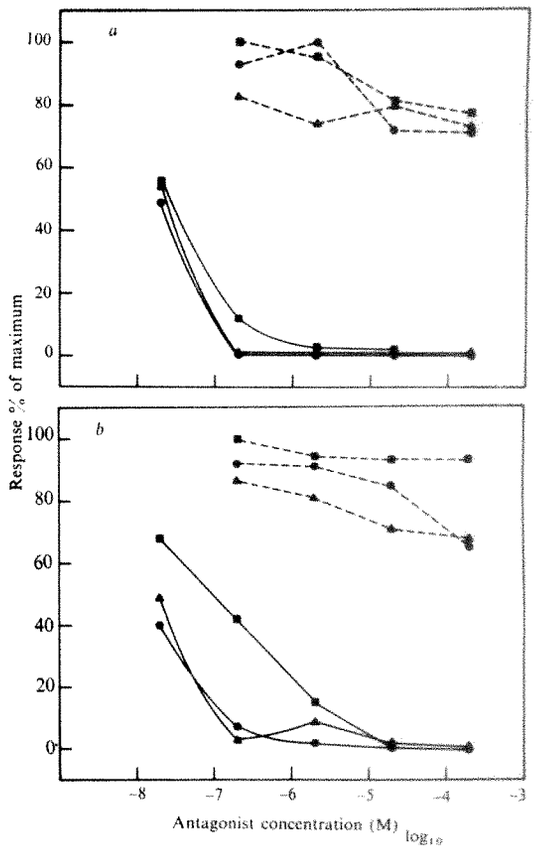


FIG. 2 Inhibition of catecholamine-induced activation of frog erythrocyte adenyl cyclase by adrenergic antagonists at (*a*) 37°C and (*b*) 10°C. Propranolol (———); phentolamine (– – – –). The agonists were present at the following concentrations: isoproterenol, 1×10^{-6} M (●); adrenaline, 1×10^{-5} M (■) and noradrenaline $1\text{–}5 \times 10^{-5}$ M (▲). Methods of membrane preparation and adenyl cyclase assay are described in the legend to Fig. 1. Incubations were carried out at 37°C for 15 min and at 10°C for 90 min. Each assay contained 250–310 μg of membrane protein. Maximum response refers to enzyme activity, in the presence of each agonist, at the stated concentration. 0% maximum response refers to activity in the absence of agonist, that is basal enzyme activity. In these experiments the mean basal activity was 207 pmol per mg protein and the concentration of agonists used gave usually a two to three-fold stimulation over basal activity. The results shown are the mean of four determinations.

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1 Kunos, G., and Szentivanyi, M., *Nature*, **217**, 1077 (1968).
2 Buckley, G. A., and Jordan, C. C., *Br. J. Pharmac.*, **38**, 394 (1970).
3 Kunos, G., Yong, M. S., and Nickerson, M., *Nature*, **241**, 119 (1973).
4 Price, J. M., Swann, J., and Nayler, W. G., *Archs int. Pharmacodyn. Ther.*, **168**, 296 (1967).
5 Robison, G. A., Butcher, R. W., and Sutherland, E. W., in *Cyclic AMP*, 146 (Academic Press, New York, 1971).
6 Lefkowitz, R. J., Sharp, W. G., and Haber, E., *J. biol. Chem.*, **248**, 342 (1973).
7 Krishna, G., Weiss, B., and Brodie, B. B., *J. Pharmac. exp. Ther.*, **163**, 379 (1968).
8 Rosen, O. M., Erlichman, J., and Rosen, S. M., *Molec. Pharmac.*, **6**, 521 (1970).
9 Sobel, B., and Mayer, S., *Circulation Res.*, **32**, 407 (1973).

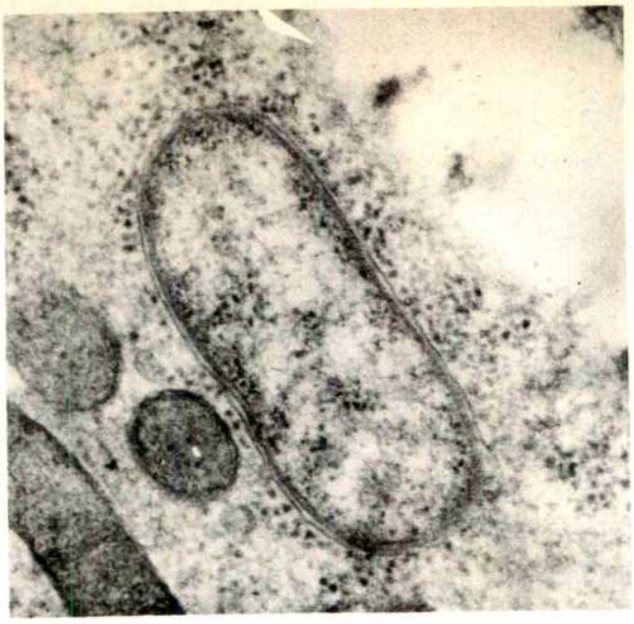


FIG 2 A bacteroid in the ooplasm of an actively vitellogenic oocyte from the active ovary of a female 4 d after emergence. Note the fibrils and ribosomes in the bacteroid. (× 54,500.)

Bacteroids in the ovaries of a tsetse fly

SYMBIONTS of *Glossina* previously described by Wigglesworth[1] as Gram-negative intracellular bacteroids are located in a group of specialised cells, the mycetome, in the midgut. Their intracellular nature is well known from studies with the light[1–3] and electron[4] microscopes (see Fig. 4). Roubaud[3] demonstrated bacteroids in the mycetome of the larval midgut of *G. palpalis*. He was unable to determine the mode of transmission, but suspected the involvement of the milk gland, as have other authors[5,6]. Recent work on the ultrastructure of the milk gland has demonstrated that bacteroids occur extracellularly in the lumen of the gland[7], thus adding support to the notion that transmission from one generation to the next may occur through the secretions of the milk gland. Bacteroids have not, however, been located in the cells of the milk gland, and it remains uncertain whether this route of transmission is used.

We report here for the first time the presence of intracellular bacteroids in the ovary of *G. austeni*, and suggest that their presence indicates the transovarian transmission of symbionts found in the larval and adult mycetome.

The ovaries of mated *G. austeni*, reared by the procedure of Tobe and Davey[8] were fixed[9] on the day of emergence and every second day thereafter up to 28 d after emergence, thereby spanning two pregnancy cycles. Both ovaries of two or three flies were fixed at each time, and tissues were embedded in Epon-Araldite[10]. Sections were cut on an LKB ultratome using glass knives, collected on uncoated copper grids, stained with uranyl acetate and lead citrate and examined in a Zeiss EM9S electron microscope.

Bacteroids were present in the cytoplasm of oocytes (Figs 1 and 2) and nurse cells (Fig. 3) of the ovary throughout the reproductive cycle. Similar bacteroids were seen occasionally in follicle cells and sheath cells of the ovary. Their surface structure and internal morphology resemble Gram-negative bacteria[11]. They are oblong, approximately 2 μm long, and contain a fibrillar network and ribosomes. They are bounded by a plasmalemma, an intermediate layer, and an outer double membrane. In addition, a host cellular membrane surrounds the whole structure (Figs 2 and 3). Dividing bacteroids were sometimes observed (Fig. 1). The presence of bacteroids in ovaries from newly emerged flies from pupae received from the Tsetse Research Laboratory at Langford in Britain ruled out the possibility of the microorganisms originating as an infection due to the culture conditions in our laboratory. The bacteroids do not elicit degenerative responses from the host tissues: they were not found to be associated with lysosomes or autophagic vacuoles. Since the viability of the subsequent offspring from our flies was not affected, we can only conclude that these are normally occurring symbionts with some functional significance. Previous studies on oogenesis in tsetse flies[12–14] have revealed no symbionts, but the methods used gave insufficient resolution.

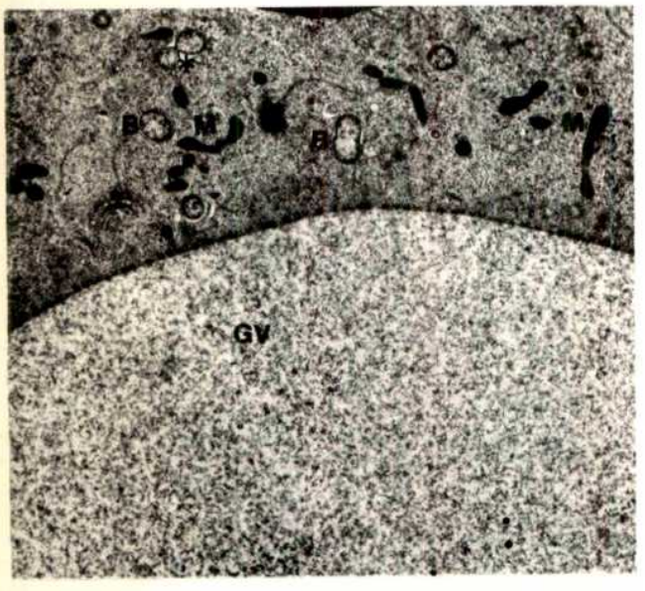


FIG. 1 An electron micrograph of part of a young oocyte from the inactive ovary of a newly emerged fly. Note the numerous bacteroids (B) in the ooplasm, which are clearly distinguishable from the mitochondria (M). A dividing bacteroid (*) is also visible. GV, germinal vesicle. (× 7,000.)

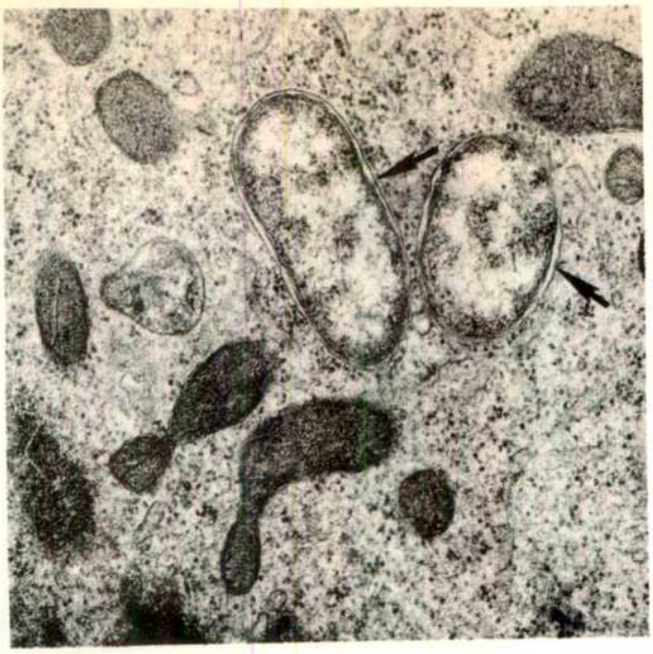


FIG. 3 Bacteroids in the cytoplasm of a nurse cell from a newly emerged female. Note the presence of host membrane (arrow) around the bacteroid. (× 39,500.)

While ovarian symbionts may be important to the processes of oogenesis or fertilisation[15], it is perhaps more likely that these organisms constitute a source for the symbionts of the larval and adult midgut. Certainly such a function is well known in other arthropods[6]. The symbionts present in the nurse cells may be transported through intracellular bridges to the oocyte, where they would be conveniently placed for localisation in the presumptive midgut cells of the embryo. The symbionts of the ovary are similar in structure to those in the midgut mycetome of the adult (Fig. 4), but are considerably smaller. Moreover, the midgut forms appear not to be enclosed in a host membrane[4]. However, the symbionts of the ovary resemble those of the midgut more closely than do the microorganisms reported in the lumen of the milk gland[7].

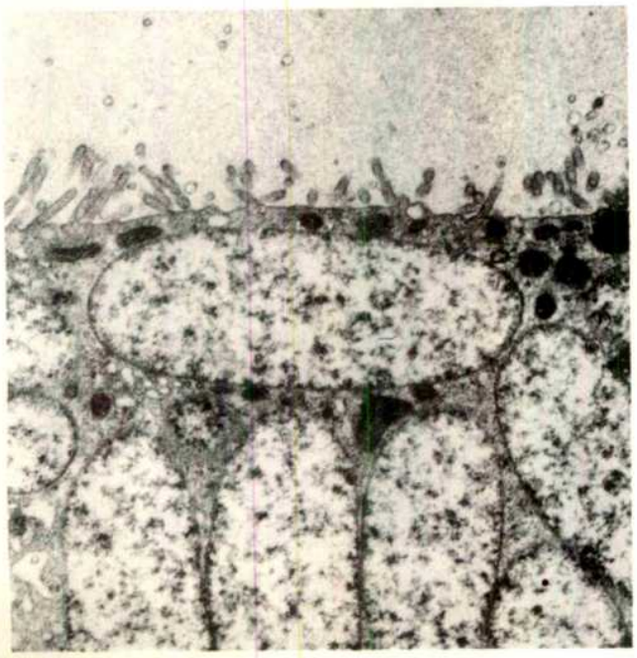


FIG. 4 Bacteroids in the apical cytoplasm of the mycetome of an adult female. (× 13,000.)

Although we do not discount the possible role of milk in the transmission of symbionts to the larva, our findings provide concrete support for transovarian transmission as an alternative or parallel mechanism. This means of transmission from one generation to the next does not require the externalisation of the bacteroids into the lumen of the milk gland, and their subsequent penetration into the cells of the gut of the larva. While the presence of symbionts in the ovaries of the adult, and hence the possibility of transovarian transmission, is clear, a decision as to whether the symbionts are the progenitors of those found in the mycetome of the larval and adult midgut awaits a detailed ultrastructural study of their fate during embryonic development.

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1 Wigglesworth, V. B., *Parasitology*, **21**, 288 (1929).
2 Stuhlmann, F., *Arb. Kaiserl. Gesundh.*, **26**, 301 (1907).
3 Roubaud, E., *Ann. Inst. Pasteur*, **33**, 489 (1919).
4 Reinhardt, C., Steiger, R., and Hecker, H., *Acta trop.*, **29**, 280 (1972).
5 Buxton, P. A., *The natural history of tsetse flies* (Lewis, London, 1955).
6 Buchner, P., *Endosymbiosis of animals with plant microorganisms* (Wiley, New York, 1965).
7 Ma, W-C., and Denlinger, D. L., *Nature*, **247**, 301 (1974).
8 Tobe, S. S., and Davey, K. G., *Can. J. Zool.*, **49**, 577 (1971).
9 Huebner, E., and Anderson, E., *J. Morph.*, **136**, 459 (1972).
10 Anderson, W. A., and Ellis, R. A., *J. Protozool.*, **12**, 483 (1965).
11 Glauert, A. M., and Thornley, M. J., *A. Rev. Microbiol.*, **23**, 159 (1969).
12 Moloo, S. M., *Acta trop.*, **28**, 334 (1971).
13 Odhiambo, T. R., *Experientia*, **24**, 738 (1968).
14 Odhiambo, T. R., *J. exp. Zool.*, **177**, 447 (1971).
15 Peleg, B., and Norris, D. M., *Nature new Biol.*, **236**, 111 (1972).

Uptake of horseradish peroxidase by frog photoreceptor synapses in the dark and the light

INDIRECT evidence suggests that vertebrate photoreceptors decrease their release of a depolarising transmitter substance in response to the absorption of light. Vertebrate photoreceptors[1–3] and most horizontal cells[2,4] hyperpolarise in response to light and show an increased membrane resistance. In addition, transretinal currents that depolarise receptor terminals also depolarise horizontal cells[5–7]. Finally, magnesium, known to decrease transmitter release in neuromuscular[8] and other synapses[9], hyperpolarises skate[10] and turtle[11] horizontal cells. We now report more direct evidence that the photoreceptors are synaptically active in the dark and that this activity can be diminished by light.

Active synapses can be labelled with macromolecular tracers. This has been established using horseradish peroxidase to study lobster neuromuscular junctions[12]; by work with vertebrate neuromuscular junctions studied with peroxidase[13,14] and dextrans[15]; by investigations of cultured central



FIG. 1 Phase contrast photomicrograph of a thick section of a retina exposed to peroxidase for 60 min in the dark. At R, a rod cell synapse is seen, made dark by its content of peroxidase reaction product; note the thin characteristic stalk (arrows) by which the synapse is attached to the nuclear region (this latter region is not seen at this plane of focus). C indicates a cone cell synapse, also containing reaction product; as expected for this cell type, the synapse is quite close to the nuclear region (N). OS indicates the level of the outer segments. S indicates the level of the synaptic layer. The bar represents 10 μm.

nervous tissue[9], and by studies of neurosecretory systems[16]. The essential findings in these studies and some related work (for review see ref. 9), were that synaptic terminals could be shown to take up tracers into small synaptic vesicles and other membranous structures when stimulated to release transmitters by electrical or chemical means. Uptake into synapses that were not active in transmitter release was slight.

For the present work, isolated frog retinae were prepared by standard procedures and maintained under various conditions of illumination in either Ringer or Na-aspartate Ringer solution[17]. Aspartate depolarises the postsynaptic membranes and thus isolates receptor potentials[18–20]. The retinae were placed in a chamber of an extracellular recording system so that we could monitor the electrical activities of the receptors. Horseradish peroxidase (Sigma Type II) at a concentration of 0.5% was included in the bathing medium. After suitable periods of exposure, the retinae were rinsed briefly in Ringer, fixed in 2.5% glutaraldehyde in cacodylate buffer, cut in small pieces, incubated to demonstrate peroxidase activity[21], postfixed in osmium tetroxide, embedded in Epon and prepared for light and electron microscopy. Cytochemical controls, such as preparations incubated in a peroxide-free cytochemical medium or retinae never exposed to exogenous tracers but incubated in full medium, showed no reaction product.

The synapses of frog photoreceptors appear to be active in the dark. When retinae were maintained in the dark for 30-90 min, extensive uptake of peroxidase by the synapses was observed. This was readily detectable by light microscopy of thick sections cut from the Epon blocks (Fig. 1) where reaction product is seen as dark brown diffuse or granular deposits within the synapses. It was often possible to determine whether a particular synapse belonged to a rod or a cone receptor by focusing up and down carefully, and thus following the cell from the synapse to its outer segment. Furthermore, the position of the nucleus of the cell making the synapse also helped show whether it was a cone or rod synapse. Most rod nuclei are located in the distal layer of the photoreceptor nuclear region while cone nuclei are found in the proximal layer[22]. (Most of the green rod nuclei are also located among the cone nuclei. These cells, however, comprise only about 8% of the receptor population while cones make up about 42% and red rods 50% (ref. 23). In the electron microscope, most, if not all, the synapses from dark retinae were seen to have taken up peroxidase into numerous vesicles (Fig. 2).

When retinae were maintained in the light (for 30–90 min) at an intensity that decreased rod sensitivity substantially relative to cone sensitivity, markedly fewer synapses were observed to have taken up extensive amounts of tracer. It was clear from careful light microscopy that many of the active synapses were of cone cells and many of the inactive ones were of rod cells. (It was impossible to designate all synapses as rod or cone because of such factors as inappropriate plane of section. We are confident, however, that few rods had appreciably active synapses under these conditions.) In the electron microscope (Fig. 3), many synapses were observed to have contents of peroxidase comparable with that found in dark synapses (synapse A) while many others (synapse B), took up little tracer. Synapse A is quite close to its nucleus, indicating that this is probably a cone.

Another procedure used is known to decrease the frog's rod sensitivity relative to that of the cones, but it does not require the continuous presence of light. Retinae were exposed for 5 min to very high intensities of light that bleach about 90% of the visual pigment[24]. The preparations were allowed to recover for 30 min in the dark and then for the first time they were exposed to peroxidase (for 30–45 min in the dark). Under these conditions, the rods of an isolated retina permanently lose sensitivity because they cannot regenerate their visual pigment in the absence of the pigment epithelium[25–28]. Cones, on the other hand, can regenerate their pigment[29] and recover nearly all their sensitivity[17]. The results we obtained were similar to those found in the continuous light condition. In the light microscope, many of the active synapses seemed to belong to cone cells and many of the inactive ones were from rod cells. In the electron microscope, the preparations were essentially similar to the continuous light preparations (compare Fig. 3).

The previous work on tracer uptake by neuronal synapses[9,12–16] strongly suggests that such uptake reflects the membrane rearrangements attendant on transmitter release. As always, the presence of a tracer molecule could conceivably

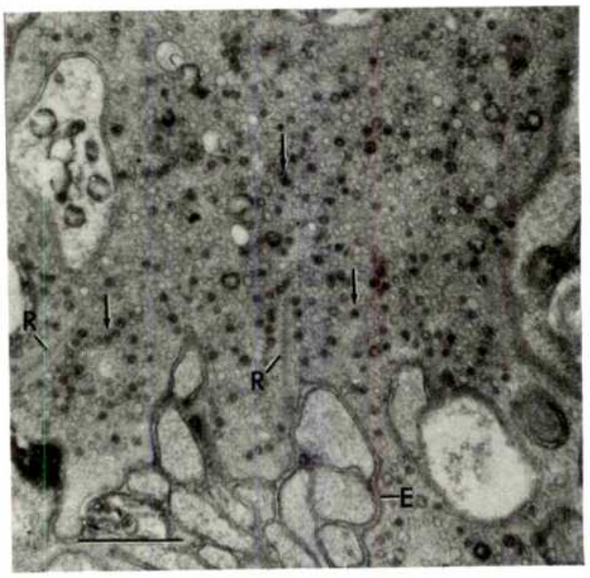


FIG. 2 Synapse from a retina exposed to peroxidase in the dark for 90 min. Reaction product is present in about 35% of the small vesicles. A few labelled vesicles are indicated by arrows; it may eventually prove of interest that some of these vesicles are clustered near the synaptic ribbons (R). Reaction product in extracellular space is seen at E. The bar represents 0.5 μm.

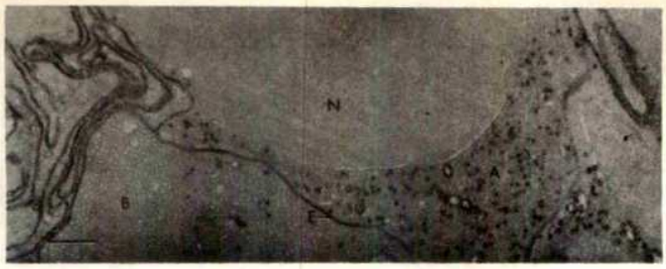


FIG. 3 Synapses from a retina exposed to peroxidase in the light for 45 min. Synapse B shows reaction product at its surface (E) but only 3% of the vesicles are labelled. In synapse A, 30% of the vesicles show reaction product. Note the edge of the nucleus (N) abutting on the synaptic region of A. The bar represents 0.5 μm.

alter the normal behaviour of the cells. However, our electrophysiological recordings indicate that the presence of peroxidase had little or no effect on the receptor responses. In addition, since the rods do not take up peroxidase in the light and bleach conditions, uptake cannot be explained as simply an artefact of the presence of the tracer.

Thus, our findings indicate that, at least in the isolated retinae, the photoreceptors are synaptically active in the dark. On exposure to lights (continuous or brief high intensity) that have been shown to desensitise frog rod receptors[24,30], rod synapses become quiescent but cone synapses remain active. If we make the reasonable assumption that synaptic activity is controlled by membrane potential, then our results are consistent with the evidence indicating that rod receptors are relatively depolarised in the dark and hyperpolarised during continuous light exposure[31,33]. However, more than 30 min after light exposures that bleach substantial rod pigment, we found little or no rod synaptic activity while physiological experiments on skate[34] and fish[35] retinae can be interpreted as indicating that synaptic activity returns fairly quickly to dark levels. Further work on potential changes in the frog retina is needed to determine the time course of changes under the conditions we have used.

Preliminary reports of these findings have been presented already[36,37]. We thank Fe Reyes for technical assistance. This work was supported by a grant from the National Institute of Neurological Disease and Stroke and by a US Public Health Service training grant.

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1 Tomita, T., *Q. Rev. Biophys.*, **3**, 179–222 (1970).
2 Toyada, J., Nasaki, H., and Tomita, T., *Vision Res.*, **9**, 453–463 (1969).
3 Hagins, W. A., Penn, R. D., and Yoshikami, S., *Biophys. J.*, **10**, 380–412 (1970).
4 Svaetichin, G., and MacNichol, E. F., *Ann. N.Y. Acad. Sci.*, **74**, 385–404 (1958).
5 Trifonov, J. A., and Byzov, A. L., *Biophysica*, **10**, 673–685 (1965).
6 Byzov, A. L., and Trifonov, J. A., *Vision Res.*, **8**, 817–822, (1968).
7 Trifonov, J. A., *Biophysica*, **13**, N5 (1968).
8 Katz, B., *Nerve, muscle, and synapse* (McGraw-Hill, New York, 1966).
9 Holtzman, E., Teichberg, S., Abrahams, S. J., Citkowitz, E., Crain, S. M., Kawai, N., and Peterson, E. R., *J. Histochem. Cytochem.*, **21**, 349–385 (1973).
10 Dowling, J. E., and Ripps, H., *Nature*, **242**, 101–103 (1973).
11 Cervetto, L., and Piccolino, M., *Science*, **183**, 417–419 (1974).
12 Holtzman, E., Freeman, A. R., and Kashner, L. A., *Science*, **173**, 733–736 (1971).
13 Ceccarelli, B., Hurlbut, W. P., and Mauro, A. J., *J. Cell Biol.*, **54**, 30–38 (1972).
14 Heuser, J. E., and Reese, T. S., *J. Cell Biol.*, **57**, 315–344 (1973).
15 Ceccarelli, B., Hurlbut, W. P., and Mauro, A., *J. Cell Biol.*, **57**, 499–524 (1973).
16 Douglas, W. W., Nagasawa, J., and Schulz, R., *Mem. Soc. Endocr.*, **19**, 353–383 (1970).
17 Hood, D. C., and Hock, P. A., *Vision Res.*, **13**, 1943–1951 (1973).
18 Sillman, A. J., Ito, H., and Tomita, T., *Vision Res.*, **9**, 1435–1442 (1969).
19 Dowling, J. E., and Ripps, H., *Biol. Bull.*, **141**, 384–385 (1972).
20 Cervetto, L., and Macnichol, E. F., *Science*, **178**, 467–468 (1972).
21 Graham, R. C., and Karnovsky, M. J., *J. Histochem. Cytochem.*, **14**, 291–302 (1966).
22 Cajal, S. R-y., *The structure of the retina* (trans. by Thorpe, S. A., and Glickstein, M.) (Thomas, Springfield, Illinois, 1972).
23 Liebman, P. A., and Entine, G., *Vision Res.*, **8**, 761–775 (1968).
24 Hood, D. C., Hock, P. A., and Grover, B. G., *Vision Res.*, **13**, 1953–1963 (1973).
25 Kuhne, W., *Handbuch der physiologishen methodik* (edit. by Hermann, L.), **3**, 235–242 (Leipzig, 1879).
26 Zewi, M., *Acta Soc. Sci., fenn. N.S.B.*, **2**, 1–57 (1939).
27 Baumann, C., *Vision Res.*, **5**, 425–434 (1965).
28 Goldstein, E. B., *Vision Res.*, **7**, 837–845 (1967).
29 Goldstein, E. B., *Vision Res.*, **10**, 1065–1068 (1970); *Vision Res.*, **7**, 837–845 (1967).
30 Hood, D. C., and Hock, P. A., *Am. J. Opt.*, **49**, 888 (1972).
31 Kleinschmidt, J., *Biochemistry and physiology of visual pigments* (edit. by Langer, H.), 219–224 (Springer, Berlin, 1973).
32 Normann, R. A., and Werblin, E. S., *J. gen. Physiol.*, **63**, 37–61 (1974).
33 Grabowski, S. R., Pinto, L. H., and Pak, W. L., *Science*, **176**, 1240–1243 (1972).
34 Dowling, J. E., and Ripps, H., *J. gen. Physiol.*, **58**, 163–189 (1971).
35 Naka, K. I., and Rushton, W. A. H., *J. Physiol., Lond.*, **194**, 259–269 (1968).
36 Schacher, S., Holtzman, E., and Ebrey, T., *J. Histochem. Cytochem.*, **21**, 410 (1973).
37 Schacher, S., and Holtzman, E., *Abstracts Third Ann. Meet. Soc. for Neuroscience*, San Diego, 107 (1973).

Human lymphocyte-dependent antibody mediated cytotoxicity and direct lymphocyte cytotoxicity against non-HL-A antigens

EXPRESSION of the major transplantation antigens in humans is generally considered to be controlled by the genes of the *HL-A* region[1–4]. Skin, kidney and bone marrow transplants usually survive longer when donors and recipients are HL-A identical than when they are not[5–7]. However, the fact that grafts are still rejected with HL-A identity and negative mixed lymphocyte reactivity (MLR)[8–11] strongly suggests the presence of minor transplantation antigens. The major problem to date has been the development of *in vitro* assay systems to detect these antigens.

Attempts to identify presensitisation of potential graft recipients to non-HL-A antigens have generally failed when they have involved testing for complement-dependent antibody to donor cells[12,13]. On the other hand, there are reports of cytotoxicity, mediated by sensitised lymphocytes from multiply transfused patients, against non-HL-A antigens[13,14]. We have now found that patients who have received multiple transfusions develop lymphocyte-dependent antibody (LDA) as well as cytotoxic lymphocytes against non-HL-A antigens. These responses occur in spite of negative MLR.

Three patients, two males and a female, were studied: two suffered from aplastic anaemia and one from a preleukaemic state with pancytopoenia. They all received multiple blood and platelet transfusions from unmatched

TABLE 1 Family *HL-A* genotypes

Family No.	Father	Mother	Patient	Siblings†	
1	1, 8/2, 8	(10, *W*10/3, *W*10)*	2, 8/10, *W*10	2, 8/10, *W*10;	1, 8/10, *W*10; 2, 8/3, *W*10
2	3, 7/?3, *W*5	(2, *W*22/?3*W*17)	3, 7/2, *W*22	3, 7/2, *W*22;	3, 7/2, *W*22; ?3, *W*5/?3, *W*17
3	3, *W*5/10*W*18	(10, 12/9, 22)	3, *W*5/10, 12	3, *W*5/10, 12;	3, *W*5/9, 22

* Parentheses indicate a deduced genotype in a parent who was not typed.
† HL-A typing was performed for families 1 and 2 by Dr P. Terasaki and the NCI Human Histocompatibility Typing Center maintained at Microbiological Associates, Inc. with identical results. Family 3 was typed only by Dr P. Terasaki

TABLE 2 Mixed lymphocyte culture stimulation indices for HL-A identical siblings*

Responder	Stimulator	Ratio†
Patient 1	Sibling	1.1
	Unrelated	5.8
Patient 2	Sibling	1.0
	Unrelated	28.8
Patient 3	Sibling	1.3
	Unrelated	7.6

* MLR was performed as previously reported[16]. There were 2×10^5 responding lymphocytes per tube. Stimulating cells ($2 - 10 \times 10^5$ per tube) were treated with mitomycin C.
† Results are expressed as the ratio of d.p.m. in cultures stimulated with allogenic cells to d.p.m. of cultures stimulated with autologous cells, that is A + Bm/A + Am. A ratio ≥ 2 represents stimulation. Incubation time was 4–7 d and the ratio was determined from the time at which the peak stimulation was achieved with that pair of individuals.

donors and from their HL-A identical sibling. HL-A typing data (Table 1) demonstrated genotypic identity between each patient and sibling.

Cytotoxicity mediated by sensitized lymphocytes and by LDA was measured by rapid release of ^{51}Cr as described before[13,15]. Target and attacking cells were obtained by passing heparinised blood over nylon columns and by incubating nonadherent cells with Plasmagel. Target cells were treated with NH_4Cl-Tris buffer to eliminate red blood cells and then were labelled with ^{51}Cr (200–400 μCi given to 10^7 cells for 30–60 min). To test for direct cellular cytotoxicity, 5×10^4 ^{51}Cr-labelled target cells were incubated with 3×10^6 attacking lymphocytes (1 ml) in 35mm plastic dishes for 4 h on a rocking platform in a CO_2 incubator. To test for LDA-mediated cytotoxicity, ^{51}Cr-labelled target cells were incubated with serum containing LDA for 30 min, and then washed by centrifugation. Target cells (10^4) were placed in dishes with 10^6 normal attacking lymphocytes (1 ml) (test group) or media alone (control group). After 4 h the contents of the dishes were transferred to tubes; 1 ml of cold balanced salt solution + 10% foetal calf serum was added and tubes were centrifuged (800*g*, 10 min). Aliquots were taken for counting of ^{51}Cr release. Immune percentage lysis was calculated by comparing the ^{51}Cr released from target cells during the 4 h incubation with the maximum amount released by freezing and thawing target cells. The following equation was used to calculate immune percentage lysis:

$$\frac{\text{Average test } ^{51}\text{Cr release} - \text{Average control } ^{51}\text{Cr release}}{\text{Freeze-thaw } ^{51}\text{Cr released} - \text{machine background}} \times 100$$

Standard error:

$$\frac{1}{\text{freeze-thaw } ^{51}\text{Cr release}} \left[\left(\frac{\text{variance}}{\text{sample No.}} \right)_{\text{test group}} + \left(\frac{\text{variance}}{\text{sample No.}} \right)_{\text{control}} \right]^{1/2}$$

Lymphocytes from each patient failed to be stimulated in MLR by cells from his other HL-A identical sibling (Table 2). MLR tests were technically satisfactory as demonstrated by stimulation of these patients' lymphocytes by cells from a third party. On the other hand, serum from each of these patients supported LDA-mediated cytotoxicity against target cells from his HL-A identical sibling and against third party target cells from a donor not matched with the patient for HL-A antigens (Table 3). LDA-mediated cytotoxicity against non-HL-A antigens was directly proportional to the relative antibody concentration, and the activity was stronger against unrelated targets. All three sera were negative when tested against autologous freshly explanted lymphocytes (Table 3). These target cells were lysible in LDA reaction as demonstrated with LDA-containing serum from unrelated multiply transfused donors.

The three pairs of donors were tested for direct lymphocyte cytotoxicity, and in each case the patient's lymphocytes lysed ^{51}Cr-labelled target cells from the HL-A matched sibling and from an unrelated donor (Table 4). These attacking lymphocytes were not cytotoxic for autologous targets, thus demonstrating specificity.

We have demonstrated that three patients who were supported by blood and platelet transfusions from their HL-A identical siblings developed LDA against non-HL-A

TABLE 3 LDA-mediated cytotoxicity against targets from HL-A identical siblings

Family	Source of target lymphocytes*	Source of coating serum†	Source of normal attacking lymphocytes‡	Target cell immune damage % lysis ± s.e.§
1	Identical sibling	Media	Father-1	−0.6 ± 1.7
	Identical sibling	Patient-1	Father-1	10.1 ± 1.1
	Father-1	Media	Father-1	1.9 ± 3.4
	Father-1	Patient-1	Father-1	17.6 ± 3.6
	Patient-1	Media	Patient-1	−0.6 ± 1.2
	Patient-1	Patient-1	Patient-1	0.8 ± 1.6
2	Identical sibling	Media	Unrelated donor	1.4 ± 1.1
	Identical sibling	Patient-2	Unrelated donor	20.2 ± 0.9
	Patient-2	Media	Unrelated donor	1.3 ± 1.0
	Patient-2	Patient-2	Unrelated donor	−0.6 ± 1.0
	Patient-2	Patient-1	Unrelated donor	15.6 ± 0.7
3	Identical sibling-3	Media	Unrelated donor	1.1 ± 1.7
	Identical sibling-3	Patient-3	Unrelated donor	16.1 ± 1.7
	Patient-3	Media	Unrelated donor	1.0 ± 0.8
	Patient-3	Patient-3	Unrelated donor	1.6 ± 0.3
	Patient-3	Patient-2	Unrelated donor	50.0 ± 0.9

* The concentration of target cells was 10^4 per dish; all numbers identify a specific family.
† Serum dilutions were: family1,1:20; families 2 and 3, 1:2.
‡ There were 10^6 attacking lymphocytes per dish.
§ Damage after a 4-h assay. Values were corrected for ^{51}Cr releases from target cells incubated in media alone. ^{51}Cr releases from antiserum-treated target cells varied between 7% and 30%; ^{51}Cr releases from untreated cells varied between 6% and 13%.

TABLE 4 Direct cellular cytotoxicity against targets from HL-A identical siblings

Experiment	Source of target lymphocytes*	Source of attacking lymphocytes†	Target cell damage % lysis ± s.e.‡
1	Identical sibling-1*a*	Patient-1	22.3 ± 1.1
	Identical sibling-1*a*	Identical sibling-1*b*	−0.4 ± 0.8
	Identical sibling-1*b*	Patient-1	22.6 ± 1.9
	Identical sibling-1*b*	Identical sibling-1*a*	−1.9 ± 1.5
	Father	Patient-1	29.1 ± 2.3
	Father	Father	−5.9 ± 2.3
	Patient-1	Patient-1	−0.8 ± 0.4
2	Identical sibling-2	Patient-2	8.04 ± 0.7
	Identical sibling-2	Identical sibling-2	1.9 ± 0.6
	Non-identical sibling-2	Patient-2	13.2 ± 1.0
	Non-identical sibling-2	Non-identical sibling-2	−0.5 ± 0.9
	Patient-2	Patient-2	0.03 ± 0.8
3	Identical sibling-3	Patient-3	6.4 ± 0.4
	Identical sibling-3	Non-related donor	−1.9 ± 0.7

* There were 5×10^4 target cells per dish. Numbers refer to specific families. Siblings 1*a* and 1*b* were HL-A identical with the patient. Results from family 2 were published in ref. 13.

† There were 3×10^6 attacking cells per dish. Numbers identify specific families.

‡ Damage after a 4-h assay. Values were corrected for 'spontaneous' ^{51}Cr release from target cells incubated in media alone. These spontaneous ^{51}Cr releases varied from 5.6% to 25.1% lysis. Media (Eagle's essential) contained 10% heat-inactivated foetal calf serum.

antigens on their sibling's target cells. In addition, these patients developed cytotoxic lymphocytes against cells from the HL-A matched siblings. Together with two other cases we described before[13], we have now five patients who demonstrated direct cellular cytotoxicity against non-HL-A antigens. LDA and cytotoxic lymphocytes did not mediate damage to autologous target cells, demonstrating the specificity of the assays.

It was not surprising that lymphocytes from each patient were not stimulated in MLR when incubated with his or her sibling's cells, while patients' lymphocytes were adequately stimulated by third party cells; cell donors presumably shared the same HL-A-linked *MLR* genes. This suggests that HL-A-linked *MLR* gene differences are not essential for development of cellular and humoral immune responses to transplantation antigens. In support of this concepts are results reported with humans[17,18], and dogs[19], involving transplants between siblings genotypically matched for major histocompatibility antigens and MLR negative. In the one human pair, a skin graft was rejected by the recipient[17]. In a separate human study, severe graft versus host (GvH) disease developed in the recipient of a bone marrow graft[18]. Likewise, six of sixteen dogs developed fatal GvH disease following marrow grafting[19]. The recipient of the skin graft and the donors of bone marrow in all these cases were not preimmunized by blood transfusions. The possibility that MLR differences are not essential for cellular immune responses does not agree with reports that HL-A-linked MLR genetic differences are necessary for primary *in vitro* sensitisation of human peripheral blood lymphocytes against allogeneic transplantation antigens[20,21]. The studies assessed sensitisation by both MLR and cell-mediated cytotoxicity tests. Perhaps the differences can be explained because our patients were hyperimmunised *in vivo*.

We conclude that cellular and humoral immune responses may develop *in vivo* in the absence of HL-A-linked MLR differences and detectable MLR responses. These responses can be detected by lymphocyte-dependent antibody and by the existence of cytotoxic lymphocytes, both of which react with non-HL-A antigens. These minor histocompatibility differences can be detected *in vitro* by rapid ^{51}Cr release assays. Thus transplantation behaviour across minor histocompatibility antigens *in vivo* can now be correlated with an *in vitro* cytotoxic reaction.

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1 Dausset, J. I., Rapaport, F. T., Ivanyi, P., and Colombani, J. in *Histocompatibility testing* (edit. by Amos, D. B., and van Rood, J. J.), 63 (Munksgaard, Copenhagen, 1965).
2 Amos, D. B., *Adv. Immun.*, **10**, 251 (1969).
3 Cepellini, R., Curtoni, E. S., Mattiuz, P. L., Leigheb, B., Visseti, M., and Colombi, A., *Ann. N. Y. Acad. Sci.*, **129**, 421 (1966).
4 Terasaki, P. I., and Mickey, M. R., *Transplant. Proc.*, **2**, 105 (1971).
5 Amos, D. B., Seigler, H. F., Southworth, J. G., and Ward, E. F., *Transplant. Proc.*, **1**, 342 (1969).
6 Ceppellini, R., in *Organ transplantation today*, (edit. by Mitchison, N. A., Creep, J. R., and Hattinga-Verschure) 26 (Excerpta Medica Foundations, Amsterdam) (1967).
7 Stickler, D. L., Siegler, H. F., Amos, D. B., Ward, F. E., Gunnels, J. C., Price, R. A., and Anderson, E. E., *Ann. Surg.*, **172**, 160 (1970).
8 Lundgren, G., Magnussen, G., Muller, E., Mordenstam, H., Werner, B., and Westberg, G., *Tissue antigens*, **2**, 32 (1972).
9 Seigler, H. F., Gunnells, J. C., Robinson, R. R., Ward, F. E., Amos, D. B., Rowlands, D. P., Burkholder, P. M., Klein, W. J., and Stickle, D. L., *J. clin. Invest.*, **51**, 3200 (1972).
10 Kruger, G. R. F., Berard, G. W., Delellis, R. A., Graw, R. G., jun., Yankee, R. A., Leventhal, B. G., Rogentine, G. N., Herzig, G. P., Halterman, R. H., and Henderson, E. S., *Am. J. Path.*, **63**, 179 (1971).
11 Salvin, R. E., and Santos, G. W., *Clin. Immun. Immunopathol.*, **1**, 472 (1973).
12 Terasaki, P. I., Kreisler, M., and Mickey, R. M., *Postgrad. med. J.*, **47**, 89 (1971).
13 Wunderlich, J. R., Rogentine, G. N., and Yankee, R. A., *Transplantation*, **13**, 31 (1972).
14 Garovoy, M. R., Franco, V., Zschaeck, D., Carpenter, C. B., Storm, T. B., and Merril, J. P., *Lancet*, i, 573 (1973).
15 Wunderlich, J. R., Rosenberg, E. B., Connolly, J. M., in *Prog. Immun.* (edit. by Amos, D. B.), 473 (Academic Press, New York, 1971).
16 Leventhal, B. G., Buell, D. N., Yankee, A., Rogentine, G. N., and Terasaki, P., in *Proc. Fifth Annual Leukocyte Culture Conference* (edit. by Harris, J.), 473 (Academic Press, New York, 1970).
17 Yunis, E. J., Seigler, H. F., Simmons, R. L., and Amos, D. B., *Transplantation*, **15**, 435 (1973).
18 Rubinstein, A., Speck, B., and Jennet, M., *N. Engl. J. Med.*, **25**, 1399 (1971).
19 Storb, R., Rudolph, R. H., and Thomas, E. D., *J. clin. Invest.*, **50**, 1272 (1971).
20 Eijvoogel, V. P., DuBois, R., Melief, C. J. M., Eijlemaker, W. P., Ratt-koning, L., and de Groot-Kooy, L., *Transplant. Proc.*, **5**, 418 (1973).
21 Bach, F. H., Segall, M., Stouber-Zier, K., Sondel, P. M., Alter, B. J., and Bach, M. L., *Science*, **180**, 403 (1973).

Pial vessels transport of substances from cerebrospinal fluid to blood

CEREBROSPINAL fluid (CSF) is formed in the choroid plexuses, in the ventricular cavities through ependymal walls[1] and in the subarachnoid space[2,3]. Some authors[3,4], consider that subarachnoid fluid production constitutes 40% or more of the total secreted CSF. The formation of this subarachnoid fluid can be attributed to the pial vessels or be considered as a collection of extracellular fluid produced by the parenchymal capillaries, passing to the subarachnoid space through the pial membrane. Endothelial cells of the respective vessels should possess specific transport properties if they produce a fluid which might be of similar composition to that formed by the plexus. Absorption of the fluid and selective transfer of substances from CSF to blood should also be accomplished. The aim of this investigation was to explore which of the two, pial vessels or parenchymal capillaries, was the preferential route.

By implanting a plastic ring on the pial surface, it is possible to create an isolated subarachnoid reservoir where substances under study can be placed[5,6]. By rapidly collecting successive samples from the sagital sinus blood, it should be possible to study the time course of the passage of substances in different experimental conditions[7]. If communication between the subarachnoid cup and the venous pial vasculature were direct, the appearance of a substance in the sinus should be immediate, whereas if the route were from the subarachnoid cup to the brain tissue and then to the parenchymal capillaries, the appearance should be delayed and probably influenced by the varying degree of metabolism or binding of the substance with the tissue constituents.

Mongrel dogs, under nembutal anaesthesia (30 mg kg^{-1}, given intravenously) weighing 12 to 20 kg, were used throughout. For access to the sagital sinus, a lucite adapter with a plastic cannula was tightly screwed into a hole drilled in the skull. The animal was heparinised to allow dropwise blood flow which could be interrupted by clamping the cannula. A plastic ring, 2 cm diameter, was placed on the pial surface of one of the hemispheres, the proximal border of the ring being 0.8 to 1.2 cm from the sinus adapter. The leak-proof plastic cylinder was kept in place by an agar gel without exerting pressure on the brain surface. Merlis artificial CSF[8], *p*H 7.4, was used as a solvent for the tracer materials studied: ^{3}HOH (water), L-^{3}H-phenylalanine and ^{22}NaCl. The radioactivity concentration in the cup varied from 12 μCi ml^{-1} (^{22}Na) to 25 μCi ml^{-1} (^{3}HOH). ^{22}Na was run simultaneously with the tritium labelled isotopes. L-^{3}H-phenylalanine was directly diluted in Merlis CSF ('without carrier', 2 μM L-phenylalanine CSF). The artificial CSF containing isotopes were poured into the cup and thereafter collection of sinus blood in successive tubes was made at intervals varying from 1 to 60 s. When the lapse was longer than 10 s, blood was collected only the last 10 s of the interval. The longest experiment lasted 10 min. Blood and cup samples were deproteinised with absolute ethanol and after centifugation an aliquot of the supernatant was used for radioactivity counting in a liquid scintillation spectrometer[7].

The results were expressed as blood to cup radioactivity concentration ratios against time, as shown in Figs. 1 and 2. Several experiments were performed in each animal, with a rest interval of at least 30 min between runs. Because of the varying characteristics of pial vessel surface in the animal preparation, blood flow, and so on, it is more convenient to introduce experimental changes in the same animal where adequate controls could be run. Blood pressure in the femoral artery and flow from the sinus cannula were measured during the experiments as an index of normal circulation. Some animals showing brain oedema with protrusion of the cortex through the pial cup, were discarded.

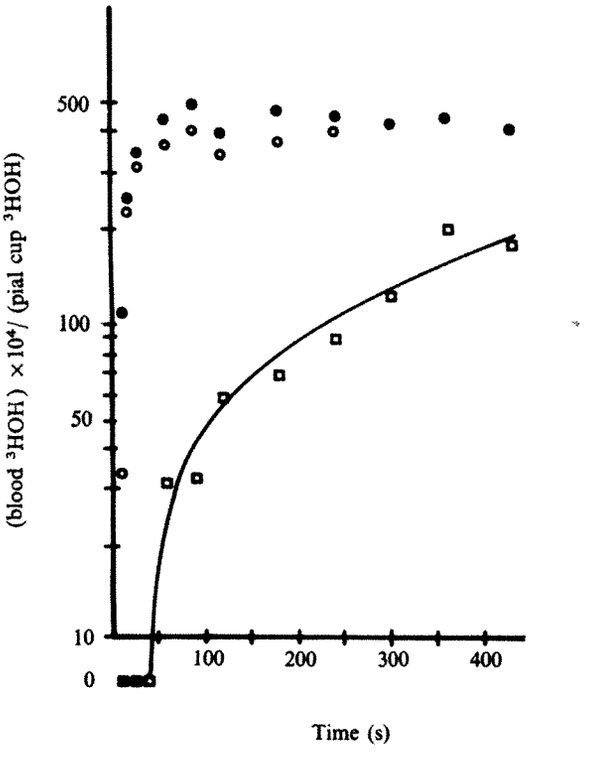


FIG. 1 Appearance of ^{3}HOH in sagital sinus blood. At zero time artificial CSF containing the isotope was poured into a subarachnoid cup and blood was collected in successive samples. ○ and ●, Two experimental runs in the same animal with the pia intact. A third run (□) was made after removing the pia, placing ^{3}HOH on the naked cortex. In this case radioactivity was not detectable in the samples corresponding to the first 30–50 s. Applying a one-compartment analysis, the calculated $t_{1/2}$ for the controls were 11 and 7 s, and for the run 'without pia', 347 s.

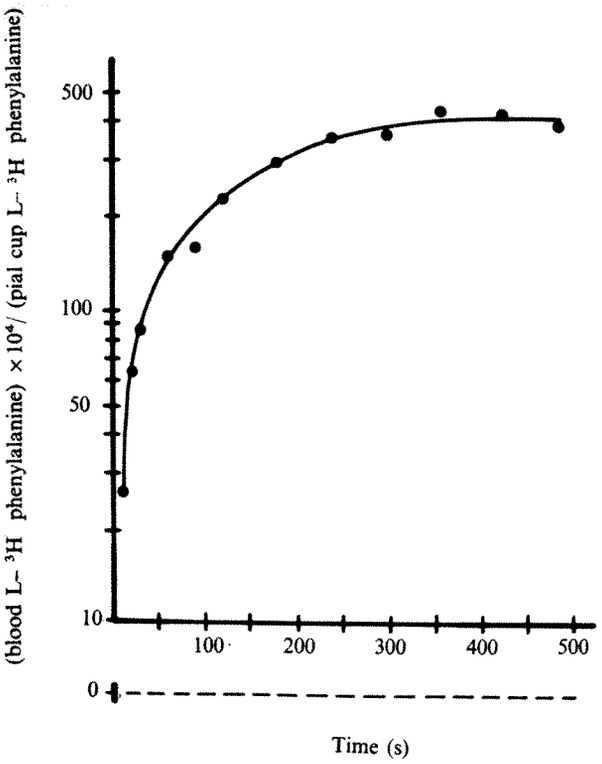


FIG. 2 Influence of removal of pia mater on the passage of L-^{3}H-phenylalanine from a subarachnoid cup to blood. With the pia intact (—), the passage is relatively rapid ($t_{1/2}$ = 92 s), whereas without pia (– – –) no significant radioactivity was found in blood throughout.

TABLE 1 Compartmental analysis of the tracer appearance curves

^{3}HOH		L-^{3}H-phenylalanine		^{22}Na
$t_{1/2}$(s)	r	$t_{1/2}$(s)	r	$t_{1/2}$
8	0.91 (9)	19	0.99 (7)	∞
5	0.99 (4)	14	0.97 (8)	∞
18	0.99 (10)	20	0.99 (11)	∞
20	0.99 (11)	22	0.96 (12)	∞
15	0.99 (14)	35	0.91 (13)	∞
11	0.99 (3)	92	0.99 (7)	∞
7	0.99 (3)			
28	0.99 (4)			
14	0.99 (4)			

Results of the linear least square regression fit for each experiment are given as $t_{1/2} = 0.693/\lambda$ (see text). The correlation coefficient r is followed by the number of experimental points in parenthesis. For ^{22}Na $t_{1/2}$ was considered infinity as no blood radioactivity was detected during the experimental time. Each result corresponds to one animal.

The rate of ^{3}HOH appearance in the sinus blood is fast during the first 30–50 s as shown in Fig. 1 ('with pia') probably indicating the passage of ^{3}HOH through the pial vessels from the cup to the pia and then to the blood. ^{22}Na placed simultaneously in the cup did not appear at all in the venous blood, indicating that pial vasculature is not permeable to Na^+. These results are similar to those obtained with the same cation introducing the tracer in the carotid[7]. The presence in parenchymal capillaries of a barrier impermeable to sodium but highly permeable to water, is indicated.

The interpretation that the way of transport is through pial vessels during the rapid ascending part of the curve in Fig. 1 ('with pia') is reinforced by experiments in which the pia mater was removed after application of $AgNO_3$ 5% solution for 5 min. It has been demonstrated that such a procedure does not affect the viability of the underlying tissue[6]. With the cup on the naked cortex another ^{3}HOH run was performed (Fig. 1, 'without pia'). No ^{3}HOH appeared in the venous blood until the 60 s sample. In the absence of the pial vessels the only route available after 60 s would be from the cup to the brain tissue and then to the blood.

Compartmental analysis of the data was performed assuming a one-compartment system (pia or brain tissue) with a constant input concentration which is the CSF in the pial cup. It can be seen in Fig. 1 that, as expected in this model, the blood concentration, b, raises approaching a plateau value, b_{max}. Therefore, a linear least-square fit of the experimental data was made according to the equation: $\ln(1 - b/b_{max}) = -\lambda t$. For data 'with pia' in Fig. 1, $t_{1/2}$ of 11 and 7 s were calculated with a correlation coefficient r of 0.99 for both. For the run 'without pia', b_{max} was assumed to be equal to that in the control experiments and the estimated $t_{1/2}$ was 347 s, $r = 0.95$. All experiments were analysed in the same way, showing high correlation coefficients. The data are summarised in Table 1. The $t_{1/2}$ for ^{3}HOH was between 5 and 28 s, with an average of 14 s. For ^{22}Na experiments in which no radioactivity could be detected, the $t_{1/2}$ was considered to be infinity.

L-Phenylalanine, an amino acid with high rate of passage through the parenchymal capillaries when introduced through the carotid in the dog[9] or rat[10] was chosen to investigate the passage from the subarachnoid space to the blood. Figure 2 shows a typical curve of blood appearance of L-^{3}H-phenylalanine 'without carrier' (2 μM L-phenylalanine in the cup). The curve is similar to that of ^{3}HOH. When in the same animal the pia is removed and L-^{3}H-phenylalanine 'without carrier' was placed on the naked cortex, no significant radioactivity was detected in sagital sinus blood during the 8 min of the experiment. Results of the compartmental analysis of the L-^{3}H-phenylalanine experiments 'with pia' are given in Table 1. As for ^{3}HOH, a high correlation coefficient was found. The $t_{1/2}$ values for five experiments averaged 22 s and in a sixth experiment $t_{1/2}$ was 92 s.

The possible transport saturation of L-phenylalanine was tested comparing the rate of appearance of L-^{3}H-phenylalanine in sinus blood, when the cup contained ^{3}H-phenylalanine without carrier and 20 mM L-phenylalanine. These experiments are presented in Table 2. An inhibitory effect of about 50% is apparent, indicating that a mediated transport system is involved. A similar saturation phenomenon for disappearance of L-leucine from the subarachnoid fluid has been reported[11]. In dog 1, the L-^{3}H-phenylalanine passage in the first 60 s is higher for 20 mM L-phenylalanine than in the experiment 'without carrier'. In this animal, the 20 mM experiment was performed after the control, whereas the opposite order was used in dogs 2 and 3. It is possible that labelled L-phenylalanine incorporated in the brain after the first assay, exchanges (or counter-transports), with the cold L-phenylalanine of the second run, increasing the amount of label appearing in the sinus. From 120 s on, the inhibitory effect is evident.

Our results point to the participation of pial vessels in the transfer of substances from the subarachnoid CSF to blood. This transfer is selective for some substances, water, L-phenylalanine, and not for sodium for example. The transport is susceptible to saturation by the substrate in the case of the amino acid. These results are similar to the results of the experiments on dog brain where the molecules were introduced through the blood[7,9]. Sodium, like sucrose, remains in the vascular space in a single passage through the brain circulation, whereas water is extracted from the blood almost completely[7]. L-phenylalanine, L-leucine and other neutral amino acids cross the blood–brain barrier by a mediated transport mechanism[9]. We suggest that endothelial cells of both pial vessels and tissue capillaries can maintain the differential composition of the CSF and blood.

There are several references pointing to the permeability of pial vessels to different substances[12,13] and to their potential ability to form and to reabsorb CSF[14]. At least morphologically, the secretory conformation of choroid plexuses is not necessary for the formation of fluid. Perhaps their main function would be the active clearance of substances from CSF, the production and regulation of the fluid composition being reserved to endothelial cells of all the cerebral vasculature: pial vessels, parenchymal capillaries and choroidal capillaries.

TABLE 2 Influence of carrier L-phenylalanine (20 mM) on the passage of L-(^{3}H)phenylalanine from subarachnoid cup to blood

Dog	30 s	60 s	120 s	240 s	420 s
1	133	95	60	48	43
2	41	40	38	46	50
3	43	58	64	61	42

Three animals were used. The values listed are % control radioactivity calculated as blood-to-cup ratio in the 20 mM run divided by that in the run 'without carrier'. Both runs in the same animal. Times are intervals elapsed since the labelled solution was placed in the pial cup.

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1 Pollay, M., and Curl, F., *Am. J. Physiol.*, **213**, 1031 (1967).
2 Bering, E. A., and Sato, O., *J. Neurosurg.*, **20**, 1050 (1963).

[3] Fernstermacher, J. D., Li, C., and Levin, V. A., *Expl. Neurol.*, **27**, 101 (1970).
[4] Sato, O., and Bering, E. A., *Brain Nerve, Tokyo*, **19**, 883 (1967).
[5] Levin, E., and Kleeman, C. R., *Proc. Soc. exp. Biol. Med.*, **135**, 685 (1970).
[6] Wright, P. M., Nogueira, G. J., and Levin, E., *Expl. Brain Res.*, **13**, 294 (1971).
[7] Yudilevich, D. L., and De Rose, N., *Am. J. Physiol.*, **220**, 841 (1971).
[8] Merlis, J. K., *Am. J. Physiol.*, **131**, 72 (1940).
[9] Yudilevich, D. L., De Rose, N., and Sepulveda, F. V., *Brain Res.*, **44**, 569 (1972).
[10] Oldendorf, W. H., *Am. J. Physiol.*, **221**, 1629 (1971).
[11] Lorenzo, A. V., and Snodgrass, S. R., *J. Neurochem.*, **19**, 1287 (1972).
[12] Bertler, A., Falck, B., Owman, Ch., and Rosengreen, E., *Pharmac. Rev.*, **18**, 369 (1960).
[13] Van Harreveld, A., and Ahmed, N., *Brain Res.*, **11**, 32 (1968).
[14] Kaplan, H. A., and Ford, D. H., *The Brain Vascular System*, 152 (Elsevier Press, Amsterdam, 1966).

Replacement of acidic phospholipids by acidic glycolipids in *Pseudomonas diminuta*

PHOSPHOLIPIDS are characteristic components of the membranes of living cells, where in many cases they represent the major polar lipid components. The common phospholipids in Gram-negative bacteria are phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol[1]. *Pseudomonas diminuta* NCTC 8545, however, contains high proportions of glycolipids and relatively low proportions of phospholipids when grown on solid media or in submerged culture[2]. The glycolipids are α-glucosyldiglyceride, α-glucuronosyldiglyceride and β-glucosyl (1→4)-α-glucuronosyldiglyceride[3], and the phospholipids are phosphatidylglycerol[3] and 6-phosphatidyl-α-glucosyldiglyceride[4]. It has been suggested that the acidic glycolipids (containing glucuronosyl residues) might behave as substitutes for acidic phospholipids in the membranes of this pseudomonad[5]. Here we describe studies on the lipid composition of *P. diminuta* grown in continuous culture in conditions of phosphate and magnesium limitation which support this suggestion. Moreover, in conditions of phosphate limitation the cells contain barely detectable amounts (0.3% of total polar lipids) of phospholipid, thereby suggesting that appreciable amounts of these compounds are not obligatory for the maintenance of membrane function.

Continuous cultures of *P. diminuta* were prepared aerobically at 30°C in a 600 ml chemostat constructed essentially as described by Baker[6]; the *p*H was automatically controlled at 7.0 ± 0.2 and a dilution rate of 0.2 h^{-1} was employed. Media were prepared using a standard mineral base[7] with the addition of cystine (50 mg/l) and 1 ml/l of a solution of vitamins (40 mg each of pantothenic acid, biotin and vitamin B_{12} in 100 ml) as recommended previously[8]; the carbon source was ammonium acetate (4 g/l). Phosphate-limited media contained KH_2PO_4 (25 mg/l) as the only phosphate-containing component and magnesium-limited media contained $MgSO_4$ (8 mg/l). Samples (250 ml) of steady state cultures were withdrawn from the chemostat vessel; the cells were collected by centrifugation, washed with NaCl (0.85%) and freeze dried.

Lipids were extracted from dry cells with chloroform-methanol (2:1, v/v) and the polar lipids examined by two-dimensional thin-layer chromatography followed by densitometry[9,10]. The proportions of the polar lipids obtained from representative cultures of phosphate- and magnesium-limited chemostat growths are shown in Table 1. The chemical nature of the individual lipids was indicated by their reaction with specific spray reagents for lipid phosphates[11] and α-glycols[12]; the identity of each lipid was confirmed by chromatographic isolation and analysis of the products of hydrolysis as described previously[3,4]. In addition to the expected polar lipids, a small amount of an unidentified component was detected; its staining properties on thin-layer chromatograms indicated that it was a glycolipid.

The amounts of phospholipids in the phosphate-limited culture were extremely small; only a trace (maximum 0.3%) of phosphatidylglycerol was detected and phosphatidylglucosyldiglyceride was absent. The lipids of the magnesium-limited culture contained significant proportions of phospholipids, the overall lipid composition being similar to that of batch cultures grown in a complex medium (Oxoid Nutrient Broth No. 2). The greatly reduced proportion of phospholipids in cells grown in phosphate limitation compared with those grown in magnesium limitation supports Wilkinson's suggestion that acidic glycolipids might have a similar function to acidic phospholipids in the membranes of some bacteria. This suggestion was also supported by the discovery[9] that in batches of *Bacillus cereus* T acidic phospholipids are partially replaced by an acidic glycolipid under certain growth conditions.

Table 1 Relative proportions of individual polar lipids in phosphate- and magnesium-limited chemostat cultures of *Pseudomonas diminuta* (expressed as % of the total polar lipid).

	Growth limitation	
	PO_4^{3-}	Mg^{2+}
Glucuronosyl diglyceride	33.0	27.0
Glucosylglucuronosyl diglyceride	24.0	21.0
Phosphatidylglycerol	0.3	22.0
Phosphatidylglucosyl diglyceride	—	7.0
Glucosyl diglyceride	40.3	18.0
Unknown glycolipid	2.4	5.0

P. diminuta is unusual in several respects; it can be distinguished clearly by DNA homology studies from other psuedomonads[13]. It has relatively smaller proportions of phosphates in its cell envelope and is remarkable in that it does not synthesise phosphatidylethanolamine, an almost universal component of the membranes of Gram-negative bacteria[1]. It is possible that glucosyldiglyceride may behave as a substitute for phosphatidylethanolamine in this organism as an interrelation between this phospholipid and diglucosyldiglyceride has been established in the lipids of Gram-positive bacilli[9,14,15]. Diphosphatidylglycerol is not synthesised by *P. diminuta*, *P. pavonacea* and *P. rubescens*[2]. In at least the first organism, phosphatidylglucosyldiglyceride might assume the function of diphosphatidylglycerol; both of these lipids contain four fatty acid residues and thus show a superficial structural similarity.

It is apparent, therefore, that the presence of phospholipids as major components of at least some biological membranes is not obligatory provided that other polar lipids, such as acidic glycolipids, are present. It is interesting that an acidic glycolipid, glucosyloxyoctadecenoic acid, has been found[16] to comprise a much higher proportion (6%) of the total lipids of *Aspergillus niger* than does the sum of all the phospholipids (2%).

These results suggest limited interchangeability between polar lipid types within substitution classes[17] which are essentially based on the overall charge of the lipids. Acidic phospholipids and acidic glycolipids form a major substitution group and zwitterionic phospholipids, ornithine amides[18] and neutral glycolipids form another.

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[1] Goldfine, H., in *Advances in Microbial Physiology*, **8**, 1-58 (edit. by Rose, A. H., and Tempest, D. W.) (Academic Press, London and New York, 1971).

[2] Wilkinson, S. G., Galbraith, L., and Lightfoot, G. A., *Eur. J. Biochem.*, **33**, 158-174 (1973).
[3] Wilkinson, S. G., *Biochim. biophys. Acta*, **187**, 492-500 (1969).
[4] Wilkinson, S. G., and Bell, M.E., *Biochim. Biophys. Acta*, **248**, 293-299 (1971).
[5] Wilkinson, S. G., *Biochim. biophys. Acta*, **164**, 148-156 (1968).
[6] Baker, K., *Lab. Prac.*, **17**, 817-824 (1968).
[7] Stanier, R. Y., Palleroni, N. J. and Doudoroff, M., *J. gen. Microbiol* **43**, 159-271 (1966).
[8] Ballard, R. W., Doudoroff, M., Stanier, R. Y., and Mandel, M., *J. gen. Microbiol.*, **53**, 349-361 (1968).
[9] Minnikin, D. E., Abdolrahimzadeh, H., and Baddiley, J., *Biochim. biophys. Acta*, **249**, 651-655 (1971).
[10] Nutter, L. J., and Privett, O. S., *J. Chromatogr.*, **35**, 519-525 (1968).
[11] Dittmer, J. C., and Lester, R. L., *J. Lipid Res.*, **5**, 126-127 (1964).
[12] Baddiley, J., Buchanan, J. G., Handschumacher, R. E., and Prescott, J. F., *J. chem. Soc.*, **2818**, (1956).
[13] Palleroni, N. J., Ballard, R. W., Ralston, E., and Doudoroff, M., *J. Bact.*, **110**, 1-11 (1972).
[14] Minnikin, D. E., Abdolrahimzadeh, H., and Baddiley, J., *Biochem. J.*, **124**, 447-448 (1971).
[15] Minnikin, D. E., Abdolrahimzadeh, H., and Baddiley, J., *FEBS Lett.*, **27**, 16-18 (1972).
[16] Laine, R. A., Griffin, P. F. S., Sweeley, C. C., and Brennan, P. J., *Biochemistry*, **11**, 2267-2271 (1972).
[17] Rouser, G., Yamamoto, A., and Kritchevsky, G., *Arch. int. Med.*, **127**, 1105-1121 (1971).
[18] Wilkinson, S. G., *Biochim. biophys. Acta*, **270**, 1-17 (1972).

New approach to study of oxygen transport in plants using chemiluminescent method

THE possibility that roots are aerated by molecular oxygen transported from the shoots continues to attract attention. A number of methods have already been involved in the study of this phenomenon, namely, polarography[1,2], labelled oxygen[3,4], enzyme investigations[5,6], and electron microscopy[7–9]. Nevertheless, the physiological role of this factor in the oxygen regime of the plant's roots remains unresolved and the data obtained in different laboratories vary.

Here we introduce a chemiluminescent method for tackling this problem. The phenomenon of an ultraweak glow (UG), or biochemiluminescence, discovered by Italian scientists[10], takes place in plant and animal tissues only in the presence of oxygen[11]. We chose this glowing of the roots, or the lack of it in anaerobic conditions, as a test for the transport of oxygen from the shoots to the radical zone of the pumpkin.

The roots of 25 pumpkin seedlings, 4–5 d old with expanded cotyledons, were submerged in Knop's solution. They were about 7–8 cm long and the solution level came to about 0.5–1 cm above the point of root attachment to the shoot—the rest of the shoot remaining in air. The intensity of the root UG (450–650 nm with a maximum of 530 nm) was measured, mainly from the middle and lower parts of the roots, by means of a quantometer apparatus. Experimental details will be described in full later.

When air was bubbling through the solution, a UG of the root was seen but when nitrogen was bubbled through the solution the intensity of the UG began to decrease sharply and after 2 h had practically ceased (Fig. 1). Stopping the passage of nitrogen failed to change the picture even though the shoots remained in air. Only the repeated bubbling of air through the solution led to a rapid increase of the roots' UG.

It is worth noting that other investigators have demonstrated that in the pumpkin[12] and other mesophytes[13,14], oxygen is not only transported from the parts of the plant above the ground to the roots but also partially flows out from the roots to the environment. Further studies of the factors responsible for these discrepancies are being made.

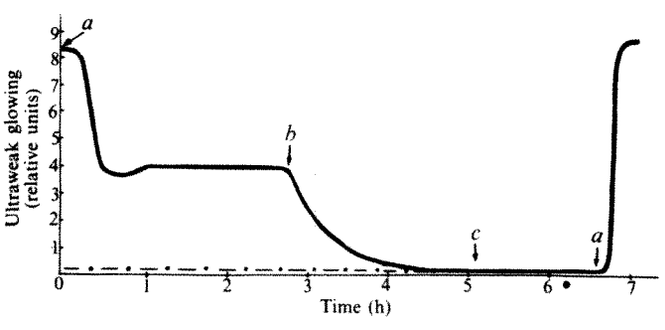


Fig. 1 Ultraweak glowing of pumpkin roots in different conditions of radical zone aeration. *a*, Bubbling by air; *b*, bubbling by N_2; *c*, stopping bubbling by N_2. o–o–o–o, Noise of apparatus (background).

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[1] Vartapetian, B. B., *Soviet Pl. Physiol.* **11**, 659 (1964).
[2] Armstrong W., *Nature*, **204**, 801 (1964).
[3] Jensen, C. R., Letey, J., and Stolzy, L. H., *Science*, **144**, 550 (1964).
[4] Barber, D. A., Ebert, M., and Evans, N. T. S., *J. exp. Bot.*, **13**, 397 (1962).
[5] Crawford, R. M. M., *J. Ecol.*, **54**, 405, (1966).
[6] McManmon, M., and Crawford, R. M. M., *New Phytol.*, **70**, 299 (1971).
[7] Vartapetian, B. B., Andreeva, I. N. Davtian N. G., and Maslova I. P., *Soviet Pl. Physiol.*, **15**, 13 (1968).
[8] Vartapetian, B. B., *Molecular oxygen and water in cell metabolism*, (Nauka, Moscow, 1970).
[9] Vartapetian, B. B., Andreeva, I. N., Maslova, I. P., and Davtian, N. G., *Agrochimica*, **15**, 1, (1970).
[10] Colli, U., Facchini, G., Guidotti, G., Dygnani-Lonati, R., Orsenigo, M., and Sommariva, O., *Experientia*, **11**, 12 (1955).
[11] Tarusov, B. N., Ivanov, I. M., and Petrusevich, V. M., *Ultraweak glowing in biological systems* (Moscow State University Publishers, Moscow; 1967).
[12] Brown R., *Ann. Bot.* (new series), **11**, 417 (1947).
[13] Greenwood, D. J., *New Phytol.* **66**, 337 (1967).
[14] Healy, M. T., and Armstrong, W., *Planta*, **103**, 302 (1972).

Cell-free studies of developmental changes in synthesis of α-foetoprotein and albumin in the mouse liver

ALPHA foetoprotein (αFP) is an α-globulin present in foetal and early postnatal sera of various animal species. It is not normally detectable in adult serum by the immunodiffusion test, but in several pathological conditions, particularly primary hepatomas, the level of αFP increases to a significant level. The potential use of this phenomenon in early diagnosis of hepatomas has been explored. These and related studies on αFP and other 'foetal-tumour antigens' have been reviewed recently[1–5].

In addition to the possible clinical application, αFP may provide an unique opportunity for the understanding of the relationship between embryonic development and malignant transformation. It has been shown that αFP is synthesised in the yolk sac and liver, but the basic mechanisms for the synthesis and its regulation are essentially unknown. This

problem would best be approached by the use of cell-free systems in which all the requirements for the synthesis of αFP can be analysed.

As an initial step toward this goal, we attempted the synthesis of αFP in cell-free liver extracts derived from foetal and postnatal mice. We describe here that such extracts can produce αFP and albumin in relative amounts which vary depending on the stage of development of mice from which the liver extract was prepared.

Foetuses were removed from Swiss albino mice by caesarean section and killed by decapitation. The liver was quickly removed and ground manually in a glass homogeniser in 20 mM Tris-HCl buffer, *p*H 7.8, containing 5 mM $MgCl_2$, 100 mM KCl, 6 mM β-mercaptoethanol, 0.5 mM dithioerythritol (DTE), 1 mM EDTA and 0.25 M sucrose. The homogenate was centrifuged at 3,000*g* for 15 min and the supernatant was applied to a Sephadex G-25 column in 20 mM Tris-HCl, *p*H 7.8, containing 4 mM $MgCl_2$, 55 mM KCl, 6 mM β-mercaptoethanol, 0.5 mM DTE and 1 mM EDTA. The void volume fraction was incubated with radioactive amino acids and other components necessary for protein synthesis as described in the legend of Fig. 1.

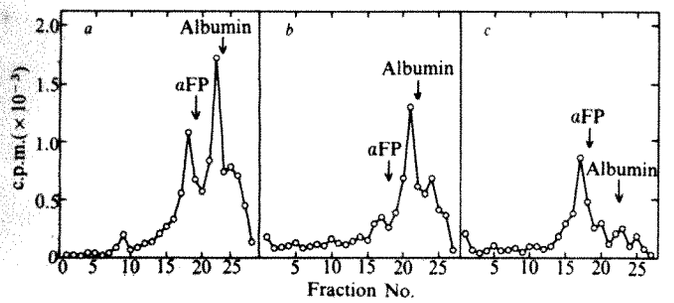


Fig. 1 Polyacrylamide gel electrophoresis of αFP and albumin produced by foetal liver lysate and their identification by immunoprecipitation. *a*, A lysate of 18 d-old foetal liver was incubated with 20 μCi each of ^{3}H-leucine (1 Ci mmol^{-1}), ^{3}H-valine (2 Ci mmol^{-1}) and ^{3}H-phenylalanine (6.15 Ci mmol^{-1}) (Amersham/Searle, Oakville, Ontario), 0.025 μmol each of 17 other amino acids, 1 μmol ATP, 0.3 μmol GTP, 10 μmol phosphocreatine, and 50 μg creatine phosphokinase ml^{-1} at 30° C for 60 min. At the end of incubation the reaction mixture was centrifuged at 149,000*g* for 90 min. The pellet was resuspended in water, frozen and thawed and then sonically disrupted in a Bronson Sonifier at setting 5 for 5 min. This was then centrifuged at 149,000*g* for 90 min and the supernatant was analysed by polyacrylamide gel electrophoresis (7% gel, *p*H 8.9) according to Davis[12]. The gel was stained with Amido Black, scanned at 650 mμ and then sliced into 2 mm fractions which were dissolved in 30% H_2O_2 at 60° C overnight. Radioactivity was measured using Aquasol scintillation liquid (NEN Canada, Dorval, Quebec). *b*, The sample shown in *a* was incubated with rabbit antiserum against mouse αFP at 37° C for 60 min and then at 0° C overnight. The precipitate was removed by centrifugation and the supernatant analysed by polyacrylamide gel electrophoresis. *c*, The sample shown in *a* was incubated with rabbit antiserum against adult mouse serum (Nutritional Biochemical Corporation, Cleveland, Ohio) as described in *b*. The precipitate was removed by centrifugation and the supernatant analysed by polyacrylamide gel electrophoresis. Arrows indicate the carrier αFP and albumin bands.

There was active protein synthesis in these conditions as measured by hot trichloroacetic acid-precipitable counts. Preliminary analysis showed, however, that newly-synthesised radioactive αFP was not released free into the supernatant. This suggested that αFP was synthesised by membrane-bound polysomes and the completed polypeptide chains remained bound to the membrane or in microsomal cisternae. Several treatments were tested to disrupt the membrane structure. A combination of freeze-thawing and sonication was found to be among the most effective for this purpose. This procedure also permits the release of newly formed albumin from inside the microsomal cisternae.

Polyacrylamide gel electrophoretic analysis of proteins synthesised by 18 d foetal liver extract and subsequently released by sonication is shown in Fig. 1*a*. Two main radioactive fractions were observed which showed slightly lower mobility than carrier αFP and albumin. To identify these proteins the sample was divided into two portions; one received antiserum against αFP and the other antiserum against adult mouse serum (to precipitate albumin). These antisera possessed the required specificity to αFP and adult serum proteins as tested by double immunodiffusion and immunoelectrophoresis.

After incubation, antigen–antibody precipitates were removed and the supernatant containing proteins not bound to antibody was analysed by polyacrylamide gel electrophoresis. In the sample which received antiserum against αFP the faster-moving fraction remained as the major radioactive peak (Fig. 1*b*). In the other sample which received antiserum against adult mouse serum, the slower moving fraction remained as the major radioactive peak (Fig. 1*c*). This indicated that the faster moving radioactive protein was albumin and the slower moving protein αFP.

Mouse αFP has been shown to exhibit microheterogeneity or several electrophoretic forms due to a variation in the amount of sialic acid residues[6]. As the sialation process is distinct from the synthesis of polypeptide chains it is possible that αFP produced in the cell-free system was not fully sialated and thus exhibited a lower electrophoretic mobility than carrier αFP. The exact cause for the slightly slower migration of radioactive albumin than carrier albumin is not clear at present, but a similar observation has been reported by others[7].

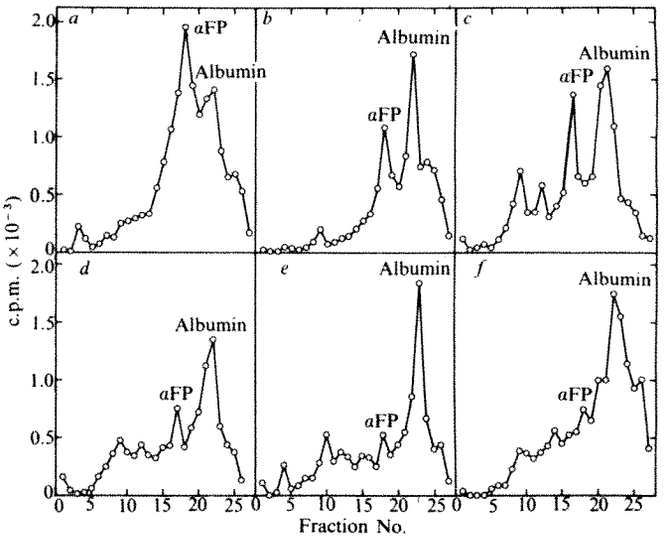


Fig. 2 Polyacrylamide gel electrophoresis of proteins produced by liver lysates from foetal and postnatal mice. Conditions for cell-free protein synthesis and gel analysis are described in the legend of Fig. 1. *a*, 13-d-old foetus; *b*, 17-d-old foetus; *c*, 20-d-old foetus; *d*, 1-d-old mice; *e*, 3-d-old mice; *f*, 6-d-old mice.

Figure 2 shows the synthesis of αFP and albumin in liver extracts from developing mice ranging in age from 13 d of gestation to 6 d after birth. It is seen that the synthesis of αFP decreased with time, whereas that of albumin increased. We found that, although the total radioactive proteins released by sonication varied from experiment to experiment, the % of αFP and albumin and their relative amounts remained relatively constant. The molecular weight of mouse αFP, as estimated by sodium dodecyl sulphate (SDS) gel electrophoresis, was 70,000, which was similar to that of mouse albumin (unpublished data). The radioactivity in the αFP and albumin fractions shown in Fig. 2 is, therefore,

directly proportional to the total amounts synthesised in the cell-free extracts.

The changes in the synthesis of αFP and albumin shown above were calculated as relative amounts and are presented in Fig. 3. in the 13-d-old foetus, the synthesis of αFP was twice as active as that of albumin. The subsequent decrease in αFP production and increase in albumin synthesis showed that at about day 15 of gestation they were produced at about an equal rate. By the time the mice were born the αFP:albumin ratio decreased to less than 1:2. After birth, the synthesis of αFP continued to decline and seemed to cease after about one week as analysed by the present method.

The level of albumin synthesis was remarkably high in the foetus of 13 d gestation. Analysis of the liver in the earlier gestation was not feasible because of its small size.

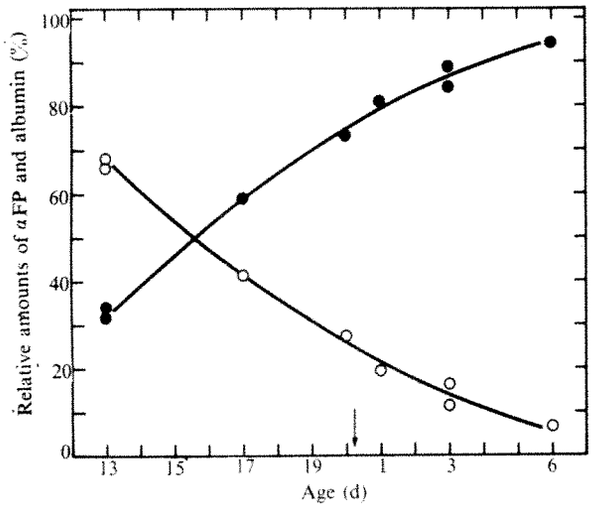


Fig. 3 Relative amounts of αFP and albumin synthesised by liver lysates from foetal and postnatal mice. The sum of the counts under αFP and albumin peaks in Fig. 2 was used as 100%. ○, αFP; ●, albumin. Arrow indicates birth.

The results described above show that the present cell-free system enables the analysis of relative synthetic activity for αFP synthesis and albumin production in the liver at various stages of development. The observed shift in the synthetic activities with maturation is in agreement with the changes in their total serum contents based on immunological analysis[8]. It is likely, therefore, that the present cellfree synthesis of αFP and albumin reflects the pattern of protein synthesis operative *in vivo*. A similar approach can be applicable to other mammalian species, and may prove to be of value as an aid in diagnosis of hepatomas.

The present study suggests that mRNA for αFP may be isolated from foetal liver and translated in a defined cell-free system. In fact, the study in progress has strongly supported this possibility. The synthesis of albumin using exogenous mRNA has recently been accomplished[9–11]. The study of requirements for the translation of these mRNAs, such as initiation factors, should help us to understand the molecular basis for the regulation of the αFP and albumin synthesis during embryonic development. The knowledge gained in such studies may also provide a basis for possible engineering of the synthetic events in tumour cells to arrest or reverse neoplastic transformation.

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1 Abelev, G. I., *Adv. Cancer Res.*, **14**, 295 (1971).
2 Uriel J., *Path. Biol.*, **17**, 877 (1969).
3 Alexander, P., *Nature*, **235**, 137 (1972).
4 Masseyeff, R., *Path. Biol.*, **20**, 703 (1972).
5 Anderson, N. G., and Coggin, jun., J. H., in *Embryonic and Fetal Antigens in Cancer* (edit. by Anderson, N. G., Coggin, jun., J. H., Cole, E., and Holleman, J. W.), 2, 361 (National Technical Information Service, U.S. Department of Commerce, Springfield, Virginia, 1972).
6 Gustine, D. L., and Zimmerman, E. F., *Biochem. J.*, **132**, 541 (1973).
7 Hill, H. Z., Wilson, S. H., and Hoagland, M. B., *Biochim. biophys. Acta*, **269**, 477 (1972).
8 Abelev, G. I., Perova, S. D., Khramkova, N. I., Postnikova, Z. A., and Irlin, I. S., *Transplantation*, **1**, 174 (1963).
9 Taylor, J. M., and Schimke, R. T., *J. biol. Chem.*, **248**, 7661 (1973).
10 Shafritz, D. A., *J. biol. Chem.*, **249**, 81 (1974).
11 Faber, A. J., Miall, S. H., and Tamaoki, T., *Can. J. Biochem.*, (in the press).
12 Davis, B. J., *Ann. N.Y. Acad. Sci.*, **121**, 404 (1964).

Blocking of syngeneic effector T cells by soluble tumour antigens

THE cellular immune response to murine sarcoma virus (MSV)-induced tumours in mice involves lymphoid effector cells of different origins[1,2]. When the chromium release test (CRT)[3–5] is used to study this immune response, only thymus-derived lymphoid cells (T cells) show any appreciable lytic activity towards the appropriate tumour target cells[6,7]. Other lymphoid cells display no lytic activity in CRT, nor are they required for T cell-dependent tumour cell lysis to occur[7]. When the microcytotoxicity assay (MA) of Takasugi and Klein[8] is used to study the cellular immune response of the same MSV tumour-bearing mice, both T and non-T lymphoid cells are seen to be active[1,2].

A time-course study with these two assays using the same lymphoid cell preparations[1] showed that, at the moment of maximum tumour size, neither T nor non-T lymphoid cell activity was detected in MA, whereas a peak value of T cell activity was detected in CRT. It has also been shown that sera from MSV tumour-bearing mice (progressor sera) are able to completely inhibit the immune activity measured in MA[1,9], although the same sera have no effect when tested in CRT[1,4,6]. These facts indicated that a different cytotoxic T cell subpopulation might be involved in each test, the effector T cells active in MA probably being blocked by the soluble tumour antigens present in progressor sera[9,10] and the effector cells active in CRT not being at all affected. Here we present further arguments in favour of such an hypothesis.

We tested the effect of progressor sera or soluble tumour-cell antigen extracts on the immune activity of spleen cells from MSV-infected C57BL/6 (B6) mice, as revealed by MA and CRT. The Moloney isolate of MSV, regularly maintained by acellular transmission in newborn B6-mice, was used in all experiments to induce experimental sarcomas in adult B6 mice. Spleen cells were taken between the 15th and 25th days after MSV inoculation because the T cell subpopulation shows an appreciable immune activity in both MA and CRT during this period[1,2], and the tumour has regressed. CRT and MA were performed as previously described[1,4], and syngeneic conditions were maintained throughout all experiments. Spleen lymphocytes

from normal B6 mice were used as negative controls in all tests.

The experiments summarised in Table 1 investigated the blocking activity of B6 progressor serum (PrSm) on spleen effector cells. The cytostatic activity detected in MA could be blocked at a limited range of serum dilutions (1/6-1/12), while no effect was evident in CRT with the same lymphocyte samples and serum dilutions. Normal B6 serum (N1 Sm), at similar dilutions, did not affect the lymphocyte activity in either test.

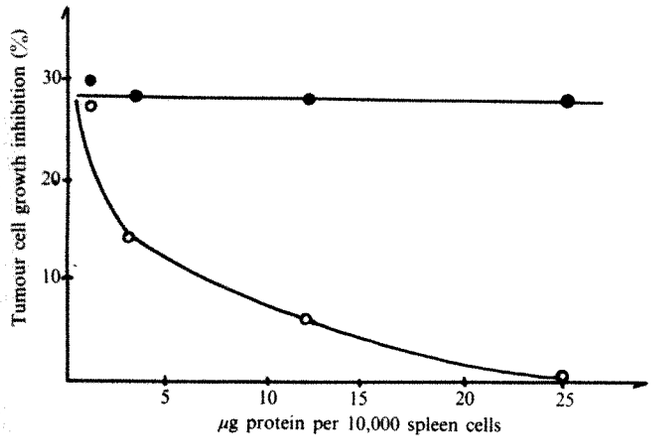


Fig. 1 The effect of GiL4 antigen (O) and of normal B6 antigen (●) on the immune activity, as detected in MA, of spleen cells from B6 mice infected with MSV 20 d previously.

Baldwin *et al.*[11] have shown that the blocking fraction of serum from tumour-bearing rats contains antigen-antibody complexes, and that solubilised tumour antigen seems to be the main blocking agent[10,11]. Sjögren *et al.*[12] had previously reached the same conclusion concerning the blocking factor in sera from MSV-infected mice. We therefore solubilised tumour cell antigens and checked whether they could block specifically the spleen cell activity detected in MA and in CRT.

The procedure developed by Mettzer *et al.*[13] and Reisfeld *et al.*[14] was chosen; this involves antigen solubilisation by 3 M KCl, *p*H 7.4, and gives high yields of functional antigen. Soluble antigens were prepared from normal spleen cells or from several different tumour cell lines belonging to the FMRGi antigen group[15] (MSV-tumour cells are FMRGi-positive). Normal or immune spleen lymphocytes were incubated for 18 h (CRT) or 48 h (MA) in the presence of target tumour cells and of antigen extract. As shown in Table 2 the cytostatic activity of immune spleen cells in MA was inhibited by the tumour antigen extracts prepared from GiL_4, MSV-tumour, or Friend leukaemia cells. No blocking effect was detected in CRT at equivalent antigen doses; indeed, in some cases immune lysis actually increased in CRT in the presence of soluble antigen.

Inhibition by the soluble antigen preparations in MA is specific because: first, the soluble extracts prepared from normal B6 or BALB/c spleen cells do not block the cytostatic activity of anti-MSV tumour lymphocytes at any of the doses used; second, when an allogeneic immune system is studied in MA, the B6 'normal' extract blocks BALB/c lymphocytes (H2d) immunised against B6 normal spleen cells (H2b) (Table 3); and third, the BALB/c soluble extract prepared from Friend virus-induced leukaemia cells blocks the activity in MA of B6 lymphocytes immunised against an MSV-induced tumour (Table 2), but it does not affect the activity in MA of BALB/c anti-B6 lymphocytes (Table 3). This also indicates that the inhibitory activity of our antigen extracts is not the result of a toxic effect on spleen cells.

The blocking effect displayed by the soluble antigen extracts in MA follows a dose-response curve (Fig. 1). The extract prepared from GiL4 cells blocks lymphocyte activity completely at doses equivalent to or higher than 25 μg protein per 10,000 spleen cells; blocking activity can still be detected at 3 μg protein per 10,000 spleen cells, but disappears at the 1 μg protein level.

The experiments discussed above show that the immune cytostatic activity of lymphoid cells in MA can be blocked when functional T and non-T effector cells are present[1,2]. As the effector cells involved in CRT are exclusively T cells[4,6,7] and cannot be blocked, it seemed important to determine if the effector T cells active in MA, when purified, could be blocked or not. Cell populations enriched in T cells were consequently obtained by passing whole spleen cell suspensions through anti-immunoglobulin coated glass bead columns[1,16] and were subsequently used to study the blocking effects of progressor sera and of soluble antigen extracts. Table 4 shows that column-purified cells can be effectively blocked in MA when they are incubated with progressor serum or with the adequate soluble tumour extract. No decrease in immune lysis is detected in CRT when the same lymphocytes are incubated with these reagents at comparative doses.

Our column-purified cell preparations contained 89 ± 2% θ_{C3H} positive cells and 1 ± 0.5% immunoglobulin-positive cells[7]. To study the role played in MA by the θ_{C3H} negative cells present in the column-purified cell suspensions, column-passed cells were treated, with AKR anti-θ_{C3H} serum and rabbit complement and dead cells were removed by incubation with 0.05% trypsin. A pure population of viable column-passed cells not carrying the θ_{C3H} antigen was thus obtained and was adjusted to the appropriate cell number before distribution in the MA plates. These spleen cells possessed no ability to inhibit tumour cell growth; experimental results published recently[1,2] show that non-T cells do not require a T cell collaboration to exert a cytostatic effect in MA. Control cells trypsinised in the same manner after incubation with normal AKR serum and complement showed no decrease in their immune cytostatic activity. Consequently, the immune activity displayed by such column-purified cells in MA seems to be due exclusively to thymus-dependent lymphocytes.

In a different set of experiments, cytostatic non-T cells were purified by treating whole spleen cell preparations with anti-θ_{C3H} serum and complement. The immune activity of 10,000 anti-MSV non-T cells was completely inhibited in MA by B6 progressor serum diluted 1/6 or by 25 μg GiL4 soluble antigen. Normal B6 serum and normal B6 antigen had no effect on the immune activity of these cells.

Our results provide further proof of the hypothesis that CRT and MA, as performed here, do not measure the same immune phenomena. As suggested in a recent communication[1], CRT reveals an exclusively T cell-mediated cytolysis, while MA seems to detect a complex cytostatic effect in our experimental conditions. This segregation of effector cell functions is probably because the target cell used in MA is highly resistant to immune cytolysis, while the target cell used in CRT is easily lysed[1]. We have provided evidence here which shows that soluble antigens prepared from MSV-induced tumours or from antigenically related tumours can block the activity of anti-MSV effector T cells in MA, whereas no inhibition of the effector T cells active in CRT can be detected. The possibility of blocking immune activity in MA with soluble tumour antigen preparations has already been indicated by Baldwin *et al.*[10,11] in other tumour systems. C. Neauport (unpublished) has recently obtained results in our laboratory showing that the activity of anti-allogeneic immune lymphocytes in CRT is very difficult to block with soluble antigen preparations, differing with results published by Wagner and Boyle[17] and agreeing with previous observations of Cerottini *et al.*[18]. We have also provided evidence that progressor sera can only block the activity of effector T cells in MA and not in CRT, thus confirming previous observations[1,4,9]. This blocking effect could result from the presence of soluble tumour antigens in the serum of progressor mice, and it might explain why no activity can be detected in MA when lymphocytes are taken at the time of maximum tumour size[1]. The fact that the effector T cells de-

Table 1 Effect of B6 progressor serum on spleen cell immune activity.

Days after MSV inoculation	Cells incubated with:	MA: Normal spleen cells	MA: Immune spleen cells	MA: Tumour cell growth inhibition (%)	MA: Degree of blocking (%)	CRT: Immune cytolysis (%)
15	MEM	199 ± 25	148 ± 18	26*		24.0 ± 3.8
	NlSm (1/6)	205 ± 29	168 ± 26	18†		24.1 ± 3.5
	PrSm (1/6)	205 ± 19	188 ± 9	8‡	54†	20.2 ± 3.9
19	MEM	229 ± 20	180 ± 21	21*		17.8 ± 1.3
	NlSm (1/6)	224 ± 27	155 ± 21	31§		11.0 ± 1.5
	PrSm (1/6)	192 ± 50	173 ± 35	10‡	72*	12.0 ± 3.3
	NlSm (1/12)	225 ± 26	183 ± 14	19*		12.3 ± 2.6
	PrSm (1/12)	199 ± 33	197 ± 22	0	100*	12.2 ± 3.7
	NlSm (1/18)	248 ± 36	205 ± 23	17†		14.9 ± 3.0
	PrSm (1/18)	248 ± 31	187 ± 28	24*	0	12.8 ± 3.4
	NlSm (1/24)	239 ± 22	184 ± 22	23*		Not done
	PrSm (1/24)	255 ± 43	204 ± 24	20†	0	
21	MEM	163 ± 24	119 ± 14	27§		15.9 ± 3.6
	NlSm (1/6)	152 ± 23	122 ± 14	20*		11.7 ± 2.8
	PrSm (1/6)	160 ± 22	159 ± 26	0	100*	18.5 ± 1.5
24	MEM	121 ± 17	76 ± 13	37§		13.2 ± 3.1
	NlSm (1/6)	167 ± 17	127 ± 18	24*		13.9 ± 3.8
	PrSm (1/6)	180 ± 18	171 ± 10	5‡	75*	13.8 ± 3.5

Normal or immune B6 spleen cells were incubated with normal B6 serum (NlSm) or B6 progressor serum (PrSm) for the duration of each test. In MA, 3034 Falcon Plastics tissue culture microplates were seeded with 100 to 200 B6 MSV tumour cells growing in monolayers and were incubated overnight; 5µl Eagle's MEM, NlSm or PrSm plus 10,000 lymphocytes in 5 µl were then added, and the plates were further incubated at 37°C for 48 h. After one wash, the remaining cells were fixed with methanol, stained with giemsa and counted. The numbers shown represent the arithmetic mean of target cell counts ± s.d. and the significance of the relative inhibition of cell growth was calculated according to Student's *t* test. The degree of blocking in MA was evaluated by taking into consideration the difference between the number of target cells counted in the presence of immune spleen cells and of normal spleen cells (ΔTC) following incubation with progressor serum (ΔTC_{PrSm}) or with normal B6 serum (ΔTC_{NlSm}): Degree of blocking (%) = $(\Delta TC_{NlSm} - \Delta TC_{PrSm})/(\Delta TC_{nlsm}) \times 100$.

In CRT 20,000 ^{51}Cr-labelled B6 GiL4 ascitis lymphona cells in 0.05 ml were mixed with 0.1 ml MEM, NlSm or PrSm, and 500,000 normal or immune B6 spleen cells in 0.05 ml were then added. After 18 h of incubation the reaction mixture was adjusted to 1.0 ml and percent chromium release was determined as described elsewhere[7]. The results shown represent the differences between chromium released in the presence of immune spleen cells and of normal spleen cells ± s.d., and are statistically significant. A low lymphocyte to target cell ratio (25:1) was chosen to give low levels of immune lysis which theoretically should be easily blocked. No blocking effects were detected with CRT in any of the samples studied.

* $P < 0.01$
† $P < 0.05$
‡ $P > 0.05$
§ $P < 0.001$

Table 2 Effect of soluble antigen preparations on spleen cell immune activity.

Days after MSV inoculation	Cells incubated with:	MA: Normal spleen cells	MA: Immune spleen cells	MA: Tumour cell growth inhibition (%)	MA: Degree of blocking (%)	CRT: Immune cytolysis (%)
20	MEM	220 ± 20	163 ± 14	26*		14.9 ± 3.8
	B6NlAg (25 µg)	234 ± 28	168 ± 13	28*		12.2 ± 3.2
	GiL4Ag (25 µg)	139 ± 14	138 ± 26	0	100†	14.8 ± 3.6
21	MEM	354 ± 37	272 ± 26	23*		14.1 ± 2.0
	B6NlAg (25 µg)	370 ± 39	271 ± 35	28*		12.7 ± 2.5
	GiL4Ag (25 µg)	327 ± 58	322 ± 54	2‡	95†	19.6 ± 3.0
	B6NlAg (12 µg)	450 ± 24	348 ± 47	23*		13.8 ± 2.2
	GiL4Ag (12 µg)	441 ± 61	454 ± 61	0	100†	16.5 ± 1.4
	B6NlAg (12 µg)	412 ± 37	342 ± 31	17†		13.8 ± 2.2
	B6MSVAg (12 µg)	348 ± 43	371 ± 69	—6‡	100†	15.2 ± 1.8
22	MEM	161 ± 17	131 ± 13	19*		13.1 ± 2.6
	CNlAg (25 µg)	156 ± 10	116 ± 17	26*		12.8 ± 3.8
	FVLAg (25 µg)	89 ± 12	83 ± 18	7‡	85‡	11.0 ± 3.5

Normal or immune spleen cells were incubated with each soluble antigen preparation during the whole duration of each test at the ratio of 25 or 12 µg protein for 10,000 lymphocytes. MA and CRT were performed as described in Table 1. Soluble antigens were prepared from B6 normal spleen cells (B6NlAg), BALB/c normal spleen cells (CNlAg), GiL4 lymphoma cells of B6 origin (GiL4Ag), solid B6 MSV-induced tumours (B6MSVAg), YC8 lymphoma cells of BALB/c origin (YC8 Ag), and Friend virus-induced leukaemia cells of BALB/c origin (FVL Ag). Following solubilisation in 3M KCL, *p*H 7.4, the crude soluble antigen preparations were filtered through Sephadex G-200 columns; the fractions presenting antigenic activity were detected by the specific inhibition of cytotoxic antisera in the presence of complement, and their protein content was evaluated with the Folin-Ciocalteu reagent.

* $P < 0.001$
† $P < 0.01$
‡ $P > 0.05$

Table 3 Effect of soluble antigen preparations on H2d anti-H2b immune spleen cells.

Cells incubated with:	MA				CRT
	Normal spleen cells	Immune spleen cells	Tumour cell growth inhibition (%)	Degree of blocking (%)	Immune cytolysis (%)
MEM	142 ± 22	111 ± 8	22*	0	30.9 ± 3.1
CNlAg (25 μg)	126 ± 15	103 ± 11	18*	0	32.4 ± 3.5
B6NlAg (25 μg)	106 ± 11	97 ± 13	8†	61*	34.4 ± 3.9
FVLAg (25 μg)	98 ± 4	64 ± 13	35‡	0	35.1 ± 3.1

Immune H2d anti-H2b spleen cells were obtained 6 d after inoculating adult BALB/c mice with 5×10^7 B6 normal spleen cells intraperitoneally. Target cells were the same as those used to test anti-MSV tumour immune lymphoid cells. Spleen cells from normal BALB/c mice were used as negative controls.

*$P < 0.01$
†$P > 0.05$
‡$P < 0.001$

Table 4 Effect of progressor serum and soluble antigens on column-purified T cells.

Experiment	Days after MSV inoculation	Cells incubated with	MA				CRT
			Normal spleen cells	Immune spleen cells	Tumour cell growth inhibition (%)	Degree of blocking (%)	Immune cytolyiss (%)
1	20	MEM	152 ± 19	129 ± 16	15*		18.9 ± 1.1
		NlSm (1/6)	195 ± 35	162 ± 31	17*		19.7 ± 2.5
		PrSm (1/6)	178 ± 20	182 ± 29	—2†	100‡	18.0 ± 3.8
2	20	MEM	122 ± 18	86 ± 19	30‡		10.6 ± 3.2
		B6NlAg (25 μg)	138 ± 12	104 ± 18	25§		13.0 ± 2.8
		GiL4Ag (25 μg)	111 ± 16	105 ± 20	5†	82‡	18.1 ± 3.5
3	21	MEM	170 ± 15	149 ± 13	12*		15.6 ± 3.3
		NlSm (1/6)	192 ± 18	169 ± 14	12*		14.3 ± 2.6
		PrSm (1/6)	192 ± 14	201 ± 26	—5†	100‡	16.7 ± 3.0
4	21	MEM	221 ± 19	173 ± 14	22§		17.8 ± 1.8
		B6NlAg (25 μg)	259 ± 32	196 ± 25	24§		16.2 ± 2.3
		GiL4Ag (25 μg)	216 ± 35	202 ± 32	6†	78‡	24.8 ± 3.6

*$P < 0.05$
†$P > 0.05$
‡$P < 0.01$
§$P < 0.001$

tected in CRT are not sensitive to such blocking factors further indicates that two different effector T cell subpopulations are involved here. These two effector T-cell subpopulations probably differ in function as well as in sensitivity to blocking factors, since the subpopulation detected in CRT displays a cytolytic activity and the subpopulation detected in MA displays a cytostatic activity. These observations recall the functional heterogeneity of T cells detected in other immune systems[19-21]. It cannot be definitely excluded, however, that in our experimental system effector T cell subpopulations of similar nature are involved which react with different antigens of the tumour cell membrane.

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[1] Plata, F., Gomard, E., Leclerc, J. C., and Levy, J. P., *J. Immun.*, (in the press).
[2] Lamon, E. W., Wigzell, H., Klein, E., Anderson, B., and Skurzak, H. M., *J. exp. Med.*, **137**, 1472 (1973).
[3] Cerottini, J. C., Nordin, A. A., and Brunner, K. T., *Nature*, **228**, 1308 (1970).
[4] Leclerc, J. C., Gomard, E., and Levy, J. P., *Int. J. Cancer*, **10**, 589 (1972).
[5] Laverin, D., Nunn, M., and Herberman, R. B., *Proc. Am. Ass. Cancer Res.*, **13**, 66 (1972).
[6] Leclerc, J. C., Gomard, E., Plata, F., and Levy, J. P., *Int. J. Cancer*, **11**, 426 (1973).
[7] Plata, F., Gomard, E., Leclerc, J. C., and Levy, J. P., *J. Immun.*, **11**, 667 (1973).
[8] Takasugi, M., and Klein, E., *Transplantation*, **9**, 219 (1970).
[9] Hellström, I., and Hellström, K. E., *Int. J. Cancer*, **5**, 195 (1970).
[10] Baldwin, R. W., Price, M. R., and Robins, R. A., *Int. J. Cancer*, **11**, 527 (1973).
[11] Baldwin, R. W., Bowen, J. G., and Price, M. R., *Br. J. Cancer*, **28**, 16 (1973).
[12] Sjögren, H. O., Hellström, I., Bansal, S. C., and Hellström, K. E., *Proc. natn. Acad. Sci., U.S.A.*, **68**, 1372 (1971).
[13] Mettzer, M. S., Leonard, E. J., Rapps, H. J., and Borsos, T. W., *J. natn., Cancer Inst.*, **47**, 703 (1971).
[14] Reisfeld, R. A., Pellegrino, M. A., Kahan, B. D., *Science*, **172**, 1134 (1971).
[15] Boyse, E. A., Old, L. J., and Stockert, E., *Nature*, **201**, 777 (1964).
[16] Wigzell, H., Sundqvist, K. G., and Yoshiva, T. O., *Scand. J. Immun.*, **1**, 75 (1972).
[17] Wagner, H., and Boyle, W., *Nature new Biol.*, **240**, 92 (1972).
[18] Brunner, K. T., Nordin, A. A., and Cerottini, J. C., in *Cellular Interactions in the Immune Response* (edit. by Cohen, S., Cudkowicz, G., and McCluskey, R. T.), 220-230 (S. Karger, Bassel, 1971).
[19] Cantor, H., and Asofsky, R., *J. exp. Med.*, **135**, 764 (1972).
[20] Segal, S., Cohen, I. R., and Feldman, M., *Science*, **175**, 1126 (1972).
[21] Stobo, J. D., Paul, W. E., and Henney, C. S., *J. Immun.*, **110**, 652 (1973).

Table 2 Relative cytotoxic potency and blast cell content of cultures following *in vitro* cultivation of C57BL/6J lymph node cells with different stimulating spleen cell populations

Composition and density of stimulating spleen cell population*		Blast cell content after 4 d in culture	Relative cytotoxic potency†
8×10^6 ml^{-1}	2×10^6 ml^{-1}		
γ-irradiated BALB/c	Ultraviolet-irradiated C57BL/6J	+++	300
Ultraviolet-irradiated BALB/c	γ-irradiated C57BL/6J	—	⩽1
Ultraviolet-irradiated BALB/c	γ-irradiated CBA/H	++	50
Ultraviolet-irradiated C57BL/6J	γ-irradiated CBA/H	+	10

* Stimulating spleen cell populations were prepared by mixing equal volumes of each component at the densities shown. *In vitro* cultures were then prepared by mixing 5 ml of the stimulating cell population with 5 ml of C57BL/6J lymph node cells at a density of 4×10^6 ml^{-1}.

† Relative potencies were calculated from Fig. 2 by measuring the displacement along the abscissa of the different dose response relationships. The potency of the cultures stimulated with ultraviolet-irradiated BALB/c spleen cells was arbitrarily taken to be equal to or less than unity.

The displacement along the abscissa of the dose response relationship obtained from different cell populations is a measure of their relative potency.

These experimental findings were reproducible. γ-Irradiated BALB/c spleen cells produce a vigorous blast cell response when cultured with C57BL/6J lymph node cells, and generate a high titre of cytotoxic cells specific for the H-2^d antigen. Ultraviolet-irradiated BALB/c spleen cells, in contrast, produce no detectable cytotoxic response and no blast transformation. When γ-irradiated CBA/H spleen cells were added to cultures of ultraviolet irradiated BALB/c spleen cells and C57BL/6J lymph node cells, however, blast transformation was evident and the cytotoxic cell response to the H-2^d antigen was increased by five-fold over the CBA/H background.

The *in vitro* production of cytotoxic lymphocytes has been shown to be a function of activated T cells [8]. Thus, the above data indicate that transplantation antigens presented on the surface of the ultraviolet-irradiated lymphoid cells are poorly immunogenic for T cells *in vitro*. The *in vitro* immunogenicity of this antigen is significantly enhanced, however, if the responsive cells receive an allogeneic stimulus at the same time as they are confronted with antigen. These findings confirm our earlier *in vivo* studies showing that allogeneic transplantation antigens are relatively weak in the absence of allogeneic stimulation[1, 9]. They also raise the possibility that antigenic differences between donor and recipient may not constitute a major barrier to allotransplantation.

According to our hypothesis[1], lymphoid cells carried in the transplanted organ or tissue may effectively constitute the major transplantation barrier because of the way in which they influence the immunogenicity of transplantation antigen.

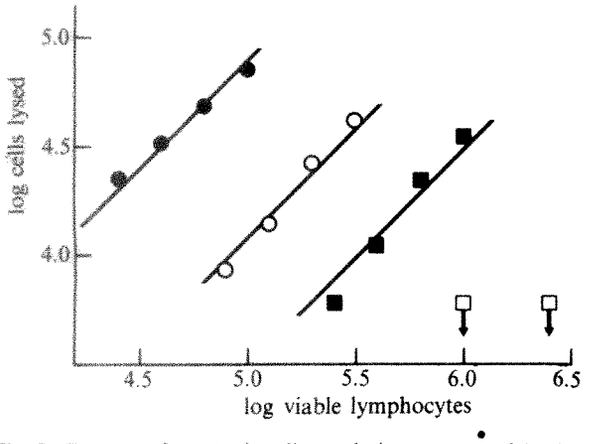


Fig. 2 Potency of cytotoxic cell populations generated *in vitro*. C57BL/6J lymph node cells were stimulated with the following spleen cell populations: ●, γ-irradiated BALB/c + ultraviolet-irradiated C57BL/6J; □, ultraviolet-irradiated BALB/c + γ-irradiated C57BL/6J; ○, ultraviolet-irradiated BALB/c + γ-irradiated CBA/H; ■, ultraviolet-irradiated C57BL/6J + γ-irradiated CBA/H. Cell populations were assayed against 2×10^6 P815 mastocytoma target cells.

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[1] Lafferty, K. J., Walker, K. Z., Scollay, R., and Killby, V. A. A., *Transplant Rev.*, **13**, 198 (1972).
Lafferty, K. J., and Jones, M. A. S., *Aust. J. exp. Biol. med. Sci.*, **47**, 17 (1969).
[3] Schellekens P. Th., and Eijsvoogel, V. P., *Clin. exp. Immun.*, **7**, 229 (1970).
[4] Lindahl-Kiessling, K., and Safwenberg, J., *Proc. 6 Leucocyte Culture Conf.* (edit. by Schwartz, M. R.), 623-638 (Academic Press, New York, London, 1972).
[5] Lafferty, K. J., Ryan, M., and Misko, I. S., *J. immun. Meth.* (in the press).
[6] Brunner, K. T., Mauel, J., Cerottini, J. C., and Chapuis, B., *Immunology*, **14**, 181 (1968).
[7] Henney, C. S., *J. Immun.*, **107**, 1558 (1971).
[8] Wagner, H., Harris, A. W., and Feldman, M., *Cell. Immun.*, **4**, 39 (1972).
[9] Scollay, R., Lafferty, K. J., and Poskitt, D. C., *Transplantation* (in the press).

Cannabinoid content of some English reefers

THIS paper reports the results of qualitative and quantitative analysis of 36 reefers or 'pro-reefers' (samples of herbal or resin cannabis sufficient for one reefer) produced in London and Leeds. As far as we know it is the first time reefers in actual use have been so analysed. The reefers were obtained from three different groups: group A, regular smokers in the London area, some of whom had asked for psychiatric help; group B, regular users in Leeds, none of whom had sought medical advice about their cannabis smoking; group C, casual users in the London area. The results show a very wide variation in potency and indicate that more than half were below the threshold dose.

The contents of each reefer were weighed, examined macroscopically and microscopically and analysed[1] quantitatively for the main cannabinoid THC (Δ^1-tetrahydrocannabinol), CBN (cannabinol) and CBD (cannabidiol) (Table 1). Almost all the reefers contained tobacco mixed with varying proportions of resin or herbal cannabis which, when possible, was separated and weighed.

We found a very high variation in potency, the content of the psychoactive substance THC varying from 0.15 mg to 41.1 mg.

Allogeneic stimulation modulates the *in vitro* response of T cells to transplantation antigen

THE theory of allogeneic stimulation[1] postulates that transplantation antigens, such as H-2 in the mouse or H-LA in man, are not strong immunogens for allogeneic animals, but are rendered highly immunogenic when presented to the immune system in conjunction with an allogeneic stimulus. The capacity to provide an allogeneic stimulus is the property of metabolically active[2-4] immunocompetent cells[1], and it has been suggested that allogeneic stimulation requires the transfer of some cytoplasmic component from the stimulating to the responsive cell[1]. According to this hypothesis, any treatment that inactivates the metabolic activity of lymphoid cells will reduce their immunogenicity for other members of the same species.

A prediction of this hypothesis is that transplantation antigen alone, either soluble or cell bound, will be poorly immunogenic when presented to potentially responsive immunocompetent cells. If an allogeneic stimulus is provided at the same time, however, this second stimulus will activate the potentially reactive cell and facilitate its response to the test antigen. Here we show that lymphocytes inactivated with ultraviolet light are unable to stimulate allogeneic lymphocytes in mixed leukocyte culture (MLC), and do not generate a significant cell-mediated cytotoxic response in such cultures. Cytotoxic activity can, however, be generated if an allogeneic stimulus is provided by a third party, γ-irradiated lymphocyte that is antigenically different from the ultraviolet-irradiated cell.

MLC reactivity was assayed by mixing responsive lymph node cells (C57BL/6J) with γ- or ultraviolet-irradiated stimulating spleen cells (BALB/c) in Linbro multi-dish culture trays Linbro FB 24-TC). The culture medium used was Eagle's Minimum Essential Medium (Grand Island Co., F15) containing penicillin (100 μg ml^{-1}), streptomycin (100 μg ml^{-1}) and neomycin (100 μg ml^{-1}). The medium was supplemented with 10% foetal calf serum and 2-mercaptoethanol to a final concentration of 10^{-4} M. Stimulating spleen cells, at a density of 1×10^7 ml^{-1}, were incubated in culture medium for 1.5 h at 37°C before irradiation[5]. ^{60}Co γ-irradiation (800 r) was given at a dose rate of 280 r min^{-1} and ultraviolet irradiation was given by exposing 5 ml of cell suspension in open 80 mm Petri dishes for 4 min, at a distance of 20 cm from a 30 W germicidal ultraviolet lamp. Following irradiation, the cells were washed once and resuspended in culture medium.

To control the specificity of MLC activation, and any nonspecific effects resulting from the addition of ultraviolet-irradiated cells to the test cultures, the following spleen cell mixtures were used as the stimulating population in MLC: γ-irradiated BALB/c + ultraviolet-irradiated C57BL/6J; ultraviolet-irradiated BALB/c + γ-irradiated C57BL/6J; and ultraviolet-irradiated C57BL/6J + γ-irradiated C57BL/6J. These stimulating cell populations were prepared by mixing equal volumes of each component at a cell density of 2×10^6 ml^{-1}. In MLC assays the stimulating spleen cell population was mixed with an equal volume of C57BL/6J lymph node cells at a density of 1×10^6 ml^{-1}. Each mixture (2.5 ml) was added to three replicate wells of Linbro culture trays and cultured for the required time at 37°C in an atmosphere of 10% CO_2, 7% O_2 and 83% N_2. Aliquots (0.2 ml) were taken from each culture over a 5 d period and added to Linbro 16 mm microtitre plates with 3 μCi of ^3H-thymidine (Amersham, TRA 120, specific activity, 5 Ci $mmol^{-1}$). Thymidine uptake was estimated 5 h later by collecting the cultured cells on glass fibre filter disks, using an automatic cell collecting device (MASH-11, Microbiological Associates), and counting the amount of thymidine incorporation in a liquid scintillation counter.

The kinetics of thymidine uptake are shown in Fig. 1. Ultraviolet irradiation of BALB/c spleen cells, in contrast to γ-irradiation, inactivates their capacity to provide an allogeneic stimulus for C57BL/6J lymph node cells as measured by MLC activation. These findings confirm those of Lindahl-Kiessling *et al.*[4]. Moreover, ultraviolet irradiation did not affect the expression of alloantigen on the surface of irradiated cells (Table 1), as determined by antibody absorption. Antigenic activity was estimated by microcytotoxicity titration before and after absorption of alloantiserum with γ- or ultraviolet-irradiated BALB/c spleen cells.

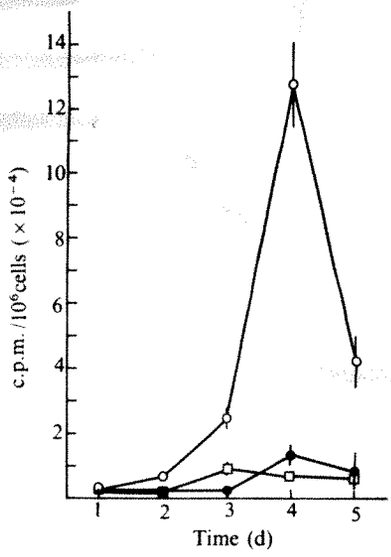


Fig. 1 Stimulation of ^3H-thymidine uptake by C57Bl/6J lymph node cells in mixed cultures containing the following spleen cell populations: ○, γ-irradiated BALB/c + ultraviolet-irradiated C57BL/6J; ●, ultraviolet-irradiated BALB/c + γ-irradiated C57BL/6J; □, γ-irradiated C57BL/6J + ultraviolet-irradiated C57BL/6J. ^3H-thymidine incorporation is expressed as c.p.m./10^6 lymph node cells and the vertical bars show the standard error of the mean of three replicate assays.

The *in vitro* generation of cytotoxic lymphocytes was carried out in Falcon plastic tissue culture flasks (30 ml), using the MLC medium described above. Ultraviolet-irradiated BALB/c ($H\text{-}2^d$) spleen cells, either alone or mixed with γ-irradiated CBA/H ($H\text{-}2^k$) spleen cells, were used to stimulate C57BL/6J ($H\text{-}2^b$) lymph node cells *in vitro*. After 4 d, the cultures were examined microscopically and the blast cell content scored semiquantitatively. These cultures were then collected and

Table 1 Antibody binding capacity* of γ- and ultraviolet-irradiated BALB/c spleen cells

Absorbing spleen cell population (10^7 cells)	Cytotoxic titre
γ-irradiated BALB/c	1 : 10
Ultraviolet-irradiated BALB/c	1 : 10
γ-irradiated C57BL/6J	1 : 160
Ultraviolet-irradiated C57BL/6J	1 : 160

* Cytotoxic anti-BALB/c antibody was prepared in C57BL/6J mice. 0.5 ml of antiserum was absorbed with the different cell populations for 2 h at room temperature. Residual cytotoxic activity was assayed against BALB/c lymph node cells, in the presence of guinea pig complement. Antibody titrations were carried out with doubling dilutions of serum and the 50% cytotoxicity end point was estimated by trypan blue exclusion.

cytotoxic cells specific for the $H\text{-}2^d$ antigen assayed against ^{51}Cr-labelled P815 mastocytoma ($H\text{-}2^d$) target cells[6]. Table 2 shows the detailed experimental protocol, including controls for specificity and any nonspecific effects of ultraviolet-irradiated cells, and the relative potencies of the resultant cytotoxic cell populations. Cytotoxic potency was obtained by plotting the logarithm of the number of target cells lysed against the logarithm of the number of sensitised cells used in the assay (Fig. 2). Such plots have a linear dose response relationship with unit slope to a level of approximately 35% specific lysis[7].

As this variation has been found in only 36 samples, it is almost certain that similar or greater variation occurs regularly. In view of the known variability in the THC content of the plant (*Cannabis sativa L.*) from which the drug is obtained, the instability of the active constituents, especially in badly prepared and stored material and the 'unstandardised' conditions in which the drug is distributed the variation is perhaps not surprising. The lack of standardisation means that a casual smoker, used to low doses, may be accidentally exposed to highly potent material.

On average each reefer in groups A and C was smoked by two people (Table 2) so that the mean dose of THC per person in group A would be 4 mg (range 0.1 to 14 mg); and in group C would be 2.2 mg (0.5–5.0 mg). For group B, with an average of three people per reefer, the dose would be 3.5 mg (0.2–13 mg). These doses are lower than those used by such workers as Neumeyer and Shagoury[2] (2–9 mg) and Isbell *et al.*[3], who quote 3.5 mg as a threshold dose and 16.2 mg sufficient to produce distinct depersonalisation. On this basis about two-thirds of the reefers, when shared, would produce effects less than that of the threshold value of 3.5 mg THC. Casual smokers may therefore be exposed to extremely small doses and so may falsely assume that cannabis is a relatively harmless substance. Conclusions based on questionnaires to smokers[4], such as students, are probably of little value unless adequate information on the potency of the reefer is also obtained.

Table 1 Components and analytical data on reefers

Reefer No.	Contents (apart from tobacco*)	Weight including tobacco (g)	Cannabinoids (mg) THC	CBN	CBD
Group A					
1	Soft resin: (0.214 g)	0.744	28.39	traces	16.68
2	Soft resin	0.752	24.98	traces	14.65
3	†Leaf only: sessile glands (0.511 g)		11.13	traces	traces
4	†Coarse resin (0.118 g)		8.21	3.10	2.74
5†	Compact smooth resin (0.219 g)		5.99	2.73	6.60
6	Resin: fine powder	0.778	5.06	0.89	1.76
7	†Crumbly resin (0.224 g)		3.72	1.23	9.36
8	Greenish brown resin (0.102 g)	0.996	3.34	0.55	3.01
9	†Compact resins (0.327 g)		2.70	12.14	30.76
10	†Brown prism (0.278 g)		2.06	5.38	9.51
11	†Compressed herb (0.285 g)		1.97	0.36	2.85
12	†Leaf only: sessile glands (0.097 g)		1.89	traces	traces
13	Herbal: stalks and seeds	1.135	0.52	4.35	0.64
14	Herbal: tops and seeds	0.460	0.15	0.74	0.11
Group B					
15	Leaf only, numerous sessile glands	1.724	41.11	traces	traces
16	Herb: bracts, leaf, seeds	1.730	18.05	4.89	traces
17	Herb: stalk, leaf, seeds	1.309	17.56	4.50	traces
18	Leaf only: sessile glands	1.166	12.92	traces	traces
19	Herb: leaf mainly	1.096	11.85	traces	traces
20	Herb: stalks, leaf, seeds	0.698	9.56	1.89	—
21	Leaf: flowering tops, seeds	1.202	7.31	1.74	—
22	Herb: leaf with sessile glands	0.887	7.11	0.83	4.25
23	†Herb: seeds and leaf (0.317 g)		4.48	1.09	—
24	†Herb: bracts, seeds (0.440 g)		3.65	1.05	—
25	Resin: greenish brown	0.941	2.52	0.52	0.75
26	†Leaf and seeds (0.182 g)		1.38	0.16	0.35
27	Herb: bracts, seeds	0.842	0.38	0.16	0.32
28	† Fragments of green glass embedded in vegetable debris	0.208	0	0	0
Group C					
29	Resin: fine powder	1.046	9.37	0.99	5.99
30	Herbal: bracts and leaves	0.910	8.38	0.86	1.36
31	Resin: small lumps	0.553	5.59	0.67	3.64
32	Herbal: unripe floral axis, immature seeds	0.383	5.02	0.71	traces
33	Herbal: flowering tops, no seeds (0.224 g)		3.00	0.33	1.25
34	Herbal: with traces of resin	0.808	2.02	traces	traces
35	Resin: small lumps	0.637	1.38	0.36	2.82
36	Resin: greenish lumps	0.516	0.99	0.26	2.03

* All reefers contained tobacco except no. 15.
† Pro-reefers, consisting of resin or herbal cannabis sufficient for one reefer.

Table 2 Mean daily use of reefers

Reefer No.	Frequency of use (d^{-1})	No. of users	THC consumed (mg per person per d)
Group A			
1	7	1	199
2	7	1	175
3	5 to 10	1	56 to 111
6	20 to 30	2	51 to 76
7	30	1 to 2	4 to 11
8	10 to 30	2	17 to 50
9	9	1	24
13	20	2	5
14	20	2	1.5
Group B			
15	1	1	41
16	3	2	27
17	10	2 to 5	35 to 88
18	2.5	2	16
19	8	4	24
20	6	2	26
21	4	1 to 2	15 to 29
22	20	4	36
23	11	2	25
24	3	1	11
25	20 to 40	2	25 to 50
26	15 to 18	5 to 6	3 to 5
27	15 to 25	3 to 5	1 to 3
Group C			
29	4	3 2	19
30	1*	1	1
31	4	1	22
32	3	1	15
33	2	1	6
34	2*	1	0.6
35	2*	3	0.1
36	2*	3	0.1

* (per week)

All these doses refer to the actual amounts of THC in the reefers; obviously the amount reaching the blood stream will be affected by the manner in which the reefer is made and, more importantly, whether the user inhales and if he does how long he holds the smoke before expelling it[5].

A more important variable is the actual number of reefers smoked by an individual per week. By careful questioning group A was found to consume 3.8 g cannabis per person per d (range 2 g to 6 g); group C 0.3 g (range 0.1 g to 1 g). For group B drug histories were collected using the techniques of participant observation[6] and actual weighing of the amounts used. The average amount was 2.8 g per person per d (range 0.3 g to 8.3 g). As the reefers (Table 1) were collected at the time this information was obtained, it is possible to calculate the daily intake of THC on the assumption that the reefer analysed represented those being smoked at that time. The results (Table 2) show that for group A the average daily dose of THC is 60 mg per person, for group B 26 mg per person and for group C 8 mg. If the first two extremely high values of group A are removed the average for the remainder is 37 mg per person. Although these first two values are high they are not dissimilar from the values of 150 mg THC d^{-1} quoted by Miras for regular users in Greece[7]. Some users evidently compensate for low potency reefers by smoking 10–20 reefers d^{-1} and in these circumstances the reported carcinogenic effects[8,9] may become significant.

Observation of some individuals, recorded before the analytical results were known, confirms a dose-response relationship. For reefer 15 (41 mg THC per dose) the user admitted he could not smoke more than 1 d^{-1}, otherwise he was unable to co-ordinate his movements. Reefer 22 (36 mg THC d) was from two females whose most characteristic feature was a persistent hilarity; neither was in full time employment. The suppliers of reefers 3 and 8 (17 to 111 mg) were all referred for mild cannabis psychoses, and were depressed and paranoid when smoking heavily. These symptoms disappeared within

2 months of giving up the drug. Reefer 27 (1–3 mg THC d) was from two small subgroups; none of their members exhibited noticeable disorganisation of the cognitive processes and all seemed entirely capable of holding full time employment. They seemed similar to the members of group C (casual smokers) who were in full time study or employment. It is significant that students reduced their intake or gave up the drug altogether before examinations. Subjects who used reefer 28 (glass fragments plus incense) made it from what they had bought as Turkish pollen hash at £18 per ounce. Although they obviously expected a good high they experienced no subjective euphoria; most reported serious headaches instead.

We found no evidence of adulteration except in reefer 28. This indicates that there is currently no shortage of genuine cannabis. Since THC decomposes into the inactive CBN due to faulty preparation and prolonged storage, the relatively high proportion of CBN in the group A reefers compared with group C indicates significant breakdown. This may come as a surprise to group A as they claimed to be nearer the sources of supply of cannabis, and therefore assumed they were using fairly fresh and active material.

Reefer 3 was grown out of doors in Brixton, London, and collected in July. Reefer 12 was grown in Dublin from Nigerian seeds and "harvested too early". Reefer 15 was from seeds taken from Zambian 'bush' and grown on a railway embankment near Leeds; the plants were harvested in September. None of the samples contained the relatively potent flowering tops, yet they had quite high THC content (21.8, 19.8 and 23.9 mg g^{-1} air-dried leaf respectively). These results should therefore dispose of the idea that potent material cannot be grown in a cool climate on unobtrusive sites sometimes with poor lighting conditions. We hope to publish soon details of work on plants grown in this country over several years, which confirm this conclusion. We have already pointed out[10] the defect in the present international definition of cannabis as "the flowering or fruiting tops of the cannabis plant (excluding the seeds and leaves when not accompanied by the tops)"[11].

Some of the reefers used by experienced smokers were low in THC content but nevertheless were claimed by the users to be satisfactory. One possible explanation is the higher average CBD figures for group A (7.0 mg) than for group C (2.1 mg). CBD is said to enhance the effect of THC[12]. Furthermore, since this work began, evidence has accumulated that the GLC peak corresponding to CBD sometimes includes small amounts of other cannabinoids[13]. Synergism cannot be ruled out, and another possible explanation is the presence of noncannabinoid active material in cannabis.

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[1] Fairbairn, J. W., and Liebmann, J. A., *J. Pharm. Pharmac.*, **23**, 150–155 (1973).
[2] Neumeyer, J. L., and Shagoury, R. A., *J. pharm. Sci.*, **60**, 1433–1457 (1971).
[3] Isbell, H., Gorodetzky, C. W., Jasinski, D., Claussen, U., Spulak, F. von G., and Korte, F., *Psychopharmacologia*, **11**, 184–188 (1967).
[4] Goode, E., *Nature*, **234**, 225–227 (1971).
[5] Agurell, S., and Leander, K., *Acta pharm. suecica*, **8**, 391–402 (1971).
[6] Hindmarch, I., *Drugs and Society*, **1**, 19–24 (1971).
[7] Miras, C. J., in *Cannabis and its derivatives* (edit. by Paton, W. D. M., and Crown, J.), 150 (Oxford University Press, London, 1972).
[8] Magus, R. D., and Harris, L. S., *Fedn. Proc.*, **30**, 279 (1971).
[9] Leuchtenberger, C., Leuchtenberger, R., and Schneider, A., *Nature*, **241**, 137–139 (1973).
[10] Fairbairn, J. W., Liebmann, J. A., and Simic, S., *J. Pharm. Pharmac.*, **23**, 558–559 (1971).
[11] *Single Convention on Narcotic Drugs*, Article 1 (United Nations, New York, 1961).
[12] Jones, G., and Pertwee, R. G., *Br. J. Pharmac.*, **45**, 375–7 (1972).
[13] Turner, C. E., and Hadley, K., *J. pharm. Sci.*, **62**, 251–255 (1973).

Evolutionary divergences between populations of Australian wild rabbits

The first successful introduction of the European wild rabbit, *Oryctolagus cuniculus*, into Australia took place in 1859 when two dozen animals were imported into Geelong, Victoria[1]. Subsequent minor introductions may have occurred but the evidence indicates that existing populations are derived mainly from the Geelong colony[2]. Rabbit populations now occupy varied environments across the southern two-thirds of Australia[3]. These rabbits provide a good opportunity for the study of evolutionary divergences.

Twenty-six measurements were taken on the skulls of adult rabbits collected from five localities in Australia (Fig. 1). The numbers in each sample were: Tero Creek, 22; Manilla, 12; Tidbinbilla, 20; Holt Rock, 20; Wanneroo, 14. The environments of the localities are diverse. This diversity is reflected in the variations in mean annual rainfalls of the

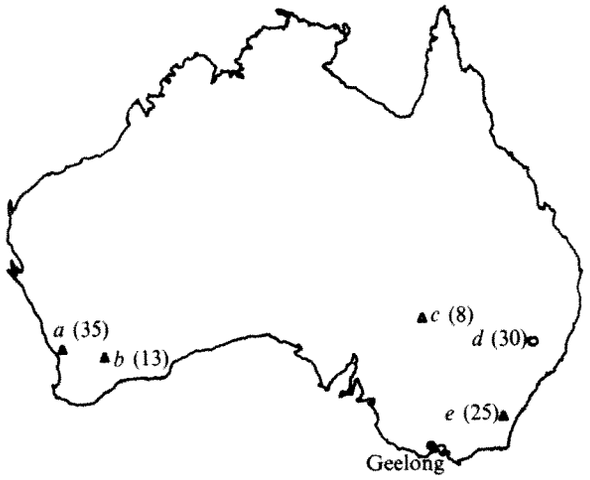


Fig. 1 Locations of collection sites. △ Site of an extant collection; ○, site of the early Manilla sample. The localities are, *a*, Wanneroo, WA; *b*, Holt Rock, WA; *c*, Tero Creek, NSW; *d*, Manilla, NSW; *e*, Tidbinbilla, ACT. The figures in parentheses indicate the approximate mean annual rainfalls (in inches) of the collection sites.

collection sites (Fig. 1). The Manilla sample was collected in 1918 (Australian Museum) and the Tero Creek sample in 1967 (CSIRO). The remainder were captured in 1972 and 1973 using ferrets, traps, spotlights and shooting. We regard all but the Manilla sample as extant.

The metrical parameters consisted largely of standard length, breadth and height measurements of the cranium, mandible and teeth. All parameters were tested for sexual dimorphism using *t* tests but only bizygomatic breadth showed significant sexual dimorphism. This measurement was eliminated from the remaining analyses. Univariate comparisons demonstrated a number of significant differences between all pairs of samples. The number of signifi-

cant t tests (0.05 level) varied from a low of 4 for the Wanneroo-Holt Rock comparison to 16 for the Wanneroo-Tero Creek and Wanneroo-Tidbinbilla comparisons. Over the 10 sample pair comparisons, the mean number of significant t tests was 10 out of 25. The parameters that showed significant differences varied considerably from one pair to the next so that most parameters showed significant heterogeneity in at least one sample pair comparison. Coefficients of variation for all characters in each of the regional samples were calculated. These coefficients generally were low[4], with a mean of 3.8 and range of 1.2–9.6. A preliminary analysis of the same data using Penrose's[5] distance statistics indicates a strong correlation between the geographic and biological distances between sample pairs. This finding is being investigated further.

The appearance of morphological heterogeneity between Australian rabbit populations in such a short time (110 yr) implies a fast rate of evolution. Skulls collected during the early stages of colonisation were not available, but some approximate evolutionary rates were calculated using the two most separated extant samples, Wanneroo and Tidbinbilla. The midpoint between the means of these two samples was taken as the ancestral value of each measurement. Our method requires the working assumption that the extant groups have diverged at the same rate from the hypothetical ancestral population.

Evolutionary rates for all measurements were calculated using Haldane's equation[6], rate $= (\log_e X - \log_e Y)/t$ where $\log_e$ is the natural logarithm, X the mean value of descendants; Y the ancestral mean and t the elapsed time (set at 110 yr). Haldane suggests that the results be expressed in darwins, where one darwin is a change of 1/1,000th part in 1,000 yr. Over all parameters a mean rate of 116 darwins was obtained, with a range of 27–406 darwins. This mean rate of evolution is 5,000–10,000 times the estimated rate in teeth of horses over a period of 5–16 million years (ref. 6). The mean for the rabbit populations however is similar to the rates found for changes in the dental dimensions in a population of the African green monkey (*Cercopithecus aethiops sabaeus*) isolated for 300 yr on the West Indies island of St Kitts[7]. In this case the mean rate was 160 darwins with a range of 10–470 darwins[7]. These rapid rates of evolution would not be expected to continue indefinitely but to slow down as populations approached a genetic equilibrium.

Our results indicate that substantial morphological divergences have developed rapidly between Australian rabbit populations.

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1 Thomson, H. V., and Worden, A. N., in *The Rabbit* (Collins, London, 1956).
2 Rolls, E. C., in *They All ran Wild* (Angus and Robertson, Sydney, 1969).
3 Myers, K., *Proc., Adv. Study Inst. Dynamics Numbers Popul.*, (Oosterbeek), 478 (1970).
4 Simpson, G. G., Roe, A., and Lewontin, C. R., in *Quantitative Zoology* (revised edition, Harcourt, Brace and Co., NY 1960).
5 Penrose, L. S., *Ann. Eugen.*, **18**, 337 (1954).
6 Haldane, J. B. S., *Evolution*, **3**, 51 (1949).
7 Ashton, E. H., and Zuckerman, S., *Proc. R. Soc.*, **B138**, 354 (1951).

Claim that two human linkage groups carry different loci for GPT and LDH withdrawn

CHEN and Giblett[1] have reported the occurrence of genetic polymorphism for human red cell glutamic pyruvic transaminase (GPT; EC 2.6.1.2). The usual phenotypes of red cell GPT (that is, GPT 1, 2-1 and 2) could be identified on cellogel by adapting the starch gel electrophoretic method of Chen and Giblett to cellogel (Fraser *et al.*, unpublished). When a buffer system which separates the human and Chinese hamster forms is used, however, the isozyme patterns formed by fibroblasts were found to be distinctly different from those by red cells. We have employed the fibroblast isozymes as markers in human gene linkage studies using man–Chinese hamster somatic cell hybrids and have reported that the enzyme with GPT activity is probably a tetramer determined by two genes named *GPT B* and *GPT C* located on different chromosomes and linked to *LDH B* and *LDH A* respectively[2].

In a later series of experiments involving further characterisation of the GPT isozymes in the hybrids and their parental cells, we have found strong evidence that the screening procedures used do not score different enzymes (GPT and LDH) but the same gene product (LDH) by different methods.

The principle of staining for GPT is that the enzyme converts alanine and α-ketoglutarate to glutamic acid and pyruvate, and the pyruvate thus formed is detected by coupling it to LDH when NADH is oxidised to NAD[3]. The sites of enzyme activity are seen under long wave ultraviolet light as quenched areas against a fluorescent background. If the appearance of bands was caused by the action of GPT isozymes, an omission of any of the substrates in the reaction mixture should prevent the formation of bands.

It turned out that an omission of alanine alone or alanine and LDH in the reaction mixture did not prevent the appearance of any of the bands seen after staining with the complete GPT reaction mixture. Moreover, a substitution of α-ketoglutarate by pyruvate in the staining mixture resulted in the same electrophoretic pattern. With pyruvate as substrate the bands appeared instantaneously with increased intensity, whereas with α-ketoglutarate the gels had to be incubated at 37°C for at least 30 min for the patterns to become visible.

These observations show that the human and Chinese hamster lactate dehydrogenase isoenzymes are capable of utilising α-ketoglutarate as a substrate analogue in the conditions used and that these conditions are obviously not optimal for the GPT of the species concerned. Therefore, the patterns observed earlier by us[2] on staining for glutamic pyruvic transaminase in the cultured fibroblasts, white blood cells and the hybrids are probably due to lactate dehydrogenase activity, indicating that the GPT-LDH linkages were spurious.

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[1] Chen, S. H., and Giblett, E. R., *Science*, **173**, 148 (1971).
[2] Someren, H. van., Meera Khan, P., Westerveld, A., and Bootsma, D., *Nature new Biol.*, **240**, 221 (1972).

Connective stability of complex ecosystems

In 1970, Garner and Ashby[1] asked an important question: "If a large system is assembled (connected) at random, or has grown haphazardly, should we expect it to be stable or unstable?" They showed on a linear model that probability of stability generally decreases with the increase of the degree of connectedness among the parts of the system. This fact was confirmed by May[2] in a study of large systems in the ecological context.

Here I now report that a wide class of linear and non-linear time-varying large systems have been identified[3,4], which are stable in spite of changes in the degree of connectedness among the parts (subsystems) of the system. A large system is considered connectively stable if it is stable (in the sense of Liapunov) for any degree of connectedness among the subsystems. The connective property of stability can be established in the systems with competitive equilibrium[5], which appear as useful models in such diverse fields as biology[6] and the arms race[7], economics[5,8] and transistor circuits[9].

The development of the connective stability concept can be best illustrated on the linear model used in (refs 1 and 2):

$$\frac{d\mathbf{x}}{dt}=A\mathbf{x}$$

where $\mathbf{x}=<x_1\ x_2\ \ldots\ x_n>$ is an n vector and $A=(a_{ij})$ is an $n\times n$ constant matrix. I assume that A has negative diagonal elements[1], and non-negative off-diagonal elements, that is

$$a_{ij}\ \begin{cases}<0\ , & i=j\\ \geqslant 0\ , & i\neq j\end{cases}$$

The above system is stable if (and only if) the matrix A satisfies the Hicks conditions:

$$a_{11}<0,\ \begin{vmatrix}a_{11} & a_{12}\\ a_{21} & a_{22}\end{vmatrix}>0,\ \ldots,\ (-1)^n\begin{vmatrix}a_{11} & a_{12} & \ldots & a_{1n}\\ a_{21} & a_{22} & \ldots & a_{2n}\\ \ldots & \ldots & \ldots & \ldots\\ a_{n1} & a_{n2} & \ldots & a_{nn}\end{vmatrix}>0$$

I allow off-diagonal elements a_{ij} to be time-varying, and replace them with $e_{ij}a_{ij}$, where $e_{ij}=e_{ij}(t)$ are arbitrary functions such that

$$0\leqslant e_{ij}(t)\leqslant 1$$

Therefore, the elements $e_{ij}(t)$ of the $n\times n$ interconnection matrix $E=(e_{ij})$ measure the coupling from x_i to x_j and represent disconnection when $e_{ij}(t)=0$ at any time t. It has been shown[4,8] that, under Hicks conditions, stability of the system is invariant to changes in any (and all) $e_{ij}(t)$ and that the system is connectively stable.

In an ecological system, $\mathbf{x}$ is the vector of the populations x_i and the elements a_{ij} of the matrix A represent the effect of the jth species on the ith species in the neighbourhood of the equilibrium. Non-negativity of the off-diagonal elements $a_{ij}(i\neq j)$ implies (gross) symbiotic interactions among species. Negativity of the diagonal a_{ii}s means that each species is density dependent or 'stabilized'[2]. Both restrictions on the sign of a_{ij}s can be removed at the expense of more refined analysis. By applying the Hicks conditions to the McKenzie's diagonal form of A, the off-diagonal a_{ij}s can have arbitrary signs and connective stability of the ecosystem with mixed (competitive – predator – symbiotic – saprophytic) interactions among species can still be established. The restriction $a_{ii}<0$ can be removed by considering species in the stable 'blocks' (subsystems) and using the decomposition–aggregation methods[3,4,8] to conclude stability of the overall system by Hicks conditions. The decomposition–aggregation analysis can take advantage of special structural 'block' properties of ecosystems[2]. Furthermore, it is possible to demonstrate[8] wide tolerances to nonlinearities in the interactions of stable ecosystems reflected by non-linear elements $a_{ij}=a_{ij}(t,\ \mathbf{x})$ of the system matrix $A=A(t,\ \mathbf{x})$.

By increasing the magnitude of the off-diagonal elements a_{ij} and thus the interaction complexity of the ecosystem, the Hicks conditions are eventually violated. Since the conditions are both necessary and sufficient for stability, by reaching the critical values of a_{ij}s as determined by the Hicks inequalities, a sharp transition from stability to instability takes place. Therefore, there is an analytical basis[3,4,8] for verification of the important Garner–Ashby conjecture[1] and the equally significant May's experiment[2].

The recursive nature of the Hicks conditions suggests that individual parts of the system can be decomposed from each other and again composed together randomly in various ways without affecting stability. It should be noted, however, that Hicks conditions are valid only for the constant matrix A. Kamke's comparison principle and vector Liapunov functions from the theory of differential inequalities[10] are needed to extend the validity of Hicks conditions to matrices with time varying elements and to give a rigorous proof[4,8] of this intuitive result.

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[1] Garner, M. R., and Ashby, W. R., *Nature*, **228**, 784 (1970).
[2] May, R. M., *Nature*, **238**, 413-414 (1972).
[3] Šiljak, D. D., *IEEE Trans.*, SMC-2, 657-663 (1972).
[4] Šiljak, D. D., *IEEE Trans.*, SMC-3, 415-417 (1973).
[5] Arrow, K. J., and Hahn, F. H., *General Competitive Analysis* (Holden-Day, San Francisco, 1971).
[6] Keyfitz, N., *Introduction to Mathematics of Population*, (Addison-Wesley, Reading, Massachusetts., 1968).
[7] Richardson, L. F., *Arms and Insecurity*, (Boxwood Press, Pittsburgh, 1960).
[8] Šiljak, D. D., *Proc. 1973 IEEE Conf. Decision and Control, San Diego*, 265–275 (New York, 1973).
[9] Sandberg, I. W., *Bell Syst. Tech. J.*, **48**, 35–54 (1969).
[10] Lakshimkantham, V., and Leela, S. *Differential and Integral Inequalities*, I, (Academic Press, New York, 1969).

Palatability dynamics of cardenolides in the monarch butterfly

The adaptive strategy of sequestering cardiac glycosides from milkweed plants (Asclepiadaceae and Apocynaceae) has evolved in several taxa of insects[1]. Since these cardenolides elicit vomiting following ingestion, birds learn to avoid the insects on sight after one or more emetic experiences[2]. By a specific assay, we found a spectrum of cardenolide concentrations in adult monarch butterflies (*Danaus plexippus* L., Danainae) collected during the autumnal migration from four areas in eastern North America[3]. In this communication we compare Atlantic with Pacific Coast monarch populations and explore quantitative relationships between cardenolide concentrations and palatability spectra. The latter were measured by our blue jay (*Cyanocitta cristata bromia* Oberholser, Corvidae) emetic dose fifty (ED_{50}) test[4].

Migrating East Coast butterflies were netted in September 1971 in the floodplain of the Connecticut River in Northampton and Hockanum, Massachusetts. Those from the West Coast came from three overwintering colonies in California during November 1971 at Muir Beach, Natural Bridges State Park at Santa Cruz, and at the Academy of Music at Santa Barbara. All butterflies were frozen until oven dried and individually ground for analysis as before[3]. Cardenolide concentrations were measured from February to June 1972. Because there were no significant concentration differences between the California populations, they were combined for statistical analyses.

The spectrophotometric assay[3] was modified as follows: (*a*) a correction in the alignment of the visible light source of the Perkin-Elmer 402 spectrophotometer was made; (*b*) absorbances were read at the peak (626 nm); (*c*) a tendency for opalescence[5] was eliminated by changing the aqueous NaOH solution to 50% EtOH : 50% H_2O. These modifications resulted, respectively, in corrections of 1.08, 1.18 and 1.11, to give a combined correction factor of 1.42. As previously, the *F* peak (536 nm) of a holmium oxide filter was used to monitor machine performance ($n=83$; $\bar{X}$ absorbance $=0.377$; s.d. $=0.003$).

ED_{50} tests were done between March 1972 and February 1973 on blue jays trapped in Hampshire County, Massachusetts between December 1971 and February 1973. They were stored for various times in an outside aviary or inside in a 12 h light: 12 h dark regime. Birds given Massachusetts butterfly material were force fed once. Thirty-three birds given the California 0.250–0.279 and 0.320–0.399 subsample material (Table 2) had been force fed once, at least 5 weeks previously. The mean weight of the 226 birds was 84.25 g ($s^2=41.83$).

After determining cardenolide concentrations in 351 California and 355 Massachusetts butterflies (Table 1, Fig. 1), we combined the residual dry powders of both sexes into nine concentration range subsamples for California and eleven subsamples for Massachusetts (Table 2); as all residual powders were used, larger butterflies contributed more than smaller ones. After mixing, we measured the absorbance per 0.1 g of each subsample.

We then determined the ED_{50} values for most subsamples (Table 2). The lower concentration groups for both coasts either were not emetic or were subemetic so that these ED_{50} values could not be determined. The ED_{50} and subsample absorbance values were run in regression analyses by the method of least squares[7] to fit the exponential equation $y=ax^c$, giving high correlation coefficients (Table 3*D*). Because of the exponential relationship in both samples, the mean values and 95% confidence limits are plotted logarithmically together with regression lines (Fig. 2).

Concentrations are summarised in Tables 1 and 2 and in Fig. 1, which also shows the distribution of total cardiac glycoside per butterfly, obtained as the product of individual absorbance × dry weight. The histograms for the Massachusetts butterflies confirm the earlier study[3] and are essentially normal distributions showing continuous variation in cardenolide content. More than 98% of the Massachusetts monarchs contained substantial amounts of cardenolide. The population of California monarchs, however, was effectively dimorphic, with 47% of the individuals containing little to immeasurable amounts.

As Fig. 1 and Table 1*A* show, California butterflies had a much lower mean cardenolide content than the Massachusetts butterflies, even when values were recalculated after excluding all butterflies with absorbances of less than 0.100 per 0.1 g (Table 1*B*). With this group excluded, the variance of the cardenolide content in both sexes was three to four times greater in the Massachusetts butterflies. In both samples females had higher concentrations than males. The two-way analyses of variance[8] indicate that both geographic and sexual differences in concentration were highly significant.

The size (right forewing length, measured with calipers from base to apex) and dry weight of the butterflies were highly correlated (Table 3*B*)[9]. The males were heavier as well as larger than the females on both coasts (Table 1*C* and *D*). California butterflies, however, although significantly smaller than their Massachusetts counterparts, were substantially heavier, no doubt because of a higher fat content (observed during grinding). Moreover, California butterflies showed greater discrepancy between the sexes for both weight and size (interactions). The differences in cardenolide content between the sexes, exaggerated in the California butterflies (Table 1*A* and *B*), support the idea[3] that there is a significant physiological

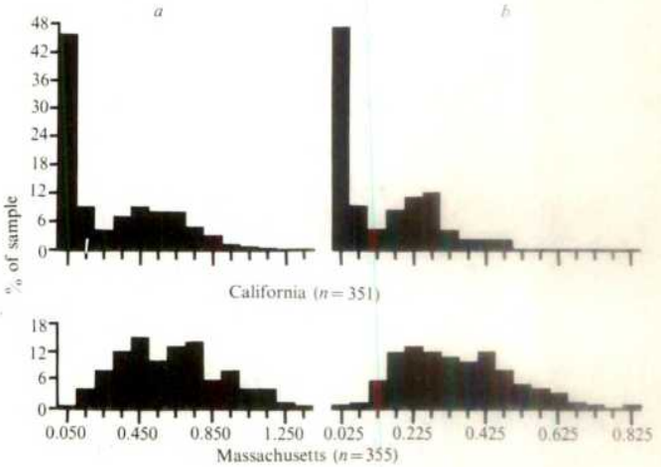


Fig. 1 Variation in cardiac gycoside content of monarch butterflies from California and Massachusetts (data are in Table 1). *a*, Total cardiac glycoside (absorbance per butterfly); *b*, cardiac glycoside concentration (absorbance per 0.1 g butterfly). The West Coast monarchs are bimodal, with nearly half containing little to immeasurable amounts of cardiac glycosides. The East Coast monarchs are unimodal and have a substantially higher mean cardenolide content. An extract of 0.1 g of butterfly in 5 ml of alcohol which gives an absorbance of 0.200 by our standard method is equivalent to a 3.16×10^{-5} M solution of digitoxin, or 241 μg per 0.2 g butterfly.

Table 1 Summary of cardenolide concentrations (absorbance per 0.1 g powdered butterfly), dry weights and right forewing lengths of Massachusetts and California butterflies

	Massachusetts			California			Two-way analysis of variance				
	Mean	Variance	*n*	Mean	Variance	*n*	Variation	ss	df	ms	*F*	*P*
(*A*) Cardenolide concentration: all butterflies											
Males	0.317	0.018	186	0.115	0.012	190	Sex	0.135	1	0.135	7.314	<0.01
Females	0.349	0.025	169	0.139	0.020	161	Coast	7.472	1	7.472	404.061	<0.001
Totals	0.332	0.021	355	0.126	0.016	351	Interact.	0.002	1	0.002	0.158	>0.25
(Ratio of Mass.: Calif. variances = 1.313, $P < 0.05$)							Residual	12.983	702	0.018		
(*B*) Cardenolide concentration: absorbances ≥ 0.100												
Males	0.323	0.017	182	0.228	0.004	84	Sex	0.192	1	0.192	12.163	<0.001
Females	0.354	0.024	166	0.281	0.008	71	Coast	0.753	1	0.753	47.600	<0.001
Totals	0.338	0.020	348	0.252	0.006	155	Interact.	0.013	1	0.013	0.827	>0.50
(Ratio of Mass.: Calif. variances = 3.33, $P < 0.001$)							Residual	7.903	499	0.015		
(*C*) Dry weight (g)												
Males	0.191	0.0007	186	0.234	0.0017	190	Sex	0.028	1	0.028	20.156	<0.001
Females	0.185	0.0007	169	0.215	0.0024	161	Coast	0.235	1	0.235	169.505	<0.001
Totals	0.188	0.0007	355	0.225	0.0020	351	Interact.	0.005	1	0.005	4.081	<0.05
							Residual	0.977	702	0.001		
(*D*) Length of right forewing (cm)												
Males	5.17	0.041	186	5.13	0.046	190	Sex	1.008	1	1.008	21.037	<0.001
Females	5.15	0.038	169	5.00	0.068	161	Coast	1.500	1	1.500	31.296	<0.001
Totals	5.16	0.040	355	5.07	0.056	351	Interact.	0.570	1	0.570	11.908	<0.001
							Residual	33.652	702	0.047		

Table 2 Subsample determinations of cardenolide concentrations and emetic dosages for California and Massachusetts butterflies

	Cardiac glycoside content of butterflies (absorbance per 0.1 g dry weight)						Emetic dosage of butterflies (g of dry butterfly per 100 g of bird)		
	No. of butterflies in subsample		% of total sample	Absorbance range of subsample	Absorbance of combined subsample	No. of birds tested	ED_{50}	95% Confidence limits of ED_{50}	Estimated no. of ED_{50} units*
	Males	Females							
California									
	66†	56†	35	0.000 to 0.024	0.014	9†	—Indeterminate—		
	24	20	12	0.025 to 0.049	0.030	9‡	—Indeterminate—		
	26	18	12	0.050 to 0.149	0.100	11§	—Indeterminate—		
	19	8	8	0.150 to 0.199	0.171	15	0.123	0.098 to 0.153	2.8
	22¶	15¶	11	0.200 to 0.249	0.228	14	0.051	0.046 to 0.057	4.1
	12	16	8	0.250 to 0.279	0.250	21	0.056	0.051 to 0.062	4.6
	16	8	7	0.280 to 0.319	0.290	15	0.047	(0.045 to 0.049)φ	5.6
	4	9	4	0.320 to 0.399φ	0.305	14	0.035	0.031 to 0.040	6.0
	1	11	3	0.400 to 0.475**	0.420	12	0.037	0.035 to 0.039	9.1
Totals	190	161				120		(maximum estimate = 10.6)	
	351								
Massachusetts									
	4	3	2	0.000 to 0.099	0.085	2††	—Indeterminate—		
	19	13	9	0.100 to 0.157	0.150	7‡‡	—Indeterminate—		
	14	17	9	0.158 to 0.199	0.195	8§§	—Indeterminate—		
	27	20	13	0.200 to 0.249	0.235	10§§	—Indeterminate—		
	24	20	12	0.250 to 0.299	0.280	12	0.259	0.218 to 0.308	0.9
	22	17	11	0.300 to 0.349	0.320	10	0.219	0.201 to 0.238	1.0
	20	15	10	0.350 to 0 399	0.385	13	0.186	0.160 to 0.216	1.2
	26	17	12	0.400 to 0.449	0.440	14	0.171	0.158 to 0.184	1.3
	14	15	8	0.450 to 0.499	0.490	12	0.147	0.128 to 0.169	1.4
	12	19	9	0.500 to 0.599	0.510	11	0.138	(0.131 to 0.145)φ	1.4
	4	13	5	0.600 to 0.815**	0.635	7	0.150	0.133 to 0.169	1.7
Totals	169	186				106		(maximum estimate = 2.0)	
	355								

* Based on regression lines in Fig. 2, the mean weight of the Californian or Massachusetts butterflies, and the mean weight of the blue jays.
† Combined sample included random selection of twenty-two males and seventeen females.
‡ No birds vomited; three birds dosed at each of three levels: 0.129, 0.192, and 0.287 g.
§ One bird each dosed at levels 0.086, 0.105, 0.129, 0.142, 0.173, 0.212, 0.287, 0.350, and 0.522 g. did not vomit; one bird each at levels 0.129 and 0.287 g vomited.
¶ Two males and five females in this range were not combined.
φ Estimate subject to error because $NB\text{-}A^2/N^2 = 0.250$ for California and 0.160 for Massachusetts[6].
‖ One male of 0.013 absorbance was mistakenly included in the combined sample.
** The maximum concentration.
†† No birds vomited; dosed at 0.287 and 0.350 g.
‡‡ One bird dosed at 0.287 g vomited; two each dosed at 0.287, 0.350 and 0.428 g did not.
§§ Two birds dosed at 0.287 g did not vomit; at 0.350 g two did and one did not; at 0.428 g one did and two did not.
¶¶ Three birds dosed at 0.368 g did not vomit; at 0.407 g, three did and two did not; one bird each at 0.449 and 0.496 g did not vomit.

cost to sequestering these compounds.

The relationships of cardenolide concentrations and ED_{50} values are shown in Fig. 2 and Table 2. Although there seems to be an upper limit for both the California and Massachusetts butterflies, generally the higher the concentration of cardenolide, the lower the dose needed for emesis. However, at all emetic concentrations, the California monarchs were more emetic than their Massachusetts counterparts. Within the range of overlapping absorbances, at 0.250 and 0.475, the Massachusetts ED_{50} values were, respectively, 0.264 g and 0.162 g, compared with California ED_{50} values, respectively, of 0.057 g and 0.025 g. In other words, within this absorbance range the California monarchs were at least 4.6 to 6.5 times as emetic. This no doubt mirrors the separate *Asclepias* floras on the East and West coasts[10], several species of which are known larval food plants of the monarch[11].

Both dry weight and size were negatively correlated with cardiac glycoside concentration in the Massachusetts females, and significance was approached for the Massachusetts males (Table 3*A* and *C*). In contrast, neither sex in California showed these correlations. In order for the Massachusetts butterflies to be sufficiently emetic, they must incorporate

Table 3 Summary of linear regression analyses of cardenolide concentration (absorbance per 0.1 g powdered butterfly), dry weight (g), and right right forewing length (cm)

	No. of data points	a	c	Correlation coefficient	Standard error	P
(*A*) Dry weight compared with concentration						
Massachusetts males	186	−0.568	+0.427	−0.118	0.132	$0.20 > P > 0.10$
Massachusetts females	169	−0.997	+0.534	−0.167	0.155	$0.05 > P > 0.02$
California males	190	−0.316	+0.190	−0.119	0.109	$0.20 > P > 0.10$
California females	161	−0.027	+0.145	−0.009	0.142	$0.50 > P > 0.40$
(*B*) Dry weight compared with right forewing length						
Massachusetts males	186	+5.120	+4.187	+0.694	0.146	< 0.001
Massachusetts females	169	+5.792	+4.079	+0.785	0.120	< 0.001
California males	190	+2.568	+4.532	+0.498	0.186	< 0.001
California females	161	+2.863	+4.381	+0.544	0.219	< 0.001
(*C*) Right forewing length compared with concentration						
Massachusetts males	186	−0.087	+0.766	−0.132	0.132	$0.10 > P > 0.05$
Massachusetts females	169	−0.192	+1.341	−0.237	0.153	$P < 0.001$
California males	190	+0.043	−0.104	+0.084	0.110	$0.40 > P > 0.20$
California females	161	+0.053	−0.126	+0.098	0.141	$0.40 > P > 0.20$
(*D*) ED_{50} compared with absorbance per 0.1 g						
Massachusetts	7	+0.092	−0.757	−0.931	—	< 0.01
California	6	+0.009	−1.313	−0.875	—	< 0.025

For *A*, *B* and *C*, $y = ax + c$; for *D*, $y = ax^c$.

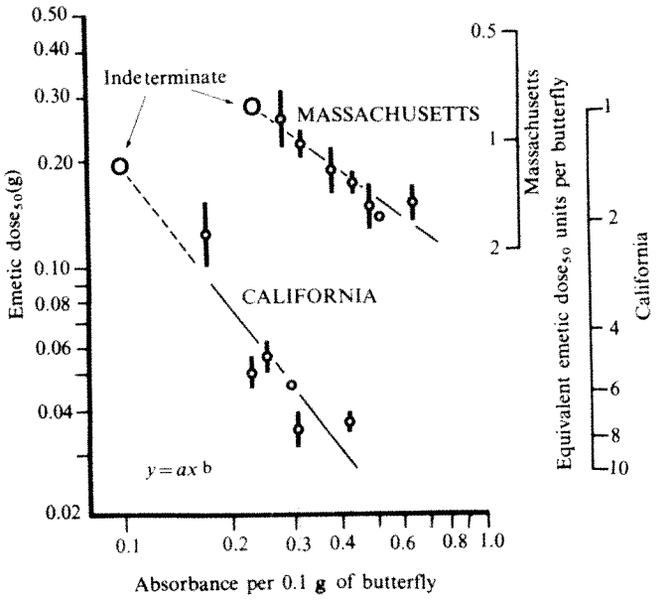


Fig. 2 Relationship of cardiac glycoside concentrations and ED_{50} values (data are in Table 2). The lines extending from the small open circles are the 95% confidence limits of the means; the dotted lines represent indeterminate to nonemetic concentrations. Emetic dose is inversely related to cardiac glycoside concentrations, thereby proving that the palatability spectrum in these wild monarch butterflies is causally related to a spectrum in cardenolide concentration. Cardenolides in the California butterflies, however, are far more emetic than those in the Massachusetts butterflies, no doubt reflecting the completely distinct *Asclepias* species on the two coasts from which the wild larvae sequester the poisons. The ranges in equivalent emetic dose 50 U per blue jay predator per butterfly are shown on the right side of the graph.

more cardiac glycoside than the California butterflies and these higher concentrations may be physiologically detrimental.

In terms of blue jay ED_{50} units, the Massachusetts butterflies ranged from an estimated maximum of 2.0 to less than 0.9, whereas the more toxic California butterflies ranged from 10.6 to 2.8. We did not resolve the number of ED_{50} units below these minima. There is, however, a concentration threshold below which the butterflies are effectively not emetic in terms of the numbers capable of being ingested per bird per unit time. For the California sample, this threshold is certainly not less than an absorbance of 0.050, so that at least 47% of the population is effectively palatable. A small percentage of the population (perhaps 5–10%) contains less than one emetic unit and is therefore of intermediate palatability, while the remaining 43–48% is unsuitable as food. For the Massachusetts sample, the threshold is not as clear-cut, but is probably near an absorbance of 0.150. Thus only about 10% of the Massachusetts population is effectively palatable, with a larger group of intermediate palatability (about 35%), leaving approximately 55% of the population with one to two ED_{50} units.

The mean absorbance per butterfly in Massachusetts reported[3] in 1970 (0.198), when corrected (0.281), was 15% lower than found in 1971, ($t = 3.42$, d.f. $= 475$, $P < 0.001$). However, the corrected absorbance values of one ED_{50} unit for the 2 yr are very similar: 0.327 for 1970 and 0.320 for 1971. In other words, the basic emetic properties per unit of absorbance remained virtually the same, but the mean concentration changed, probably because of a difference in environmental factors affecting cardiac glycoside production in the wild milkweeds during the 2 yr.

The fact that California and Massachusetts populations contain both palatable and unpalatable butterflies verifies the heretofore largely theoretical category of mimicry known as automimicry[12,13]. In this type of mimicry, which is mathematically analgous to a perfect Batesian mimicry system, the palatable individuals of the species derive mimetic protection from the unpalatable portion of the same population from which they cannot be visually distinguished. Automimicry theory predicts that the more noxious the unpalatable individuals are, the higher can be the proportion of palatables in the population. The greater percentage of completely palatable butterflies in the much more noxious California population compared with the Massachusetts population verifies this prediction. Moreover, the high degree of protection that must result from the very emetic California butterflies probably made possible the evolution of the spectacular overwintering clusters which are found on the California coast but not in the East.

The assumption that wild Danaine butterflies are uniformly unpalatable has played a key roll in the development of mimicry theory. This assumption can no longer be made, however, either in attempts to interpret older experiments[14] or in future work. Our study makes it clear that unpalatability is a dynamic situation. We must also question whether all Danaine species are equally adept at sequestering cardenolides, as well as whether they are equally able to eat the various poisonous to nonpoisonous milkweed species available to them. Because of the dynamics of palatability, certain of the inconsistencies in the mimicry literature, such as the colour polymorphism found in some populations of supposedly unpalatable butterfly species[15], now seem amenable to analysis. An interesting case is the widespread African *Danaus chrysippus* L. which is polymorphic in some parts of its range and monomorphic in others[16]. Where monomorphic, a substantial portion of the population is probably unpalatable. But in other areas, *D. chrysippus* may be effectively palatable and a polymorphic Batesian mimic.

The distinct differences in emetic properties of the cardenolides found in the Massachusetts and California butterflies suggests that more *Asclepias* species might profitably be investigated to discover new cardenolides which retain their useful attributes as heart drugs, but which have less noxious emetic side effects[17,18].

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1 Rothschild, M., *Symp. R. ent. Soc.*, **6**, 59 (1972).
2 Brower, L. P., *Scient. Am.*, **220**, 22 (1969).
3 Brower, L. P., McEvoy, P. B., Williamson, K. L., and Flannery, M. A., *Science*, **177**, 426 (1972).
4 Brower, L. P., Ryerson, W. N., Coppinger, L. L., and Glazier, S. C., *Science*, **161**, 1349 (1968).
5 Rabitzsch, G., and Tambor, U., *Pharmazie*, **24**, 262 (1969).
6 Dixon, W. J., and Massey, jun F. J., *Introduction to Statistical Analysis*, second ed., 323 (McGraw-Hill, New York, 1957).
7 Program 1317 EB/ST3, Rev. 1, 700 *Series Program Library*, **9**, 95 (Wang Laboratories, Inc., Tewksbury, Massachusetts, 1971).
8 Program 1351 EB/ST4, Rev. 1, 700 *Series Program Library*, **9**, 15 (Wang Laboratories, Inc., Tewksbury, Massachusetts, 1971).
9 Program 1316 EB/ST3, Rev. 1, 700 *Series Program Library*, **9**, 93 (Wang Laboratories, Inc., Tewksbury, Massachusetts, 1971).
10 Woodson, R. E., *Ann. Missouri Bot. Garden*, **41**, 1 (1954).
11 Urquhart, F. A., *The Monarch Butterfly*, 10 (University of Toronto Press, 1960).
12 Brower, L. P., Pough, F. H., and Meck, H.R., *Proc. natn. Acad. Sci. USA*, **66**, 1059 (1970).
13 Pough, F. H., Brower, L. P., Meck, H. R., and Kessell, S. R., *Proc. natn. Acad. Sci. USA*, **70**, 2261 (1973).
14 Rothschild, M., and Kellet, D. N., *J. Ent.* A, **46**, 103 (1972).
15 Turner, J. R. G., in: *Ecological Genetics and Evolution* (edit. by Creed, R.), 224 (Blackwell, Oxford, 1971).
16 Owen, D. F., *Oikos*, **21**, 333 (1970).
17 Goldstein, A., Aranow, L., and Kalman, S. M., *Principles of Drug Action*, 760 (Harper and Row, New York, 1969).
18 Farnsworth, N. R. *J. Pharm. Sci.*, **55**, 225 (1966).

Genetic selection of winner and loser rats in a competitive situation

GENETIC selection of behavioural characteristics has been observed, among others, in the Maudsley, Roman, and Tryon strain of rats[1]. Here we describe an experiment designed to test the hypothesis that rats could be selectively bred to win or lose in a food competition situation of the type reported by Lindzey *et al.*[2] with mice and later used by other authors with rats[3–8].

The apparatus is described in detail elsewhere[5]. Briefly, it consisted of a straight wood runway 100 cm long, connected at the extremities with two identical open-topped chambers; a guillotine door bisected the runway. The animals had food withheld for 20–22 h and were trained individually every 48 h to traverse the runway to be rewarded with food. They were then subjected to the contest situation, in which two rats were simultaneously introduced into the opposite sides of the apparatus. As the rats met in the middle of the runway, one pushed the other and gained the reward.

The offspring of three randomly selected couples of Wistar rats from our own colony, mantained by random breeding, were subjected to the competitive situation when about 80 d old. A stable hierarchy of winners and losers was obtained among seven females and seven males. The two male rats which consistently won were bred with the two females that were winners from their hierarchy. Similarly, the two loser rats of the female and male hierarchy were also bred. The offspring (F1) consisted of 15 rats which were descendants of winner couples (DW) and 14 rats which were descendants of loser couples (DL). At 80 d of age, competitive hierarchies were established independently for DW males, DW females, DL males and DL females. The two DW male rats which occupied the top of the hierarchy were mated with the two DW female rats which also occupied the first and the second positions. Similarly, the two DL males at the bottom of their hierarchy were mated with the two DL females which occupied the last two positions. Only one female and one male were put together for mating.

F2, consisting of 15 DL and 11 DW were tested against random-bred controls, using a fixed pair procedure. Each pair was made up by a DW or DL rat and a random-bred control of the same sex and age. Each pair was subjected to three experimental sessions consisting of five contests each. After that, the rats were selected for mating according to their number of wins (DW that won at least 13 out of the 15 contests and DL that lost at least 13 out of the 15 contests). F3 (31 DW and 31 DL) and F4 (32 DW and 32 DL), were subjected to the same procedure as F2. In the three generations (F2, F3 and F4) tested against randombred controls, the DW rats won about 75% of the contests while the DL rats lost nearly 80% of the contests. Throughout the generations, mating brothers and sisters was avoided when possible. At the F1 and F2 about 50% of the couples which were mated were brother and sisters while this dropped to about 10% in the following generations.

In F5, 60 pairs were formed, each consisting of a DW and a DL rat of similar weight. Thirty-seven pairs were females and the remaining 23 males. Each pair, deprived of food for about 22 h, was tested using the fixed pair procedure. Three experimental sessions were run, consisting of five contests each. The results (Fig. 1) show that DW rats consistently won more than 80% of the 15 contests. In all generations, records of weights were kept and correlations between weight and number of wins were calculated. No significant correlations were found.

These data indicate that genetic selection occurred. DW and DL rats probably have different behavioural or physiological characteristics, which are yet to be detected, that led them either to win or lose in the straight runway. The possibility that the results obtained were not due to genetic selection but to an influence of the mothers during weaning was excluded, as in the F4 and in the F5 generation, all rats were reared by foster mothers.

How winning and losing in the straight runway correlates with other competitive situations remains to be investigated.

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1 Wahlsten, D., *Behav. Biol.*, **7**, 143 (1972).
2 Lindzey, G., Winston, H., and Manosevitz, M., *Nature*, **191**, 474 (1961).
3 Hsiao, S., and Schreiber, S. C., *Psychon. Sci.*, **10**, 117 (1968).
4 Wilson, W. J., *Psychon. Sci.*, **10**, 119 (1968).
5 Masur, J., Martz, R. M. W., Bieniek, B., and Korte, F., *Psychopharmacologia*, **22**, 187 (1971).
6 Masur, J., Karniol, I. G., and Palermo Neto, J., *J. Pharm. Pharmac.*, **24**, 262 (1972).
7 Work, M. S., and Rogers, H., *J. comp. physiol. Psychol.*, **79**, 414 (1972).
8 Terada, C. W., and Masur, J., *Eur. J. Pharm.*, **24**, 375 (1973).

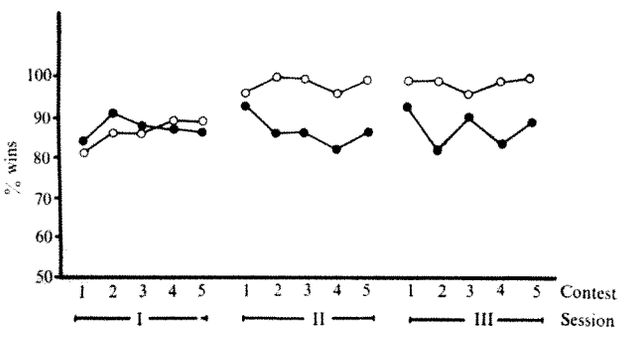


FIG. 1 Percentage of wins obtained by 23 DW males (●) and 37 DW females (○) when competing in a straight runway against 23 DL males and 37 DL females, respectively. The difference between the number of victories obtained and the casual level of 50% was significant in every contest. The statistical analysis was performed for females using the binomial test for large samples ($P \leq 0.001$) and using the binomial test for small samples for males ($P \leq 0.002$).

Infectious particles in a marine ciliate

INFECTIOUS, self-reproducing particles of unknown origin have been demonstrated in various tissues and cells[1,2], notably fresh water protozoa[3], but not in marine protozoa. Advances in the development of culture media for marine ciliates[4] and the need for simple model systems for biochemical study of intracytoplasmic particles have led us to survey marine protozoa. Of 104 isolates made from twenty-one seawater samples taken from tidal pools and brackish backwaters along the South Florida coast, twenty-six were cultivated axenically as described before[4]. Of these, four contained in their cytoplasm infectious, self-reproducing particles, which we have termed 'xenosomes' (*xenos*, alien; *soma*, body).

The xenosomes were found in aceto-orcein-stained preparations[4] under the phase microscope (Fig. 1*a*). They seemed to be distributed randomly throughout the cytoplasm. We

have maintained one xenosome-bearing stock, a small hymenostome ciliate identified as *Parauronema acutum* 110-3, for more than 2 yr, subcultured weekly, without loss of particles. The ciliates were maintained axenically in M medium[4] modified to contain 500 μg ml^{-1} asolectin as the sole source of lipid, at 22°C in the dark. From 10 l of late logarithmic phase culture containing approximately 10^6 animals ml^{-1}, we recovered as many as 8.0×10^{11} highly purified particles per attempt (Fig. 1*d*). The method of isolation and purification of the particles was essentially the same as that used with the mu and pi symbionts of *Paramecium aurelia*[5]. Xenosomes appear as rod-shaped particles, approximately 0.3 μm wide and 1.0 μm long. They occur exclusively in the cytoplasm in numbers reaching 300 per protozoon. When released from the protozoa (ruptured by gentle pressure of a glass coverslip) the particles can be seen spinning (propellor-like) and darting rapidly, suggesting the presence of a motile apparatus, perhaps flagella. Xenosomes are Gram negative and appear to divide within the cytoplasm by transverse fission, as evidenced by a furrow, seen in some particles, in a plane perpendicular to their long axis. The appearance of the particles in the electron microscope (*in situ*) suggests that they are bounded by a double-track membrane (Fig. 1*c*). Dispersed throughout the internal contents of the particle is a network of fibrous strands, which disappears after treatment with DNase and, presumably, consists of DNA. Attempts to culture the particles outside the host in media similar to that used for the ciliates themselves have not been successful. Nor has it been possible to grow particles in media supplemented with biological materials and growth factors.

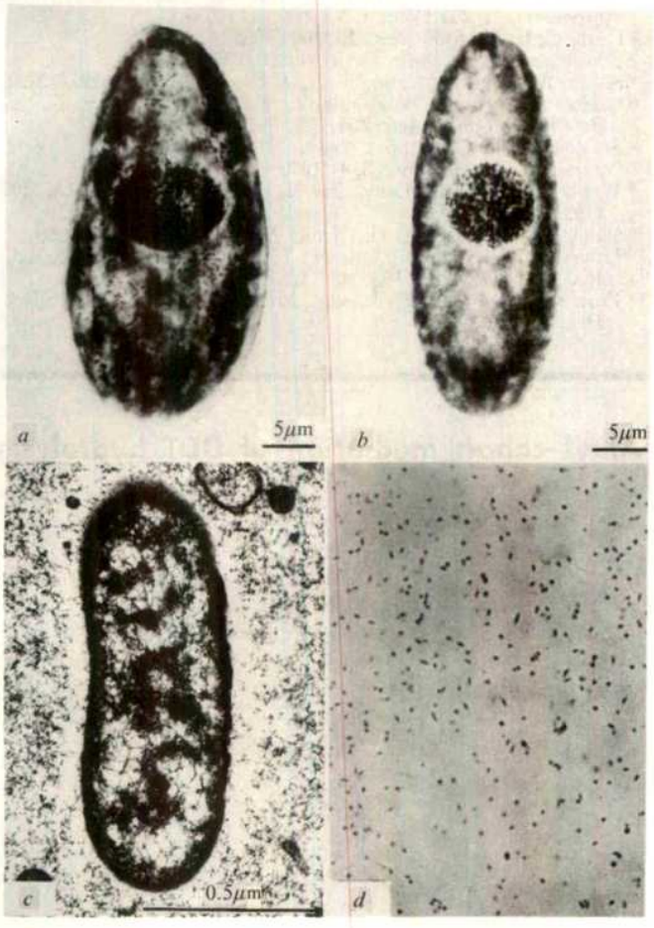


FIG. 1 Phase micrographs of xenosome-bearing (*a*) and xenosome-free (*b*) *Parauronema acutum* stained with aceto-orcein dye. (*c*), Electron micrograph of a xenosome *in situ*. Specimens were fixed in 1.33% OsO_4 buffered with 0.2 M γ-collidine, *p*H 7.4 prepared in seawater of density = 1.015 g cm^{-3} for 30 min at 0°C. Ciliates were left in buffer overnight at 0°C, dehydrated through an alcohol series and embedded in Araldite. Ultrathin sections were cut with a glass knife, doubly stained with alcohol-uranyl acetate and lead acetate and examined in a Phillips EM 300 electron microscope. *d*, Phase micrograph of isolated and purified xenosomes, unstained (×570).

TABLE 1 Comparison of infectivity of whole culture blendates (cell-free) with filtrates of xenosome-bearing *P. acutum* 110-3

Sample tested	Sample dilution: As is	1:2	1:4	1:8	1:16
Whole culture, blended and filtered	76	41	18	0	1
Filtrate only, no blending	80	39	17	3	0
Filtrate only, blended	75	52	28	7	0

Figures represent percentage of animals infected.

Cultures of xenosome-bearing *P. acutum* 110-3 in the exponential phase of growth (population density, $0.5 \times 10^6 - 1.0 \times 10^6$ animals ml^{-1}) were either blended in a sterile Waring blendor to more than 95% cell breakage and filtered through a sterile Millipore filter (8 μm pores) to remove unbroken cells, or filtered directly without blending. Samples of 0.1 ml of the filtrates, or dilutions thereof, were added to 0.9 ml each of fresh modified M culture medium contained in the wells of nine-spot depression slides, inoculated with about 200 particle-free animals, and incubated at 22°C in the dark in humidifying chambers for 7–10d. About 5 μl of the cell suspension from each depression was then placed on a glass slide, exposed to osmium vapours (2% (w/v) OsO_4 in distilled water) for about 5 s and allowed to dry at room temperature. A small drop of aceto-orcein dye was added, followed by a coverslip, and the animals were viewed under oil immersion using phase optics in the Leitz microscope. Fifty animals from each depression were observed and scored for the presence of particles. The number of animals containing particles from triplicate depression slide cultures were averaged and the data expressed as percentage of animals infected. A filtered sample of the culture, blended for the same time as the whole culture, was included as a control.

Purified particle preparations were fractionated by the Schneider procedure[6] and the nucleic acids were extracted from the dry, de-fatted residues with hot trichloroacetic acid (TCA). The extract was analysed for DNA by the diphenylamine procedure[7] or by the method of Webb and Levy[8] and for RNA by the orcinol procedure[9]. Protein was determined by solubilising the TCA-extracted residue in 0.1 N NaOH and assaying the extract by the Lowry procedure[10]. The DNA, RNA and protein content of the isolated particles (pg per particle ($\pm$ s.d.)) was 0.008 $\pm$ 0.002; 0.031 $\pm$ 0.007; and 0.041 $\pm$ 0.007 respectively. The data represent average values of nine duplicate determinations of seven preparations.

When penicillin, tetracycline or chloramphenicol were added to the culture medium at the time of inoculation, the particles within the cells were destroyed and lost irreversibly from the cytoplasm. (Optimum concentrations that eliminated particles from the cytoplasm but did not significantly affect growth or division were 50 U ml^{-1} for pencillin, 0.5 μg ml^{-1} for tetracycline and 0.25 μg ml^{-1} for chloramphenicol.) Cycloheximide, neomycin sulphate and streptomycin had no such selective effect. Xenosomes *in situ* were also destroyed by irradiation with an electron beam (Fig. 2*a*): the effective dose (ED_{50}) was about 30,000 rad, comparable with values reported for X-irradiation of certain bacteria and members of the Mycoplasma[11]. The ED_{50} for the protozoon itself was approximately 260,000 rad. Thus, xenosomes could be removed from the cytoplasm of the ciliates by treatment with the electron beam using doses of about 200,000 rad (see graph in Fig. 2*a*).

The infectious nature of the particles was demonstrated by adding aliquots of cell-free blendates or filtrates of xenosome-bearing cultures to particle-free animals and observing the uptake of the particles into the cytoplasm. The infective agent readily passed through Millipore filters with pores 8 μm

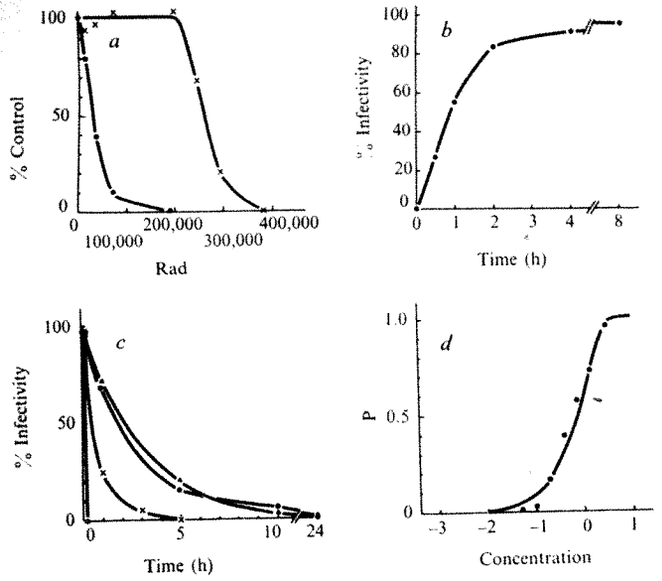


FIG. 2 *a*, Selective effect of electron beam irradiation on xenosomes *in situ*. *Xenosome*-bearing *P. acutum* 110-3 was inoculated into 5 ml of fresh medium in sterile 30-ml tissue culture flasks (No. 3012, Falcon) at a cell density of 10,000 ml^{-1}. The cell suspension was 2 mm deep. Flasks were irradiated in a CLINAC 6 MeV linear accelerator (Varian Associates) with an output of 3.5 MeV at 10,000 rad min^{-1} for doses up to 100,000 rad and 100,000 rad min^{-1} for larger doses. Flasks were then incubated for 7 d at 22° as in the dark. Both protozoon and particle populations were estimated as described previously[5]. Population densities of non-irradiated protozoons averaged 2.3 × 10^6 ml^{-1}; the number of xenosomes per protozoon was estimated at 204 ± 25 (s.d.). Thus, the 'population' of xenosomes calculated per ml of culture was 4.7 × 10^8. x, Protozoan; ●, xenosomes. *b*, Release of infectious agent into ambient culture fluid. Exponential phase xenosome-bearing animals cultured in M medium were washed free of exogenous particles by centrifugation three times at 600 *g* for 1 min each, suspended in M medium at a cell density of 500,000 ml^{-1} and left at 22°C in the dark. Samples were removed at intervals, filtered (Millipore, 8 μm pores) to remove the cells, and filtrates were assayed for infectivity as described in Table 1. *c*, Stability of infectious agent to temperature. Filtrates of exponential phase xenosome-bearing animals cultured in M medium were left at various temperatures. Samples were removed at intervals, brought rapidly to 22°C and assayed for infectivity (see Table 1) ○, 65°C; ×, 37°C; ●, 22°C; ▲, 4°C. *d*, Experimental frequency of positive response (infectivity) to various concentrations of xenosomes in filtrates of xenosome-bearing *P. acutum*. Abscissa: logarithm of concentration (empirical scale). Ordinate: *P*, frequency of infection. Each point represents the average value of four experiments. The solid line was the curve derived from the expression $P_{r,s} = 1 - e^{-s}$ where P_r is the probability of having at least one infective particle per protozoon and *s* is the average number of particles per sample. Infectivity was determined as described in Table 1.

in diameter, but not when the pores were 0.45 μm or less in diameter. We found no significant quantitative difference when whole filtered blendates were compared with cell-free culture filtrates for infectious activity (Table 1). This suggests that infectivity is associated with a particulate agent released from the protozoon into the culture medium. Figure 2*b* shows the release of the infective agent into the ambient culture fluid from particle-bearers washed free of exogenous particles. The infectious agent was relatively unstable to temperature (Fig. 2*c*). Infectious activity was lost almost immediately at 65°C; about half the infectious activity remained after 1 h at 37°C and after 2 h at 22°C or 4°C. Sonication for periods as brief as 20 s completely inactivated particles present in cell-free culture filtrates. Attempts to stabilise the infectious agent by varying conditions of ionic strength, osmotic pressure and *p*H have been unsuccessful. Statistical analysis of the mode of action of the infectious agent present in culture filtrates suggests that a single particle can infect a susceptible particle-free animal (Fig. 2*d*).

Although host range studies were limited by the lack of well-characterised strains of marine ciliates, we have found that the xenosomes of this strain can infect three other particle-free stocks of *Parauronema acutum*, but not *Uronema nigricans* or the related *Parauronema virginianum*.

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1 Buchner, P., *Endosymbioses of Animals with Plant Microorganisms*, 1–200 (Wiley, New York, 1965).
2 Lanham, U. N., *Biol. Rev.*, **43**, 260 (1968).
3 Sonneborn, T. M., *Adv. Virus Res.*, **6**, 229 (1961).
4 Soldo, A. T., and Merlin, E. J., *J. Protozool.*, **19**, 519 (1972).
5 Soldo, A. T., van Wagtendonk, W. J., and Godoy, G. A., *Biochim. biophys. Acta* **204**, 325 (1970).
6 Schneider, W. C., *J. biol. Chem.*, **161**, 293 (1945).
7 Dische, Z., *Mikrochemie*, **8**, 4 (1930).
8 Webb, J. M., and Levy, H. B., *J. biol. Chem.*, **213**, 107 (1955).
9 Ogur, M., and Rosen, G., *Archs Biochem.*, **25**, 262 (1950).
10 Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol Chem.*, **193**, 265 (1951).
11 Furness, G., Pipes, F. J., and McMurtrey, M. J., *J. infect. Dis.*, **118**, 1 (1968).

Alkyl–cobalt mediation of DDT hydrolysis

A POSSIBLE role for vitamin B_{12} in the detoxification of polyhalogenated hydrocarbon pesticides such as DDT has occasionally been postulated (ref 1 and references therein), and DDT has been shown to inhibit bacterial B_{12}-dependent methanogenesis[2]. Although reduced cobalamins and cobaloximes will react with simpler species such as polyhalomethanes[3,4], no adduct between DDT or related compounds and cobalt has previously been observed[2]. It has been suggested that alkyl–cobalt complexes of the ligand CR (Fig. 1)

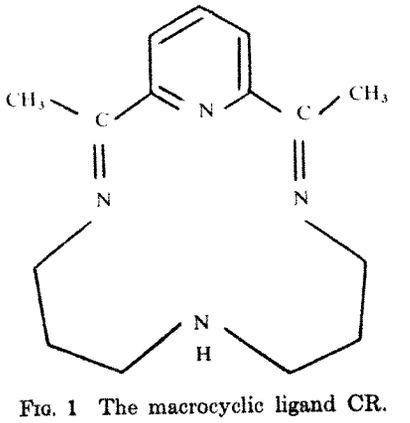


FIG. 1 The macrocyclic ligand CR.

have some chemical similarities to B_{12} (ref. 5) and zinc complexes of CR have yielded results relevant to carbonic anhydrase[6]. We report the use of cobalt–CR to mediate the hydrolysis of DDT, the isolation of an alkyl–cobalt derivative of a hydrolysis product of DDT, and the formation of di(*p*-chlorophenyl)ketone from this compound.

The Co(II) complex of CR was prepared in aqueous ethanol by the standard template method[7], and reduced *in situ* to the nucleophilic Co(I) species using borohydride at *p*H 9. This was allowed to react under nitrogen with pure *p, p'*-DDT, and the solution warmed to 40° C and stirred for 12 h in the dark. A deep red, light-sensitive solution resulted and addition of PF_6^- or ClO_4^- produced a red, tarry product. This was very difficult to purify but a small quantity of the ClO_4^- salt was isolated as a clean, maroon powder by repeated reprecipitation from ethanol/ether.

The infrared spectrum of this compound has an intense band around 1,100 cm^{-1}, confirming the presence of ClO_4^-. A broad area above 3,200 cm^{-1} and around the Nujol absorption at 2,900 cm^{-1} is attributable to a carboxylic acid group hydrogen bonding with water. The region 1,550–1,675 cm^{-1} is dominated by the C=C and C=N vibrations of the aromatic rings and these could well mask the C=O stretching vibration if this group displays some resonant single-bond character. The presence of ionic chloride in the compound was demonstrated by silver nitrate titration and the resulting solution was left in sunlight for a few days. Extraction of the solution with toluene yielded a white powder—shown to be pure by thin-layer chromatography—with a mass spectrum identical to that of an authentic sample of di(*p*-chlorophenyl)ketone. These results lead us to suggest that the complex is as shown in Fig. 2; this is supported by microanalysis (required percentages for $CoC_{29}H_{35}N_4O_9Cl_4$: C, 45.3; H, 4.6; N, 7.3; total Cl, 18.5: percentages found: C, 44.8; H, 4.5; N, 7.7; total Cl 18.1) and by mass spectrometry. Thermolysis of the compound on the mass spectrometer probe at 200° C produced many fragments but, considering only those displaying peaks at the highest values of *m/e*, groups are observed at *m/e* = 248, 250, 252 (intensities 9:6:1) and 235, 237, 239 (9:6:1). The isotopic distribution of ^{35}Cl and ^{37}Cl implies that these peaks are due to species each containing two chlorine atoms. Since the aromatic chlorines will be stable under the reaction conditions used, in contrast to the aliphatic chlorines, we assign these peaks to $(p\text{-}ClC_6H_4)_2C_2H_2^+$ and $(p\text{-}ClC_6H_4)_2CH^+$. The latter is expected from cobalt–carbon bond cleavage and loss of CO_2, and peaks attributable to $(p\text{-}ClC_6H_4)_2CH.C(OH)_2^+$ are present in the region *m/e* 281–285 but the relative intensities are obscured by the presence of other fragments. It has been pointed out to us that the former set of peaks may be due to hydrogen transfer from the CR ligand during thermolysis.

The formation of the isolated compound from Co(I)CR and DDT may be readily rationalised. Nucleophilic attack by Co(I) will displace Cl^- and the resulting complex can then eliminate H^+ and Co(I) to form 1,1-di(*p*-ClC_6H_4)-2,2-dichloroethylene; further attack on Cl by Co(I) and hydrolysis of the chlorines on C2 gives the aldehyde hydrate which is easily oxidised to the acid during workup. The type of reactions involved here have been fully documented by Schrauzer[4,8]. The production of di(*p*-chlorophenyl)ketone is explained by photolysis of the cobalt–carbon bond to form a radical, which in aqueous solution gives rise to the α-hydroxy-carboxylic acid. In the presence of Ag(I) and a radical species such as Co(II) or O_2 this will readily decarboxylate to the ketone (Fig. 3) (J. Staunton, personal communication).

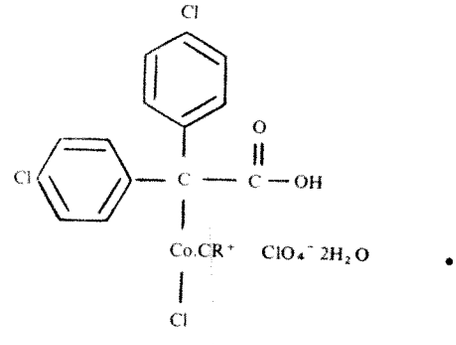


FIG. 2 Suggested structure of the isolated alkyl-cobalt intermediate.

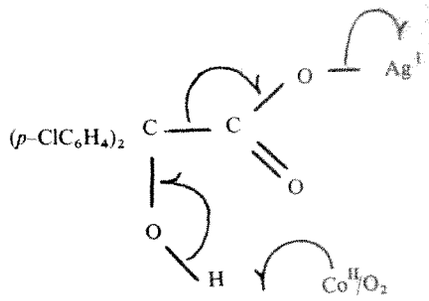


FIG. 3 Suggested decarboxylation mechanism.

These results indicate that the involvement of alkyl–cobalt species in the hydrolytic detoxification of DDT is chemically feasible. They also show that hydrolysis and partial degradation of DDT by this route requires far less vigorous conditions than the strong base and oxidising agents usually used to bring about these reactions. There are evidently many possible reaction pathways for the dechlorination of DDT in complex biological systems such as anaerobic sewage sludge[9]. The presence of microorganisms in these wastes means that B_{12}-mediated routes are available—several such routes have also been implicated in the biological fixation of mercury[10,11]—and it is thus of great interest to note that di(*p*-chlorophenyl)ketone is formed among other products from DDT in sewage sludge[12]. The possible involvement of B_{12} in the formation of this compound, rather than its being a degradation produce of di(*p*-chlorophenyl)acetonitrile[12], is open to consideration.

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1 Prince, R. H., and Stotter, D. A., *J. inorg. nucl. Chem.*, **35**, 321 (1973).
2 McBride, B. C., and Wolfe, R. S., *Nature*, **234**, 551 (1971).
3 Wood, J. M., Kennedy, F. S., and Wolfe, R. S., *Biochemistry N.Y.*, **7**, 1707 (1968).
4 Schrauzer, G. N., Ribeiro, A., Lee, L. P., and Ho, R. K. Y., *Angew. Chem.*, **10**, 807 (1971).
5 Busch, D. H., Farmery, K., Goedken, V., Katovic, V., Melnyk, A. C., Sperati, C. R., and Tokel, N., in *Bioinorganic Chemistry Adv. Chem. Ser.* **100**, 44 (American Chemical Society, 1971).
6 Prince, R. H., and Woolley, P. R., *Nature phys. Sci.*, **240**, 117 (1972).
7 Long, K. M., and Busch, D. H., *Inorg. Chem.*, **9**, 505 (1970).
8 Schrauzer, G. N., and Windgassen, R. J., *J. Am. chem. Soc.*, **89**, 1999 (1967).
9 Albone, E. S., Eglinton, G., Evans, N. C., and Rhead, M. M., *Nature*, **240**, 420 (1972).
10 Wood, J. M., in *Chem. Engng News*, **49**, 22 (1971).
11 Lewis, J., Prince, R. H., and Stotter, D. A., *J. inorg. nucl. Chem.*, **35**, 341 (1973).
12 Jensen, S., Göthe, R., and Kindstedt, M. O., *Nature*, **240**, 421 (1972).

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GENERAL

Genetics and the development of social attitudes

WHATEVER the current norms might be with regard to public opinion about various issues, and whatever factors contribute to their change with time, attitudes are far from uniform. Eysenck[1] has shown how responses to a public opinion questionnaire could be resolved into two main factors: 'radicalism' (R) against 'conservatism' and 'toughmindedness' (T) against 'tendermindedness'. Individual differences in opinion are, to some degree, the social manifestation of individual differences in personality. Significant covariation has been detected between various measures of extraversion (E) and T. Wilson[2] has suggested personality correlates of R. Such relationships, together with available evidence for the genetic determination of personality differences (see, for example, ref. 3), justify a more penetrating analysis of the extent to which variation in opinion and personality share a common genetic basis.

We consider the causes of trait covariation within and between pairs of monozygotic (MZ) and dizygotic (DZ) twins. From the responses of 708 volunteer pairs of like-sexed twins to an 80-item Personality Inventory and to a 60-item Public Opinion Inventory, scores were derived on scales of psychoticism (P), extraversion (E), neuroticism (N), R and T, together with an emphasis (Em) score which indicated any tendency of subjects to adopt more extreme opinions. The questionnaires and the scoring keys used have been published elsewhere[1,4].

On the basis of replies to questions concerning childhood similarity, the twins were diagnosed as MZ or DZ (ref. 5). The pairs differed in their ages and in the time for which they had lived apart. Our analysis is based on the raw scores and the absence of an age correction will not severely affect the conclusions from a study which can only detect gross effects.

The MZ intrapair differences in personality were not related to the duration of separation, so we have pooled separated and unseparated twins. The composition of the sample by zygosity and sex is shown in Table 1, and it can be seen that it is not representative of zygosity or sex, although the twins are representative of the population with respect to variation for the traits in question. The data were summarised by the mean squares and mean products within and between pairs for every trait and group of twins.

Twin data alone are inadequate for a detailed genetic analysis, particularly when the twins were not properly separated[6], so the structure of our data limited us to tests of comparatively simple hypotheses. We had to accept estimates of components which may be biased and tests which are not very powerful.

We wished to test, and hoped to choose between, two hypotheses of equal rank about the causes of variation and covariation for our six scales of personality and attitude. The hypotheses are: (1) that all the observed trait variation and covariation is attributable either to differences between the environmental influences shared by members of the same pair (E_2), or to differences in the environmental influences specific to individuals within the family (E_1); and (2) that all the variation and covariation is attributable either to the cumulative and additive effects of many loci for which the population is polymorphic (D_R), or to specific (E_1) environmental influences. We refer to the first hypothsis as the 'simple environmental hypothesis' and the second as the 'simple genetic hypothesis'.

For a particular pair of traits, i and j, we can write expectations for the mean products, in terms of the genetic and environmental covariance components for that pair of traits.

TABLE 1 Composition of the sample of twins (number of pairs)

		Sex		Total
		Male	Female	
Zygosity	MZ	120	331	451
	DZ	59	198	257
Total		179	529	708

These components are D_{Rij}, E_{1ij} and E_{2ij}, and they have been defined in detail and the restrictions on their interpretation discussed elsewhere[1]. The expectations derived for both simple hypotheses are given in Table 2.

The coefficients of D_R are applicable to randomly mating populations. The slight assortative mating observed for personality variables is insufficient to cause a serious departure from these expectations, since the heritability of the traits is quite low. Insel[8] reported correlations between spouses for the Wilson Conservatism Scale, finding values between 0.59 and 0.66. If this correlation had a large genetic component, its effect in our case, would be inseparable from E_2 and would contribute to failure of our simple genetic model. It is possible to obtain weighted least squares estimates of the parameters of either model[7]. A detailed analysis suggested that the genetic and environmental parameters might be inconsistent over sexes. At the risk of introducing some redundancy into our model, therefore, we allowed different values for our estimates in the two sexes and subsequently compared them. There are 168 raw statistics altogether, and we fit 84 parameters, leaving 84 d.f. for testing either model.

When the simple environmental hypothesis was tested we found that the residual $\chi_{84}^2 = 167.31$ ($P < 10^{-6}$) so we concluded that a model invoking only environmental factors fails to provide an adequate description of the variation and covariation of the six traits. For the simple genetic hypothesis, we found that the residual chi square is smaller ($\chi_{84}^2 = 111.60$, $P \simeq 0.025$) and just significant. A model in which D_R E_1 and E_2 were fitted jointly gave a very satisfactory fit ($\chi_{42}^2 = 47.98$, $P \simeq 0.24$), suggesting that some E_2 parameters might be added to our simple genetic model. By this stage, however, there were many redundant parameters in the model and in the absence of any theory about which parameters could be deleted from the more complex model, we present the results of the simple genetic model, bearing in mind that some modification might still be appropriate (Table 3). The results were obtained by pooling estimates over sexes.

The estimates for the components of variance can be used to calculate heritabilities for the six traits from

$$\hat{h}t = \tfrac{1}{2}\hat{D}_R/(\tfrac{1}{2}\hat{D}_R + \hat{E}_1)$$

and provided we are justified in accepting our model, these estimates refer to the proportion of the population variance which is genetic in origin (Table 4).

The simple environmental model is insufficient to explain the observed variation and covariation of the traits in this study. We are, however, restricted by the structure of the data available. A larger or better designed study might identify common environmental influences in the departures from expectation which, in our study, appear as error. We

TABLE 2 Expectations of mean squares (mean products) on two simple models

Model	Expectation: Environmental		Genetic	
Parameter	E_{1ij}	E_{2ij}	D_{Rij}	E_{1ij}
Mean square/product				
Between MZ pairs	1	2	1	1
Within MZ pairs	1			1
Between DZ pairs	1	2	$\frac{3}{4}$	1
Within DZ pairs	1		$\frac{1}{4}$	1

TABLE 3 Estimates of parameters of a simple genetic model*

Scale	D_R(genetic)						E_1(environmental)					
	R	*T*	*Em*	*P*	*E*	*N*	*R*	*T*	*Em*	*P*	*E*	*N*
R	12.39¶	−1.43	4.38§	0.81	−0.29	2.08	3.37¶	−1.19¶	1.37‡	0.02	0.03	0.19
T		†21.53¶	−4.19	2.11§	6.62¶	2.61		†9.18¶	−2.67§	0.71§	−0.32	0.06
Em			40.67¶	2.47‡	−0.82	2.85			34.76¶	0.44	1.65	1.73
P				3.19¶	0.48	1.80‡				2.92¶	0.67‡	0.05
E					17.61¶	−1.21					9.41¶	−0.13
N						22.04¶						11.25¶

* Values given after pooling over sexes. Where the estimates were heterogeneous over sexes they are marked †.
‡ Significant at the 0.05 level.
§ Significant at the 0.01 level.
¶ Significant at the 0.001 level.

can, however, be reasonably confident that some kind of genetic mechanism has to be invoked to account for the observations so far. That such a mechanism might involve the correlation of developmentally significant environmental factors with genetic variation should not be discounted. About half the variation between individuals for the six traits has a genetic basis (Table 4).

There are also some predictable significant covariance terms. Our study confirms the correlation between *E* and *T*, showing that it has a genetic, rather than an environmental basis, since only the D_R component is significant. There is also a significant genetic correlation between *P* and *Em*. This suggests that individuals who achieve high *P* scores share the strong conviction of being right, which is characteristic of some psychotic behaviour. The more significant tendency, for high *P* scores to be associated with 'toughmindedness' scores, is perhaps indicative of psychopathic tendencies which are known to be related genetically to psychosis and hence to *P* (ref. 9).

Although we claim to show some common basis for differences in personality and attitude, we stress that, for the scales in question, the greater part of the genetic and environmental variation is specific to particular traits. 'Radicalism' scores, for example, are totally independent of the measured personality variables, although they have significant associations with the other attitude measures.

Genotype–environmental interaction could inflate our estimates of both D_R and E_1. Some kinds of correlation between *G* and *E* may be discounted because the fit of the D_R, E_1, E_2 model implies that the total dispersions are homogeneous for the different kinds of twins. In many cases, however, genotype–environment correlation, if present, remains as a source of bias in our estimated genetic components.

Our model assumed that gene action is purely additive. Even without the complication of E_2 it would have been virtually impossible to identify any non-additive variation in our study. The bias in parameters estimated from twin studies in the presence of dominance is discussed elsewhere[7].

Extrapolation from these results to other populations and cultures is likely to be misleading because they are almost certain to differ with respect to the relative importance of different determinants of variation.

If we restrict ourselves to the British population and contemporary culture, we expect, on the basis of our simple genetic model, that attitudes in parents would be relatively poor predictors of attitudes in offspring. Parent–offspring correlations would be about $\frac{1}{2}ht$, given random mating, and thus to be roughly between 0.2 and 0.3. Many of the available familial correlations for attitudes (see for example, ref. 10) come from pre-war studies and show parent–child and sibling correlations which are much higher. The difference, if real, could reflect the consequences of cultural change during the last 30–40 yr. If individuality and mobility were not encouraged, common environmental (E_2) influences might play a significant part in maintaining the pattern of variation in attitude. In such a situation, parent–offspring correlations would resemble those for siblings and MZ twins. A culture which promotes individuality and mobility, however, may reveal a pattern of similariy between relatives which depends more obviously on genetic components and give greater significance to the environmental experiences which are unique to the individual, irrespective of his family environment. More recently, Insel[8] has obtained correlations between parents and offspring for the Wilson Conservatism Scale which are still too large to be consistent with a simple genetic model, so we cannot regard the issue as settled. The sensitivity of attitudes to cultural change may, however, make them a useful indication of the interdependence of gene expression and culture and encourage further research in this subject.

TABLE 4 Heritability estimates for personality and social attitude scales

Scale	$\hat{h}t$
R	0.65
T	0.54*
Em	0.37
P	0.35
E	0.48
N	0.49

* Based on pooling heterogeneous estimates for two sexes.

The computations were conducted on the University of Birmingham KDF-9 computer. We thank the Social Science Research Council and the Medical Research Council for grants, Mrs J. Kasriel for help in data collection, and Professor J. L. Jinks for helpful comments on the manuscript.

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[1] Eysenck, H. J., *The Psychology of Politics* (Routledge and Kegan Paul, London, 1954).
[2] Wilson, G. D., *The Psychology of Conservatism* (Academic, London, 1973).
[3] Eysenck, H. J., in *Human Behavior Genetics* (edit. by Kaplan, A. R.), (C. C. Thomas, Springfield, 1973).
[4] Eysenck, S. B. G., and Eysenck, H. J., *Br. J. Crim.*, **11**, 49 (1971).
[5] Cederlöf, R., Friberg, L., Jonsonn, E., and Kaij, L., *Acta Genet.* (Basel), **11**, 338 (1961).
[6] Jinks, J. L., and Fulker, D. W., *Psychol. Bull.*, **73**, 311 (1970).
[7] Eaves, L. J., and Gale, J. S., *Behavior Genetics*, **4**, (in the press).
[8] Insel, P. M., Thesis, Univ. London (1971).
[9] Eysenck, H. J., in *Genetic Factors in Schizophrenia* (edit. by Kaplan, A. R.) (C. C. Thomas, Springfield, 1972).
[10] Fuller, J. L., and Thomson, W. R., *Behavior Genetics* (John Wiley, New York, 1960).

Persistence of traditional beliefs among undergraduates

JAHODA[1], Pasachoff *et al.*[2], and Salter and Routledge[3] have investigated the hypothesis that university education leads to the decline of supernatural beliefs, arriving at strikingly similar results that contradict the hypothesis. We carried out an extension of these studies at our institution. Our investigations were aimed at an evaluation of the persistence of traditional, Eastern beliefs among undergraduates.

One hundred and forty science undergraduates (100 male and 40 female), chosen at random, answered 14 questions on astronomy, astrology, superstitions, evil spirits, auspicious time, pilgrimage and sacred animals (Table 1).

The returned questionnaires were first processed by the year of study and then by sex. The options to each question—certainly believe, sometimes yes, sometimes no and certainly do not—were allotted scores of 20, 11, 9 and 0 respectively (as in ref. 1 where 0 denoted 'total unqualified disbelief' and 20, 'total unqualified belief'). For each question these scores were summed and then the sum was divided by the number of respondents, giving a 'mean belief score'. There are two questions for each topic so by averaging the two mean scores an 'average belief score' for that topic is obtained.

TABLE 1 The questionnaire

This study seeks to evaluate the degree of traditional beliefs held by the students of our college. The answers shall be treated as confidential and it does not seek to evaluate individual students. Each of the questions below are provided with four options. Please indicate clearly by an 'X' the option most relevant to you.

Personal data:

(i) Year of graduate study: 1 yr, 2 yr or 3 yr.
(ii) Sex: male or female.

The respondents were then asked to answer certainly believe, sometimes yes, sometimes no or certainly do not to the following questions.

Astronomy:

1. Do you believe that the stars and planets have any influence over your daily routine?
2. Do you believe that the appearance of a comet or an eclipse brings bad luck?

Astrology:

3. Do you believe in consulting your partner's horoscope before settling for marriage?
4. Do you believe in palmistry and numerology?

Superstitions:

5. Do you believe that Friday, the 13th, is an unlucky day?
6. Do you believe that treading on one's nails would bring misfortune?

Evil spirits:

7. Do you believe that evil spirits dwell in the grave yard?
8. Do you believe in the power of witchcraft and charms?

Auspicious time:

9. Do you believe in an "auspicious time" before setting off on important ventures?
10. Do you believe that a child must be born at an "auspicious time" to be successful in life?

Pilgrimage:

11. Do you believe that the River Ganges has the ability to wash away your sins?
12. Do you believe that shaving your head fulfills your vow to God?

Sacred animals:

13. Do you believe that the peacock (bird) is a vehicle of God?
14. Do you hold snakes and cows to be sacred animals?

TABLE 2 Average belief scores by the year of graduate study

Question	First year	Second year	Third year	All years
Astrology	7.67	7.43	7.37	7.49
Astronomy	6.05	5.96	5.85	5.96
Superstitions	8.48	8.16	8.11	8.25
Evil spirits	7.77	7.75	7.73	7.75
Auspicious time	9.99	9.72	9.42	9.71
Pilgrimage	7.01	6.95	6.83	6.93
Sacred animals	7.07	6.95	6.62	6.88
Mean	7.72	7.56	7.42	7.567
No. of subjects	42	41	57	140

A score of 5 or above was taken to indicate a persistence of beliefs. For example, with the students overall, we found an average belief score of 5.96 for the traditions in astronomy and 9.71 for auspicious time (Table 2), indicating a greater persistence of the belief in auspicious time over the astronomical traditions.

The mean score for all students and for all the questions is 7.567, indicating the prevalence of traditional, Eastern beliefs to a marked degree among science undergraduates (Table 2). But, unlike the study at the University of Pennsylvania[3], there seems to be a difference in attitude between the sexes (Table 3). This may be attributable to both the environment and sociology of Indian society. Table 2 also shows a very gradual decline in beliefs with increasing time spent at the university.

TABLE 3 Average belief scores by sex

Question	Male	Female	Total
Astrology	6.77	8.21	7.46
Astronomy	5.81	6.11	5.96
Superstitions	8.16	8.34	8.25
Evil spirits	7.21	8.29	7.75
Auspicious time	9.36	10.06	9.71
Pilgrimage	6.84	7.02	6.93
Sacred animals	5.23	8.53	6.88
Mean	7.054	8.08	7.567
No. of subjects	100	40	140

High scores of 9.71 and 8.25 for auspicious time and superstitions, respectively, may reflect the deep-rooted nature of these beliefs. It was surprising that a mean score of 5.96 was recorded for astronomy, despite it being a finite science. The vast difference in scores for sacred animals between the sexes seems to be an example of the sex-differentiated attitude towards these beliefs.

In conclusion, although our study was limited to science undergraduates, the results are similar to earlier studies[1–3]. If traditional beliefs hinder scientific training, a way must be sought for their eradication to improve our activites in science.

We thank Dr George Abraham for support.

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1 Jahoda, G., *Nature*, **220**, 1356 (1968).
2 Pasachoff, J. M., Cohen, R. J., and Pasachoff, N. W., *Nature*, **227**, 971 (1970).
3 Salter, C. A., and Routledge, L. M., *Nature*, **232**, 278 (1971).

book reviews

Indian view of research

Economics of Research and Development. By P. M. Chowdhury. Pp. xii+163. (People's Publishing House: New Delhi, (1973.) Rs18.

AT a time when national expenditure on research in the United Kingdom has declined from the vigorous growth rates of the past two decades to the current provision which barely maintains the present level of activities there is a great deal of thought about the "economics of R and D". Mr Chowdhury's subject is therefore timely.

The publication, in one volume, of eleven essays chosen by the author from a decade of his writing "about the science of science" does not have the cohesion and logical development of thought which this difficult subject undoubtedly needs. Readers will wish that the author had found the time to condense some of the more generalised essays and to link to them more coherently. He does not communicate ideas very easily and has a rather irritating habit of generalising in the conditional tense, often using technical slang unnecessarily, but the persevering reader is rewarded with nuggets of down-to-earth commonsense and useful ideas.

Those who seek methods for the evaluation of any wide-ranging national programme of research will readily agree that there are "woeful gaps in the data system" and will confirm the difficulties of quantifying research output in terms of the production of scientific papers or of the registration of patents. Few of Mr Chowdhury's scientific readers are likely to share his confident assertion that the economist is nonetheless able to select research priorities and to assess research programmes.

In the early essays it becomes clear that the author is concerned mainly with industrial research, that is with product and process studies, which are more amenable to short-range planning and assessment than are the endless surprises of the biological world in agricultural research. The part which the author considers chance to play in research is revealing in this context, and most experienced research workers will be startled to read that "it is difficult to obviate the chance factor in research" and that "it has been assumed that the impact of the chance factor in R and D sector is more or less on par with that originating in the production process in other sectors". Emphasising the greater role of state involvement in R and D in developing, as compared with developed countries, the author concentrates heavily on the industrial sector and pays too little attention to the predominantly agricultural occupations of his fellow-countrymen. In both the United States and the United Kingdom the state involvement in agricultural R and D outweighs that of the private sector.

Mr Chowdhury takes a somewhat unflattering view of scientific research workers. The image of a research manager as one who "assumes the role of an erudite scientist, an efficient organiser or a smart salesman" would startle many senior scientific administrators, but it is uncomfortably near to the truth.

These essays show that India has encountered very much the same underlying problems in the organisation of science as have the more intensively developed countries, for example that "public sector research is structurally separated from industry". Indeed the major part of the reorganisation of British public research on the basis of Lord Rothschild's advice, in three major aspects of biology, those of medicine, agriculture and the natural environment, was to overcome this separation in a highly developed economy. Mr Chowdhury's solutions are similar to those of other countries: of technically efficient scientific advisory committees, with a large measure of independence and a long and stable lease of life. The British Industrial Research Associations would be in full agreement with the author's conclusion that "sponsorship of projects by industries may make them more conscious about programming aspect of the project".

The author concludes that in many developing countries research projects of national importance have failed to receive sufficient momentum for want of really capable personnel for managing them, which summarises effectively some two decades of premature campaigns for "localisation".

The eleven essays offer comments on planning, investment and programming in R and D, balance of payments and conservation of foreign exchange, employment of scientific and technical personnel and the perspectives of development.

In a brief but illuminating foreword by Dr B. D. Nag Chaudhuri, Director General of R and D in the Ministry of Defence, there is set out very clearly the dilemma of Indian scientists, cut off by their higher standard of living from the largely agricultural problems of the population, but uneasy in their uncertain relationships with the all-powerful administration.

These essays will interest all those concerned with developing countries and should be particularly valuable for those who strive to understand the vast complexities of the modern Indian scene. H. C. PEREIRA

The poetical chemist

The Wild Uncharted Country. By Deric Bolton. Pp. 48. (Outposts: Walton-on-Thames, Surrey, 1973.) 75p.

SCIENTIFIC autobiography in blank verse is a literary genre not often attempted, but this little book shows that it can be done successfully. Although the author's note tells us that the events described are imaginary, the links with real life are close. The protagonist of the poem (he is too unheroic to be called the hero) struggles to qualify at night school; then he has a spell of chemical research at South Kensington; enters industry in the 1930s; engages in "vital war work"; returns to the chemical industry after the war; retires in the 60s; and is now concerned with environmental and ecological issues, after a lifetime as

> An ignorant chemist whose ways of life had run
> Within deep narrow channels.

His life has flowed like a river, carrying him he knows not where; his career proceeded without self-guidance, seemingly by blind chance. After he retired, the stream broadened out into a flat expanse, letting him see at last the lie of the land around. The poem gains strength from our familiarity with this Neoplatonic imagery of the individual being borne as in a boat down the turbulent stream of life, used directly in Shelley's *Alastor* and obliquely in Coleridge's *Ancient Mariner*.

A poem on so mundane a topic depends crucially on the quality of the wording, a challenge which the author meets with a fair degree of success. The writing is careful and, although there are some dull patches, the narrative flows easily and has enough imaginative moments to hold the attention. The chief flaw is that the chemist himself is too much of a puppet, with

little will of his own and no private life (or none that is mentioned). But his 'discovery' of ecology at the end brings a touch of strong, almost Wordsworthian feeling:

So, finally I chiselled out this goal:
To learn to live with nature,
not oppose it,
Exploit, burn up and devastate:
To change from miners into
farmers . . .
. . . crashing down those barriers
which divide
Science from man and man from
natural
Things and man from man and all
that is
Of simple panoramic love and
permanence.

D. G. KING-HELE

Dangerous insects

Insects and Other Arthropods of Medical Importance. Edited by Kenneth G. V. Smith. Pp. xiv+561. +12 plates. (British Museum (Natural History): London, 1973.) £6.50.

THIS is a revised and expanded edition of an earlier book by Dr J. Smart, published in 1944, which dealt mainly with the fauna of the Old World and included little biological information. The present work is much larger, covers the whole world and is by 16 authors. The primary purpose of the book is still to enable non-specialists to identify to generic or subgeneric level arthropods, especially insects, of medical importance. Many species which are essentially parasites of animals are also included because many of them also attack man, if only on rare occasions. It will probably come as a surprise to the non-specialist to learn that several moths feed on eye liquids of vertebrates and that one is even capable of piercing the skin and sucking blood.

Three hundred of the 523 pages are devoted to the order Diptera whose importance has been greatly increased by the recognition of many new viruses. Of the 553 different combinations of vector and disease listed in their tables summarising the records, 10 involve bacteria, 47 nematodes, 90 protozoa, 11 rickettsias, 9 spirochaetes and 386 viruses (mostly carried by mosquitoes and recognised in the past 30 years). Many of these viruses are, of course, very localised and often quite unimportant and the long known diseases such as malaria, plague and typhus are much more serious. Nevertheless, the only arthropod groups of much importance in disease transmission besides the Diptera are the Siphonaptera (fleas) and the Acari (ticks and mites). Though disease transmission is the chief way in which insects are medically important, they may also be troublesome by biting or stinging, or by fragmenting into particles which are irritating or to which man may become allergic. Even scorpions which are relatively rare may kill 1,000 people, mostly young children, each year in Mexico.

The illustrated keys for the identification of all the more important groups are excellent and will be very useful to anyone with some knowledge of entomology. In most groups, there are also keys for the identification of the early stages (larvae and pupae). The information about the biology of the various groups and of the kind of trouble they cause is concise but sufficient, together with the long lists of references, to give a useful introduction to the subject.

Readers should note that a short mimeographed list of errata has been inserted in each copy. It is chiefly concerned with some errors in the names of groups of Acari in the table of disease vectors.

O. W. RICHARDS

Halides galore

The Chemistry of the Carbon-Halogen Bond. Edited by Saul Patai. Part 1: Pp. xiii+1-608; part 2: Pp. xiii+609-1215. (Wiley-Interscience: London and New York, December 1973.) £22 (two volumes).

Fluorine in Organic Chemistry. By R. D. Chambers. Pp. xv+391. (Interscience Morographs on Organic Chemistry.) (Wiley-Interscience: New York and London, January 1974.) £10.75.

THE first monograph deals with the thirteenth functional group to appear in Professor Saul Patai's monumental series, now within sight of completion. As with previous volumes, editorial policy has been to go to press without promised chapters that had not been received by a deadline. This time there are five casualties: formation of C–X bond; modern synthetic uses of organic halides; synthesis and uses of isotopically labelled halides; fluorocarbons (see below); and, finally, optical rotatory dispersion and circular dichroism of organic halides. Of these the first two are losses indeed.

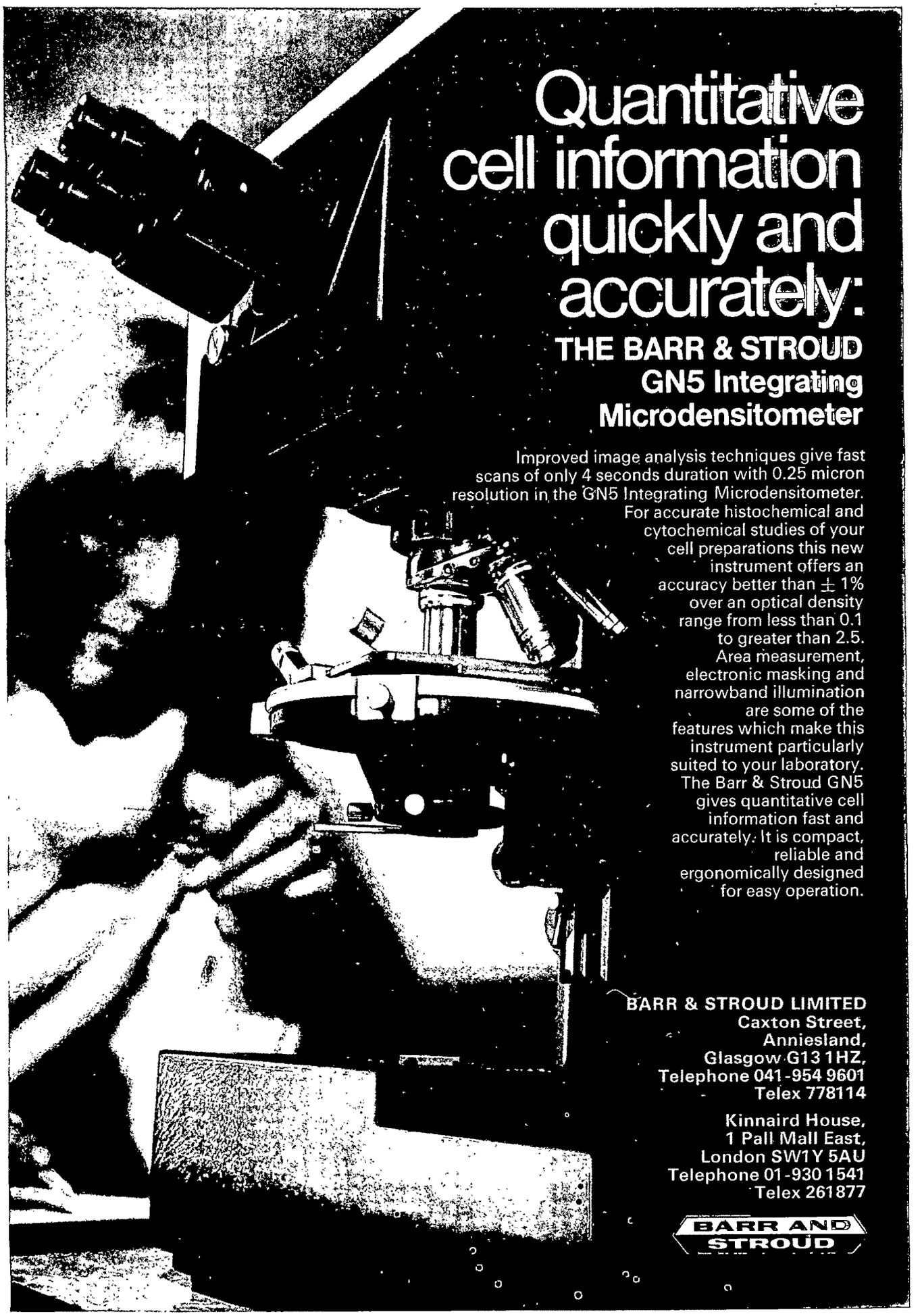
There are, however, plenty of interesting and valuable articles in what remains. Among them are: heterolytic mechanisms of substitution involving carbon-halogen bonds (P. B. D. de la Mare and B. E. Swedlund, 141 pages); directing, activating and deactivating effects (G. Modena and G. Scorrano, 106 pages); elimination reactions in solution (R. A. More O'Ferrall, 68 pages)—three excellent reviews on major aspects of the subject that are of general interest. There is also an interesting chapter on homolytic mechanisms of substitution (E. S. Huyser, 60 pages), but as this deals entirely with C–H→C–X, it seems to belong more appropriately under alkanes. Then there is a series of chapters dealing with more specialist aspects of the C–X linkage: mass spectrometry (A. G. Loudon, 42 pages), pyrolysis (K. W. Egger and A. T. Cocks, 70 pages), photochemistry (P. G. Sammes, 48 pages), radiation chemistry (R. E. Bühler, 70 pages), biochemistry (S. Doonan, 52 pages), electrochemistry (J. Casanova and L. Eberson, 70 pages) and thermochemistry (R. Shaw, 23 pages).

There is a very long chapter on analysis of organic halogen compounds (J. Zabicky and S. Ehrlich-Rogozinski, 160 pages) which, though interesting, seems to be overweight compared with space devoted to other topics. There are the usual introductory chapters on general and theoretical aspects of the carbon–halogen bond (G. H. Wagnière, 48 pages) and structural chemistry of the C–X bond (J. Trotter, 14 pages); and also one on hydrogen bonding and complex-forming properties (J. W. Smith, 36 pages). Then there are the topics that don't quite fit in anywhere else; perchloro-, perbromo- and periodo-compounds (T. Chivers, 62 pages), and, last of all, rearrangements involving halides (C. Raape, 44 pages—an interesting chapter with a good account of Favorskii and Ramberg–Bäcklund rearrangements.

All in all a good volume, though as with previous volumes one is often puzzled at the way in which space is allocated between the various topics. The reproduction of structural formulae is in general fairly good, and the layout relatively clear. Literature coverage seems to be very thorough, but to extend only into 1971 (the end?), with some citations extending into 1972 in a few articles.

The non-delivery of the fluorocarbon chapter in Professor Patai's collection is more than compensated for by the appearance of Dr Chambers' book. This does not set out to provide synoptic coverage of the subject, but seeks to place selective aspects in a general mechanistic framework, for example, total, and selected, fluorination; replacement of OH by F; nucleophilic displacement of F; eliminations; polyfluoroaliphatics and aromatics; organometallic compounds. It is an interesting, useful and well documented survey, but the author is ill served by bad layout and by poor unattractive representation of reaction schemes. Literature coverage is essentially till the end of 1971.

PETER SYKES



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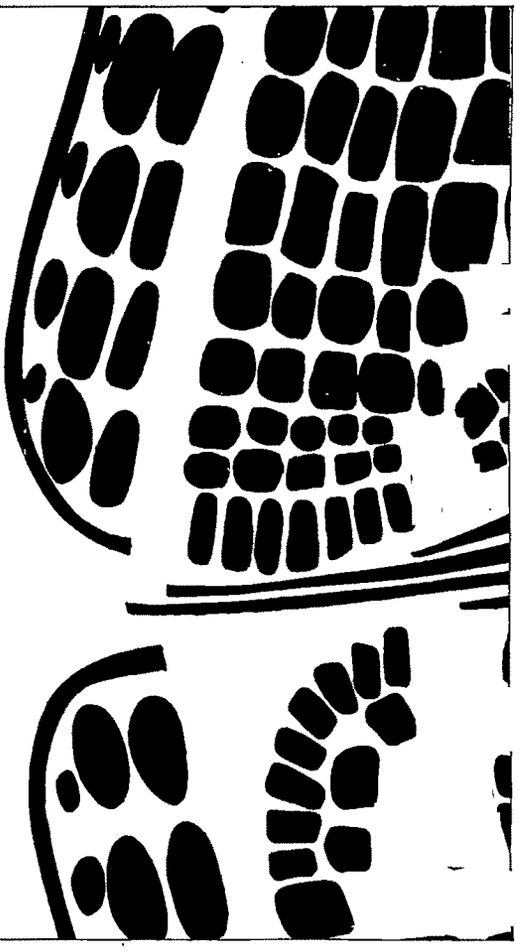
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Crystal defects

Computed Electron Micrographs and Defect Identification. By A. K. Head, P. Humble, L. M. Clarebrough, A. J. Morton, and C. T. Forwood. Pp. x+400. Defects in Crystalline Solids, vol. 7. (North-Holland: Amsterdam and London; American Elsevier, New York, 1973.) DFl 100; $35.10.

THE interpretation of electron micrographs of thin foils of crystalline material involves two radically different aspects: first, the morphology and orientation relationships of features such as precipitates, transition phases (for example, Guinier-Preston zones) and twins; second, the geometrical characteristics of crystal defects such as dislocations, stacking faults and point-defect aggregates. The first task involves simple examination of micrographs and matching of the diffraction patterns of selected areas, and offers no particular challenges to the experienced microscopist; the second is far more difficult, since the image (for instance) of a dislocation is really the image of a local distortion in the crystal lattice, and to identify the geometrical characteristics of the defect (Burgers vector, stacking parameters, and so on) requires the investigator to deduce the details of the lattice distortion from the appearance of the image under different imaging conditions. In fact, several images of the same defect are needed to interpret it securely.

The established way of identifying the Burgers vector of a dislocation is to find an imaging mode which causes the dislocation to disappear, but this method has limitations, especially with elastically anisotropic materials. The other method involves generating a theoretical image, with the aid of a computer program and pictorial print-out, for a particular set of imaging conditions, and comparing the theoretical image critically with that photographed in the electron microscope.

This book is the first detailed presentation of this computer technique, by a team of Australian authors led by A. K. Head, who originated the technique seven years ago. The book includes an outline of the two-beam dynamical theory which is the theoretical basis of the method; a full account, with many examples, of the practical steps involved in orienting a crystal, identifying the diffraction vector, dislocation line direction, and so on; the principles and details of the several computer programs, for both cubic and non-cubic crystals; and the methods for matching experimental and theoretical images. Finally the uses and limitations of the method are outlined.

The book is very much a practical operator's handbook; while its main use of course is to show the reader how to compute images, it will also be useful for the beginner who is learning how to orient foils and features in foils. Altogether, this is an impressive compilation by a group of pioneers who have stayed the course and finished the job.

R. W. CAHN

Quaternary fossil plants

Quaternary Plant Ecology. Edited by B. J. B. Birks and R. G. West. (The 14th Symposium of The British Ecological Society, University of Cambridge, 28–30 March, 1972.) Pp. ix+326. (Blackwell Scientific: Oxford and London, 1973.) £13.

THE relation between the ecology of plants, as seen today, and the study of fossil assemblages of plant remains from the Quaternary period has always been an exchange of information and ideas in both directions. It is generally realised that any attempt to interpret what an assemblage of fossil remains really represents, must depend on a knowledge and understanding of modern vegetation and the ecology of the constituent species. In return, the study of fossil assemblages leads to a much better appreciation of the extent to which the composition of present day vegetation depends on its history, both directly and indirectly through concurrent changes in the soil. The papers presented at this symposium are primarily concerned with the first relationship, that is, the ecology of the past but quite clearly a better knowledge of the past helps understanding of the present.

The first four sections, containing eleven papers, are directly concerned with methodology and the process of matching assemblages of pollen, or in one paper, other fossilised parts of plants, with modern vegetation. It is at once clear that a technological revolution has taken place as a consequence of the use of the proportions of carbon isotopes to give an independent estimate of the absolute age of a deposit. This allows the rate of deposition of a sediment to be calculated and thus pollen contents can be measured in absolute, rather than relative terms. This has stimulated a much more fundamental approach to the problem of relating the quantity of pollen deposited on unit area in unit time to the actual representation of species in the surrounding vegetation. Studies of the production of pollen, its dispersal in the air, its redistribution in water and its preservation are all included. These sections provide a fund of information and ideas which should be read by all palynologists.

The fifth section contains four papers concerned with the evidence from quaternary studies of the rates of change in vegetation over long periods, including both natural processes and changes for which man is largely responsible. The sixth section is devoted to limnological studies which demonstrate the value of interdisciplinary studies using palynology and stratigraphic analysis of a variety of aquatic organisms.

Symposia tend to be rather uneven in their coverage and often include papers of relatively light weight. The very opposite seems to be true in this case. Those who planned, organised and reported the meeting are to be congratulated on bringing together so many stars each of such magnitude into a single constellation and on producing an outstanding contribution to the literature of palaeoecology.

C. D. PIGOTT

Eccentric extracts

Strange Phenomena: A Sourcebook of Unusual Natural Phenomena, Vol. G-1. Compiled by William R. Corliss. Pp. 277. (Corliss: Glen Arm, Md 21057, 1974.) $6.95.

As the flow of letters we receive whenever *Nature* publishes anything about ball lightning testifies many of our readers are interested in bizarre phenomena at the fringe of scientific understanding. That is as it should be; only by such interest can any of these phenomena be brought within the fold of science proper, just as continental drift has gained respectability during this century. So the publication of this sourcebook is likely to meet with a warm response.

Corliss has collected what he calls "homeless facts" and given them a home, in the form of a collection of brief reports (some no more than 200 words) culled from the scientific, pseudoscientific and unscientific literature. The task is far from complete, and we are promised further volumes as the work progresses; the present edition is in the form of a ring-binder, so that necessary additions or deletions can be made conveniently.

Some examples culled from a random search of the pages suffice to give an impression of the book's scope: ball lightning, flames from the sky, earthquakes and lightning, strange detonations (barisal guns and the like), sunspots and rainfall, and so on. The march of science is such that the last of these phenomena has already been received into the bosom of 'serious' science, and most of the oddities which are mentioned are far from the lunatic fringe of flying saucers and little green men.

The net cast by Corliss has gathered a harvest from many sources, and *Nature* is lavishly represented. But it is a curious feature of the extracts from this journal in particular that they are clustered distinctly in time about two peaks, the first about 100 years ago and

the second in the past few years. This may reveal something about the collection technique used by Corliss, or about this journal, or about the general awareness of such phenomena and 'things that go bump in the night'; I doubt if it reflects a genuine distribution of such oddities. But the discovery of such a pattern shows just one example of the way in which the book can provide hours of harmless fun. At the price, it is excellent value for entertainment alone; there is also the added bonus that such a collection must inevitably include something which will become 'reputable' in the near future. Owners of the volume will then be able to say "Of course, I knew all about that back in 1974".

JOHN GRIBBIN

Plan the countryside

Rural Resource Development. By M. C. Whitby, D. L. J. Robins, A. W. Tansey and K. G. Willis. Pp. 243 (Methuen: London, January 1974.) £2.50 cloth; £1.30 paper.

THE main concern of the authors is to present ways of analysis which will lead to more effective public decision making and lead to the better use of rural resources. At present many of the decisions affecting the countryside are reached in an illogical way; because recommendations at officer level concentrate on only a selected scheme, because of political considerations, because of the influence and pressure of organisations such as the Country Landowners' Association, the National Farmers' Union, and strong amenity bodies.

This book gives the elements of the economic theory of resource allocation to provide a framework for understanding the economic processes at work in the countryside; private rural economy is well documented whereas public issues have received less attention. The authors explore in some detail the factors which do, as well as those which should, lead to decisions. They contend that most public agencies seem to pursue inappropriate objectives under incorrectly specified constraints, and that suboptimal decisions are taken because cash revenue and expenditure are used instead of the assessment of social costs and benefits.

There is a chapter on recreation, conservation and amenity which explores the general question of allocating land to physically non-productive uses and of measuring the less tangible consequences of such allocation decisions. There is discussion of the very difficult subject of estimating the amenity value of land.

There are useful chapters on the statutory British planning system and on population trends and transport in rural areas. A better understanding of all these matters would enable judgments to be made which used resources more efficiently, not only in cash terms, for society.

It can certainly be justly claimed that this is a useful source book for planners, landscape designers, students of environmental studies, agricultural economics and rural geography. There is an excellent list of references and a good index.

GEOFFREY BERRY

Free radicals

Free-radical Chemistry: Structure and Mechanism. D. C. Nonhebel and J. C. Walton. Pp. xv+572. (Cambridge University: London, January 1974.) £15; $35.

RAPIDLY growing scientific subjects periodically need exposition in comprehensive textbooks and it is many years since this subject has received an adequate broad survey that meets the requirements of both senior undergraduates and research workers. Recent quantitative measurements, mainly by gas chromatography and ESR spectroscopy, have so greatly enhanced knowledge of chemical reactions that publications of only 10 years ago are no longer factually correct and contain some theoretical deductions that are no longer fully valid.

This book from Scotland, which is now a very active region for research in free radical chemistry, meets a real need. It is a collaboration between a physical (Dr Walton) and an organic chemist (Dr Nonhebel) who have surveyed harmoniously physical measurements, gas reactions, reactions of atoms and simple alkyl radicals, as well as a wide range of more complex reactions in solution. They have paid particular attention to recent work and theories.

Polymer chemistry has been omitted for lack of space. But though polymerisation technology is still expanding its theoretical basis is changing but little and has been covered reasonably well in a long chapter on reactions of alkyl radicals.

A particularly long chapter is accorded to radical oxidations and reductions. This, a rapidly expanding sector of free radical chemistry, certainly merits its detailed treatment, but the text gives far too much space to phenol oxidation in comparison with autoxidation or possibly to reactions involving transition metal ions which, for students, deserved particular attention since they bridge the deleterious scholastic gap between inorganic and organic chemistry.

Only the price should detract from the wide use of this excellently produced book.

W. A. WATERS

Life in the sea

Biological Oceanographic Processes. By Timothy R. Parsons and Masayuki Takahashi. Pp. x+186. (Pergamon: Oxford and New York, October 1973.) £4.

THIS reads like the summary of an excellent four- or five-volume treatise that, unfortunately, has never been written. The authors insist that it is no more than an introduction to biological oceanography. A reader with no previous knowledge of the subject could, indeed, start from first principles. By page 10 he would reach the mathematics of diversity indices; four or five pages later he would cope with the application of statistics to the interpretation of samples; by page 21 he would have polished off biogeography and started on time-series variability.

The authors maintain this pace throughout. No new term is introduced without an excellent definition. One cannot even quarrel with their one non-definition: of detritus, they say that "while the word lacks definition it should not be regarded as lacking in importance" and then, having paused, off they go again and deal with the physics and chemistry of detritus in great depth—in four pages.

The book deals, almost exclusively, with near-surface pelagic populations, their environment and their ecological processes. Benthic, littoral and bathypelagic systems are excluded. Indeed, almost everything is excluded that cannot be expressed in quantitative terms—and preferably summarised as an equation.

Some sections are superficial but what else can one expect in a book of such breadth? There are chapters dealing with the distribution and chemical composition of plankton, nutrients and seawater, heterotrophic and autotrophic processes, plankton feeding and production, organic and inorganic cycles and the transfer of energy and materials in the food chain. The authors claim that it is not a literature review but nearly 700 well-chosen references, alone, justify the purchase price of the book.

The last chapter, "some practical problems in biological oceanography", is trivial and unnecessary. Otherwise, this is an excellent book for professional oceanographers, biologists or, indeed, as the authors put it, "scientists in other professions who may require some quantitative expressions of biological oceanographic phenomena".

R. S. GLOVER

obituary

O. M. B. Bulman

PROFESSOR OLIVER MEREDITH BOONE BULMAN, FRS, who died at the age of 71 on February 18 was generally recognised as the leading authority on the graptolites, the group of colonial organisms which came into being, flourished and died out in the Lower Palaeozoic.

Bulman was trained as a geologist at Imperial College where he remained to carry out research under the direction of W. W. Watts. Among fellow students investigating various aspects of the Palaeozoic rocks of Britain at that time were Stubblefield, Whittard, Mitchell, Howell and David Williams all of whom were to hold posts of distinction.

Bulman has recorded that he was first attracted to the graptolites by beautifully preserved specimens of *Dictyonema* in the Sineton shales, on which he and Stubblefield were working; and he took this as the subject for his first palaeontological investigations. From the first, he concentrated on well preserved material which he prepared and figured with great skill. In this way he was able to establish details of the external form, and in many instances, of the internal structure. With this new information he was particularly sucessful in elucidating the early stages of the growth of graptolites. When he completed his tenure of a Senior Studentship of the Royal Commission for the Exhibition of 1851, it was said that only a man of exceptional talent could have produced the results he had by then achieved.

After holding demonstratorships in the Zoology and Geology Departments at Imperial College, Bulman was appointed in 1931 to a post in the Sedgwick Museum where he remained for the rest of his academic life. In 1945 he became Reader in Palaeontology and in 1955 was appointed Woodwardian Professor of Geology. In 1945 he was elected a Fellow of Sydney Sussex College. He was President of Section C of the British Association in 1959 and received in succession the Lyell Fund and the Lyell Medal from the Geological Society of which he was President from 1962–64. He was connected with the *Geological Magazine* either as Editor or its advisor for 40 years.

Throughout his career the study of graptolites remained his principle scientific concern. He continued as he had begun with a series of meticulous accounts which covered graptolites not only from Britain, but from North America, Scandinavia and Asia. He acquired an immense knowledge of their worldwide distribution which he put to use in his discussions of their mode of life and of the steps by which the graptolites evolved. He expressed his conclusions cautiously leaving the reader in no doubt where uncertainty existed.

Whatever the future may hold Bulman's part in the elucidation of the graptolites is likely to remain as a landmark in studies of Palaeozoic life.

Announcements

Appointments

The following are the members of the government select committee on science and technology: **R. Brown, R. Carter, J. Cunningham, N. McFarlane, A. Fletcher, D. Ginsberg, F. Hooley, T. Ledbetter, I. Lloyd, A. Neave, A. Palmer, N. Tebbit, C. Tyelar and K. Warren.**

D. J. Lyons has been appointed a member of the **Science Research Council.**

E. J. Denton has been appointed **Director** of the **Plymouth Laboratory** and **Secretary** of the **Marine Biological Association of the United Kingdom.**

G. R. Bainbridge has been appointed to the Chair of Energy Studies at the **University of Newcastle upon Tyne.**

I. R. Smith has been appointed to a chair in the Department of Electronic and Electrical Engineering at **Loughborough University of Technology.**

R. N. Parkins has been appointed to the William Cochrane Chair of Metallurgy and Engineering Materials at the **University of Newcastle upon Tyne.**

C. McGreavy has been appointed to a second chair in the Department of Chemical Engineering at the **University of Leeds.**

G. T. Stevenson has been appointed a professor of the **University of Southampton.**

D. K. Bailey has been appointed Professor of Geology at the **University of Reading.**

The following have been elected **Foreign Members** of the **Royal Society: R. Dulbecco, G. H. Hitchings, G. P. S. Occhialini** and **J-P Serre.**

The following have been elected **Members** of the **National Academy of Sciences: R. D. Alexander, E. Anders, R. C. Atkinson, K. F. Austen, W. O. Aydelotte, J. W. Backus, D. Baltimore, L. M. Beidler, F. R. Boyd, Jr, M. K. Brakke, E. Braunwald, W. R. Briggs, T. C. Bruice, S. J. Buchsbaum, O. L. Chapman, J. A. Clements, G. L. Closs, W. G. Cochran, C. C. Cockerham, L. J. Cronbach, H. W. Davenport, C. A. Finch, W. H. Flygare, H. L. Fraenkel-Conrat, R. Freedman, J. Furth, D. C. Gajdusek, E. P. Gteiduschek, I. Giaever, M. Gibbs, R. R. Gilruth, H. H. Goldstine, L. A. Goodman, R. W. Gould, K. I. Greisen, J. Gross, R. C. L. Guillemin, C. F. Hockett, H. S. Houthakker, F. S. Hulse, L. Hurwicz, J. Hurwicz, J. D. Isaacs, A. Javan, E. V. Jensen, E. R. Kandel, B. Kok, N. M. Kroll, H. D. Lasswell, P. F. Lazarsfeld, W. Leontief, E. B. Leopold, A. P. Lerner, R. Levins, D. L. Lindsley, Jr, F. J. Low, C. R. Lynds, R. S. MacNeish, J. L. Margrave, C. F. Mosteller, G. D. Mostow, H. J. Müller-Eberhard, H. N. Munro, T. M. Newcomb, A. B. Novikoff, J. P. Ostriker, C. K. N. Patel, R. G. Pearson, W. G. Pfann, L. J. Postman, D. M. Prescott, C. L. Prosser, A. E. Puckett, S. Ratner, W. H. Riker, H. Ris, H. E. Robbins, A. Robinson, G. W. Salisbury, R. Schmid, T. W. Schultz, W. R. Sears,**

I. I. Shapiro, R. G. Shulman, L. T. Silver, E. M. Stein, De-W. Stetten, Jr, P. Teitelbaum, H. M. Temin, B. L. Vallee, K. M. Watson, J. S. Waugh, G. W. Wetherill, B. Widom, J. Wolfowitz, and **J. B. Wyngaarden.**

Awards

The **Wellcome Prize** for 1974 has been presented to **J. H. Wilkinson.**

The **National Academy of Sciences** has presented the **Henry Draper Medal** to **L. Spitzer, Jr;** the **United States Steel Foundation Award in Molecular Biology** to **D. Baltimore;** the **Gibbs Brothers Medal** to **P. Eisenberg;** the **Selman A. Waksman Award** to **R. Dulbecco;** and the **NAS Award in Environmental Quality** to **G. E. Hutchinson.**

The **1974 Lenin Prizes** for science and technology have been awarded to: **L. V. Keldysh, Y. G. Abov, P. A. Krupchitskii, V. M. Lobashev, V. A. Nazarenko, A. V. Kirsanov, N. V. Belov, A. I. Opanrin, I. I. Mints, V. D. Timakov, G. Ya. Kagan, A. I. Nesterov, E. M. Tareev** and **A. I. Strukov.**

Miscellaneous

The **George Von Hevesy Prize** will be awarded for papers on the subject of Nuclear Medicine at the First World Congress of Nuclear Medicine in Tokyo (Professor W. Horst, Clinic of Nuclear Medicine, University of Zurich, Ramistrasse 100, 8006 Zurich, Switzerland).

The **Edna McConnell Clark Foundation** of New York announces a programme of support for research projects on schistosomiasis (Dr Donald B. Hoffman, Jr, Vice President, The Edna McConnell Clark Foundation, 250 Park Avenue, Suite 904, New York, New York 10017).

Erratum

In the article "Additional location of metalliferous sediments in the Red Sea" by R. D. Bignell *et al.*, (*Nature* **248,** 127; 1974), as the result of a printer's error, the following corrections should be made. Page 128, line 15 should read "possibility exists of further deposits at depth. Analysis of . . ." and line 29 should read "possibility that they formed as a result of the oxidation of . . .".

International Meetings

May 22, **Herbal Remedies in Europe** (Professor J. M. Rowson, Pro-Vice-Chancellor, University of Bradford, Bradford, UK).

Reports and publications

Great Britain

Arthritis: a Vitamin Deficiency Disease. By E. C. Barton-Wright. Pp. 32. (London: United Trade Press, 1973.) £1.25. [42

National Radiological Protection Board. Code of Practice for the Display of Sources of Ionizing Radiations at Exhibitions. Pp. 13. (London: HMSO, 1973.) 18 p net. [42

Wildfowl 24. Edited by G. V. T. Matthews and M. A. Ogilvie. Pp. 176. (Oxford and London: Blackwell Scientific Publications, 1973. Published for the Wildfowl Trust, Slimbridge.) £2; 6.50. [42

Scotland Today—Economic Developments. By W. S. Robertson. (The Betts Brown Memorial Lecture). Pp. 21. (Edinburgh: Heriot-Watt University, 1974.) 50p. [42

Annual Report of the National Museum of Wales, 1972/1973, Pp. 158. (Cardiff: National Museum of Wales, 1973.) [42

Mercury as an Environmental Pollutant—a Bibliography. By D. Taylor. Pp. 135+78. (Brixham, Devon: Imperial Chemical Industries, Ltd, 1973.) [42

Natural Environment Research Council—National Institute of Oceanography. Collected Reprints Nos. 831–885. (Wormley, Godalming, Surrey: National Institute of Oceanography, 1972.) [42

The Art of Observation. By Charles H. Gibbs-Smith. Pp. 24. (London: Bedford Square Press of the National Council of Social Services, 1973. Distributed by RPS, Victoria Hall, East Greenwich, London SE10 ORF.) 30p. [62

The Wellcome Trust. Interim Report, 1972/1973. Pp. 11. (London: The Wellcome Trust, 52 Queen Anne Street, London W1, 1974.) [62

The Clean Air Year Book for 1974. Pp. 120. (Brighton: National Society for Clean Air, 136 North Street, 1974.) £1.25. [62

Freshwater Biological Association. Scientific Publication No. 18: A Key to the British Freshwater Cyclopid and Calanoid Copepods. By J. P. Harding and W. A. Smith. Second edition. Pp. 56. (Ambleside, Westmorland: Freshwater Biological Association, 1974.) [72

Other countries

Stockholm International Peace Research Institute. Report of Activities 1972/1973. Pp. 27. (Stockholm: Stockholm International Peace Research Institute, 1974.) [12

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Paper 73-5: Current Research in the Geological Sciences in Canada, 1972–1973. Compiled by Thomas E. Bolton. Pp. vi+193. $2. Paper 73-42: Fossil Dinoflagellates—Index to Genera and Species. By J. K. Lentin and G. L. Williams. Pp. 176. $2. Bulletin 225: Quaternary Stratigraphy of the Moose River Basin, Ontario. By R. G. Skinner. Pp. 77 $3. (Ottawa: Information Canada, 1973.) [12

United States Department of the Interior: Geological Survey. Professional Paper 818-A: Hydrothermal Alteration Association with Beryllium Deposits at Spor Mountain, Utah. By David A. Lindsey, Harold Ganow and Wayne Mountjoy. Pp. iii+20. (Washington, DC: Government Printing Office, 1974.) 75 cents. [42

United States Naval Observatory. Circular No. 145: Normalized Observations of Mercury 1901–1937. By P. M. Janiczek, P. K. Seidelmann and W. D. McClain. Pp. 35. (Washington, DC: United States Naval Observatory, 1973.) [42

Smithsonian Contributions to Zoology. No. 146: The Families and Genera of Hyperiidea (Crustacea: Amphipoda). By Thomas E. Bowman and Hans-Eckhard Gruner. Pp. iv+64. $1.35. No. 161: Five New Bomolochid Copepods Parasitic on Indo-Pacific Clupeid Fishes. By Roger F. Cressey and Hillary Boyle. Pp. 25. 60 cents. (Washington, DC: Smithsonian Institution Press, 1973. For sale by US Government Printing Office.) [42

Einstein's Special Theory of Relativity: Right or Wrong? By Ian McCausland. Pp. 17. (Toronto: Ian McCausland, 401 Castlefield Avenue, 1973.) [42

Canada: Ministry of State. Federal Scientific Resources 1972 to 1974—Natural and Human Sciences. Pp. 95. (Ottawa: Information Canada, 1973.) [52

Ministère de la Santé Publique et de l'Environnement. Institut d'Hygiène et d'Epidémiologie. Institute Royal Météorologique de Belgique. Publications Série A, No. 84: La Pollution Atmosphérique par les Oxydes de Soufre et al Fumée en Belgique au cours de la Période du 1er Avril 1971 au 31 Mars 1972. Par A. Derouane, J. Grandjean, M. Legrand et G. Verdyun. Pp. 65. (Bruxelles: Institut Royal Météorologique de Belgique, 1973.) [52

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Fisheries and Oceanography, 1971/1972. Pp. 59. (Cronulla, NSW:CSIRO, 1973.) [62

Stochastics, Vol. 1, No. 1, 1973. Edited by R. S. Bucy. Pp. 1–128. Subscription rates (per volume of four issues postpaid): Libraries, USA/elsewhere $41; £17.08; Great Britain £15.50. Individuals (who warrant the journal is for their own use and order direct from the publisher), USA/elsewhere $14.50; £6; Great Britain £5.50. (New York, London and Paris: Gordon and Breach Science Publishers, 1973.) [62

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Minerals Research Laboratories, 1972/1973. Pp. 56. (East Melbourne: CSIRO, 1973.) [62

Académie Royale de Belgique. Mémoires de la Classe des Sciences. T.XLI, Fasc. 1: Contribution á la Spectroscopie Photoélectronique—Effets de l'Autoionisation dans les Spectreas Photoélectroniques de Molécules Diatomiques et Triatomiques. Par Paul Natalis, Pp. 60. (Bruxelles: Académi Royal de Belgique, 1973.) [62

Australia: Commonwealth Scientific and Industrial Research Organization. Biennial Report of the National Standards Laboratory, 1971/1973. Pp. 114. (Sydney: CSIRO, 1973.) [62

The Pasteur Institute of Southern India, Coonoor. Annual Report of the Director 1971, and Scientific Report 1972. Pp. 160. (Coonoor, S. India: Pasteur Institute, 1973.) [82

Records of the Australian Museum, Sydney. Vol. 28, No. 19: Broom Cave *Cercartetus,* with Observations on Pygmy Possum Dental Morphology, Variation, and Taxonomy. By William D. Turnbull and Frederick R. Schram. Pp. 437–464. A $1.75. Vol. 29, No. 1: Observations on Sexual Behaviour in Some Australian Platystomatidae (Diptera: Schizophora). By David K. McAlpine. Pp. 1–10. 50c. (Sydney: The Australian Museum, 1973.) [82

Bulletin of the American Museum of Natural History. Vol. 152, Article 6: Morphology and Speciation of Hawaiian and Eastern Pacific Smittinidae (Bryozoa, Ectoprocta). By Dorothy F. Soule and John D. Soule. Pp. 365–440. (New York: American Museum of Natural History, 1973.) $3.50. [82

Reakcie Fotyczne u Much. By Jerzy A. Chmurzynski. Pp. 94. (Warszawa: Instutut Biologii Doswiadczalnej im. Marcelego Nenckiego Pan, 1973.) [112

Materiaux pour de Leve Geobotanique de la Suisse. Fasc. 54: Contribution a l'Etude Phytosociologique et Ecologique du Haut Jura. Par Claude Beguin. Pp. 190. (Bern: Editions Hans Huber, 1972.) [112

Towards a Definitive Solution of Zeno's Paradoxes. By F. A. Shamsi. Pp. 84. (Karachi: Hamdard Academy, 1973.) Rs. 20; US$2. [122

Options: a Study Guide to Ponulation and the American Future. Pp. ii+75. (Washington, DC: Ponulation Reference Bureau, Inc., 1973.) [142

United States Department of the Interior: Geological Survey. Bulletin 1391-A: Mineral Resources of an Area near the Popo Agie Primitive Area, Fremont County, Wyoming. By Roberrt C. Pearson, Lowell L. Patten and David L. Gaskill. Pp. 18+1 plate. $1.30. Water-Supply Paper 2124: Surface Water Supply of the United States, 1966–70. Part 9: Colorado River Basin. Vol. 1: Colorado River Basin Above Green River. Pp. viii+543. $2.95. (Washington, DC: Government Printing Office, 1973.) [152

Smithsonian Institution. Contributions from the United States National Herbarium, Vol. 38, Part 6: Studies of Fern Types, II. By C. V. Morton. Pp. 215–281. (Washington, DC: Smithsonian Institution Press, 1973.) [152

United Nations—Economic Commission for Africa. African Regional Plan for the Application of Science and Technology to Development. (World Plan of Action.) Pp. 135. (New York and Geneva: United Nations, 1973.) $3.50. [182

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Paper 73-10: Three New Lower Paleozoic Formations of the Boothia Peninsula Region, Canadian Arctic Archipelago. By R. L. Christie. Pp. v+32 (8 plates). $2. Paper 73-26: Palynology and Nannofossil Processing Techniques. By M. S. Barss and G. L. Williams. Pp. v+25 (2 plates). $2. Paper 73-35: Use of a Geological Field Data Collecting Form on Operation Bylot, North-Central Baffin Island, 1968. By E. W. Reinhardt and G. D. Jackson. Pp. viii+27. $2. (Ottawa: Information Canada, 1973.) [182

United States Department of the Interior: Geological Survey. Water-Supply Paper 1974: Water Resources of the New Jersey Part of the Ramapo River Basin. By John Vecchioli and E. G. Miller. Pp. vi+77 (4 plates). $2.20. Water-Supply Paper 2117: Surface Water Supply of the United States, 1966–1970. Part 6: Missouri River Basin. Vol. 2: Missouri River Basin from Williston, North Dakota, to Sioux City, Iowa. Pp. 612. $3.20. Professional Paper 809-B: Location and Characteristics of the Interface Between Brine and Fresh Water from Geophysical Logs of Boreholes in the Upper Brazos Basin, Texas. By W. S. Keys and L M. MacCary. Pp. iii+23. 60 cents. (Washington, DC: US Government Printing Office, 1973.) [182

US Department of Commerce: National Oceanic and Atmospheric Administration. Publication 41-1: Earthquake History of the United States. Edited by Jerry L. Coffman and Carl A. von Hake. Revised edition (through 1970). Pp. vi+208. (Washington, DC: Government Printing Office, 1973.) $2.80. [182

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APPOINTMENTS VACANT

UNIVERSITY OF LEICESTER

The Department of Physiology has a vacancy for a Laboratory Technician Grade III (salary scale £1,650 to £1,920) for research and teaching practical duties. Applicants should have ONC/OND or equivalent qualifications and have had 3 to 5 years laboratory experience. The work will largely consist of electrophysiological techniques and a good knowledge of electronics is essential.

Applications with full curriculum vitae together with the names and addresses of 2 referees should be sent to the Chief Technician, Department of Physiology, University of Leicester, Leicester, LE1 7RH, as soon as possible. (1814)

SENIOR TECHNICIAN

required to assist and supervise research haematology laboratory. Whitley Council Scales and Conditions. Applications in writing enclosing curriculum vitae and the names of two referees to the Secretary, University College Hospital Medical School, University Street, London WC1E 6JJ quoting reference ERH/ST. (1816)

ST. MARY'S HOSPITAL MEDICAL SCHOOL
RESEARCH TECHNICIAN

required in Department of Experimental Pathology to form one of team studying effects of drugs in cancer and organ transplantation. Commencing salary within scale £1,440 to £1,664 plus £126 London Weighting p.a. Apply, The Secretary, St. Mary's Hospital Medical School, Paddington, London W2 1PG quoting Ref. EPN2. (1830)

UNIVERSITY OF NOTTINGHAM
DEPARTMENT OF PHYSICS

Applications are invited for two postdoctoral Research Assistants, sponsored by the Science Research Council. The first is for theoretical work, under Dr. C. A. Bates, on Spin-Phonon Interactions, and the second is for theoretical work, under Professor K. W. H. Stevens, on Many-Electron Theory of Magnetic Materials. Experience in related fields is desirable. Salary, according to age and experience, on the Lecturer scale.

Applications in writing giving full details of qualifications and experience to Staff Appointments Officer, University of Nottingham, University Park, Nottingham NG7 2RD as soon as possible. Names of two referees should be given. (1818)

UNIVERSITY OF BERN
Switzerland
INSTITUTE OF GENERAL MICROBIOLOGY

A Post-doctoral Research Assistant is required to work on the analysis of yeast-tRNA as studied in mutants defective in the structural and modificator genes of suppressor tRNA. Previous experience in the field is necessary. The post is for up to two years with a minimal salary of Swiss Fr 2500 monthly and possibilities of renewing, commencing on or after May 1974. Write with a curriculum vitae and the names of two referees to Dr. R. Fluri, Institute of General Microbiology, Altenbergrain 21, CH-3013 Bern, Switzerland. (1836)

UNIVERSITY OF BIRMINGHAM
WEST MIDLAND REGIONAL HEALTH AUTHORITY

M.Sc., Course in Clinical Biochemistry

Applications are invited for the appointment of twelve basic grade biochemists for a two-year course for the M.Sc. Degree in Clinical Biochemistry commencing on October 1, 1974, and based on the following hospitals:

Coventry and Warwickshire Hospital, Coventry
Dudley Road Hospital, Birmingham
East Birmingham Hospital, Birmingham
General Hospital, Burton-on-Trent
Good Hope Hospital, Sutton Coldfield
Guest Hospital, Dudley
Royal Salop Infirmary, Shrewsbury
North Staffordshire Royal Infirmary, Stoke-on-Trent
Royal Hospital, Wolverhampton
Selly Oak Hospital, Birmingham
Warwick Hospital
Worcester Royal Infirmary

The course is organised by the Departments of Clinical Chemistry and Biochemistry. The course is open to probationary basic grade biochemists in the National Health Service and other selected candidates who must possess a 1st or 2nd Class Honours Degree in Chemistry or Biochemistry or hold an equivalent qualification.

The object of the course is to provide a thorough training in all aspects of clinical biochemistry than is possible in the usual two years probationary period.

Initial selection will be by the University and successful candidates will then be interviewed by the area Health Authority concerned of whose staff the candidate will be a member. The University course fee of £45 will be paid by individual candidates, otherwise salary paid in accordance with approved N.H.S. scale.

Application forms from the Assistant Registrar (Science), University of Birmingham, Edgbaston, Birmingham B15 2TT, to be returned by May 31, 1974. (1825)

At the

DEPARTMENT of PHYSICS and ASTRONOMY

a position is vacant for a

full Professor of Technical Physics

who will undertake **research** and **education** in fields related to the **energy problem,** including its **environmental aspects.**

Applicants should have conducted research of outstanding quality in physics or an allied discipline and should have shown actual interest, and preferably have participated in the above fields. Industrial or comparable experience would be an advantage. Candidates should be interested in interdisciplinary studies and have a capacity for, and preferably experience with teaching. Knowledge of the Dutch language is not a prerequisite.

Resources are available to develop an adequate research programme to be carried out by Ph.D. candidates.

Details of the salary, of the conditions of service, and of the method of application, may be obtained by writing to Professor C. Th. J. Alkemade, Physical Laboratory, Sorbonnelaan 4, University, Utrecht, The Netherlands. (Tel. No. 030-533302). (1846)

UNIVERSITY OF BRISTOL
Long Ashton Research Station

HEAD OF HOME FOOD PRESERVATION SECTION

Applications are invited for the appointment of a graduate to Head a small team, financed by the Ministry of Agriculture, Fisheries and Food, that is concerned with improving methods of home food preservation, particularly freezing, and other aspects of the domestic handling and storage of foodstuffs.

The work includes analysis, investigations and experiments leading to recommendations and advice to users, and the dissemination of information by publications, lectures and short courses.

Applicants should have had experience in the food field and hold a good science degree, preferably in Biochemistry, Food Science or Nutrition or equivalent qualifications. Lecturing and contact with the public will be the special responsibility of the successful candidate.

Appointment will be made in the Principal Scientific Officer grade (salary at present under review—£3,715 to £4,895 per annum). Superannuation under F.S.S.U. with allowance to offset contributions.

Applications to the Secretary, Research Station, Long Ashton, Bristol BS18 9AF, by June 11, 1974. (1822)

IRELAND

Biochemist Principal Grade

South Eastern Health Board

Salary: £3,039 to £4,051.

Essential: M.Sc. in Biochemistry or Physiological Chemistry or equivalent and five years experience in Clinical Biochemistry and/or Chemical Pathology in the laboratory of a hospital or allied institution.

For application forms and further particulars write to: **Secretary, Local Appointments Commission, 45 Upper O'Connell Street, Dublin 1.**

Latest date for receiving completed application forms:

30th May 1974

(1839)

UNIVERSITY OF DUBLIN
Trinity College

CHAIR OF OBSTETRICS AND GYNAECOLOGY

Applications are invited for the above full-time appointment. The Professorial Unit is situated in the Rotunda Hospital and the Professor also has facilities for Gynaecology at the Adelaide Hospital.

Further details concerning this appointment may be obtained from:

Mr. G. H. H. Giltrap,
Secretary to the College,
Trinity College,
Dublin 2.

to whom completed applications should be returned if possible, by the 5th June, 1974. (1843)

UNIVERSITY OF ABERDEEN
Research Assistant in Genetics

Applications are invited for the above post. The work involves electrophoresis, ultracentrifugal analysis and quantitative estimation of plasma lipoproteins. Appropriate experience and qualifications in Biochemistry or Chemistry are desirable.

Salary on scale: £1,662 to £2,304 with appropriate placing.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by May 25, 1974. (1837)

UNIVERSITY OF ST. ANDREWS
DEPARTMENT OF ZOOLOGY

Applications are invited for a post of RESEARCH ASSISTANT to work on the genetic control of the expression of cell-surface antigens in protozoa. Candidates should have a good honours degree in Biology. Experience in cell culturing, microbiology or immunology would be an advantage. The post is financed by the Medical Research Council and is tenable for three years from September 1, 1974; salary scale £1,500 by £150 to £1,800 (under review).

Applications, giving details of education and qualifications together with the names of two referees, should be lodged with the Establishments Officer of the University, College Gate, St. Andrews, Fife KY16 9AJ by May 31, 1974. (1832)

AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited for the following:

JOHN CURTIN SCHOOL OF MEDICAL RESEARCH
RESEARCH FELLOW IN MICROBIOLOGY

Groups in the Department of Microbiology work on different aspects of animal virology and immunology. The Department is well equipped for all types of biochemical and biological studies in these fields. Preference will be given to candidates with experience in work on the nucleic acids of viruses, particularly by electron microscopic techniques. Applicants should have a Ph.D. degree and some postdoctoral experience.

Enquiries on academic aspects of this position should be addressed to Professor G. L. Ada, F.A.A., Head of the Department of Microbiology in the University. Closing date: **June 7, 1974.**

SENIOR RESEARCH FELLOW IN PHYSIOLOGY

Appointment will be in the Department of Physiology (Head: Professor P. O. Bishop, F.A.A.). The successful applicant will be expected to work in the general field of comparative neurophysiology of vision. Closing date: **June 7, 1974.**

FACULTY OF SCIENCE
POSTS IN THE DEPARTMENT OF FORESTRY

Two posts are available in the Department. (Head: Professor J. D. Ovington), one at the level of **Lesturer** and the other at the level of **Tutor.** The appointees will be required to take up duty as soon as possible. Applicants should be graduates in science or forestry having appropriate experience in one of the following topics: Dendrology, Fire, Soils and some aspect of Tropical Forestry. Closing date: **June 10, 1974.**

SALARIES: Salary on appointment will be in accordance with qualifications and experience within the ranges: Senior Research Fellow $A12,778 to $A15,116 p.a.; Research Fellow $A8,698 to $A11,901 p.a.; Lecturer $A8,698 to $A11,982 p.a.; Tutor $A5,740 to $A7,015 pa.; current exchange rates are approximately $A1: 67p; $US1.49.

OTHER CONDITIONS: Tenure—Senior Research Fellow and Research Fellow normally for three years in the first instance with the possibility of extension to a maximum of five years; Lecturer for three years in the first instance with provision for reappointment to retiring age; Tutor normally for twelve months in the first instance with normal expectation of yearly renewal up to five years.

Reasonable travel expenses are paid and assistance with housing is provided for an appointee from outside Canberra. Superannuation is on the F.S.S.U. pattern with supplementary benefits.

Travel expenses from overseas (except New Zealand) are not provided for posts at the level of tutor but assisted passage can be considered for a person offered appointment from the United Kingdom who intends to settle permanently in Australia.

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should write to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF for further particulars before applying. (1828)

Senior Microbiologist

Chemotherapeutic Research from £4000

This is a research management appointment for a graduate who has several years' chemotherapeutic experience in an industrial environment, and who now feels ready and able to take on a more senior position.

Our client is a large and well-known pharmaceutical company with a firmly established reputation in the field of antibiotics research. The present appointment is a completely new one, and it arises at a time when the Company is committed to a substantial research expenditure programme over the next few years.

The laboratories are extensive and well-equipped, and are located in a pleasant country area of Southern England.

The man (or woman) appointed will manage a team of about four people who are responsible for the detailed evaluation of potential new human antimicrobial agents – after they have successfully passed the Company's primary screening programme. His group will progress promising compounds to the stage where the Project Committee, of which he will be a member, can decide whether or not human trials are justified. When they are, he will play an important role in coordinating laboratory studies with the human volunteer and patient studies.

The work will involve extensive interdisciplinary cooperation with chemists, clinicians and other research teams, and may necessitate some travel within Europe.

Applicants should be graduates within the approximate age range 30–40 and with several years' experience of antimicrobial research in the pharmaceutical industry. A higher degree is not essential, but evidence of good research ability will certainly be sought. A wide knowledge of *in vivo* as well as *in vitro* test systems is called for, together with the capacity to develop new models and procedures.

Starting salary is likely to be at least £4000, but could be substantially higher for a particularly suitable candidate. Other benefits include a bonus scheme, contributory pension, free life assurance, four weeks' holiday and generous removal and relocation expenses.

If you meet our requirements, please write or 'phone in complete confidence for additional information and an application form:

A. E. F. Chandler, M.A., Ph.D., D.I.C., M.I.Biol.,
Talentmark Limited, King House,
5/11 Westbourne Grove, London W2 4UA.
Tel: 01-229 2266

Talentmark
Biomedical and Scientific Consultants

1823

RESEARCH TECHNICIAN

required for research haematology laboratory. B.Sc., or equivalent. Whitley Council Scales and Conditions. Applications in writing enclosing curriculum vitae to the Secretary, University College Hospital Medical School, University Street, London WC1E 6JJ quoting reference HAEM/R.T. (1817)

UNIVERSITY OF OXFORD
INORGANIC CHEMISTRY LABORATORY
Post-doctoral Research Assistantship

Applications are invited for an S.R.C.-supported post from physical or inorganic chemists with an interest in biochemistry or biochemists with a strong physical bias to work with Dr. H. A. O. Hill (in collaboration with Dr. R. Ambler) on the structure and function of some metalloproteins, initially the plastocyanins, by n.m.r. spectroscopy and allied techniques.

The appointment would be for one year in the first instance, renewable for a second year, beginning on October 1, 1974. Starting salary according to age within the range £1,821, age 24, to £2,058, age 26, with F.S.S.U. membership.

Applications, with a **curriculum vitae** and the names of two referees, should be sent as soon as possible to the Administrator, Inorganic Chemistry Laboratory, South Parks Road, Oxford. Preliminary enquiries should be sent to Dr. Hill at the same address. (1861)

THE UNIVERSITY OF LEEDS
DEPARTMENT OF PLANT SCIENCES

Applications are invited for a post of RESEARCH ASSISTANT in PLANT PHYSIOLOGY which is sponsored by the A.R.C. for work on the potential of C4 photosynthesis in crops of maritime temperate regions. The appointment will be made for one year in the first instance with possible renewal for a further period of two years. Salary on the scale £1,449 to £1,692 plus superannuation.

Applications together with the names of two referees should be sent to Professor H. W. Woolhouse, Department of Plant Sciences (Baines Wing), University of Leeds, Leeds LS2 9JT, by May 24, 1974. (1862)

AGRICULTURAL RESEARCH INSTITUTE OF NORTHERN IRELAND
HILLSBOROUGH CO DOWN

Assistant Experimental Officer

Applications are invited for the post of Assistant Experimental Officer to assist with experimental work with beef animals.

Salary scale:- £1,435 – £2,329 plus Superannuation Allowance.

Candidates should possess—a pass degree; H.N.D.; or O.N.D. in Agriculture, or equivalent.

Previous experience of supervisory duties and working with animals would be an advantage.

Application forms and further particulars may be obtained from the Secretary to whom applications should be submitted by Wednesday, 5th June, 1974.

Canvassing will disqualify. (1847)

LIVERPOOL POLYTECHNIC

Department of Chemistry

LECTURER II in CHEMICAL PROCESSING/ or INDUSTRIAL CHEMISTRY

Applications are invited for the appointment of a chemical engineer or chemist to join a team involved in the teaching and development of Industrial Chemistry to honours degree level. The successful candidate will have a first or higher degree in chemical engineering or chemistry and the necessary experience of semi-technical scale plant to exploit fully the extensive facilities in the recently completed Industrial Chemistry Block. Research and consultancy will be encouraged.

SALARY: £2700 — £3474 per annum

Application forms available from Staff Officer, E.C.N. Dept., Liverpool Polytechnic, Richmond House, Rumford Place, Liverpool L3 9RH. (1905)

FLINDERS UNIVERSITY OF SOUTH AUSTRALIA

School of Medicine

LECTURERS IN CLINICAL BIOCHEMISTRY, HUMAN MORPHOLOGY AND HUMAN PHYSIOLOGY

Applications are invited for lectureships in the new School of Medicine currently being established in Adelaide at the Flinders University of South Australia in each of the following fields:

Clinical Biochemistry, Human Morphology and Human Physiology.

The School of Medicine will form an integral part of the Flinders Medical Centre, a 710 bed teaching hospital located on campus. The first medical students began their course this year and will enter the Centre in February 1975. It is anticipated that appointees will take up their positions by October 1974 in order to help plan the integrated curriculum.

Suitably qualified appointees will have responsibility in the areas of teaching, research and clinical service. Lecturers with medical qualifications registrable in South Australia will receive a clinical loading, the amount depending on their qualifications and the degree of clinical responsibility undertaken.

Salary Scales: $A8,698 — $A11,982

Further information about the positions, including conditions of appointment and details required of applicants, may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Applications should be lodged, **in duplicate,** with the Registrar, The Flinders University of South Australia, Bedford Park, South Australia, 5042 by **June 7, 1974.** (1868)

CHAIRMAN DEPARTMENT OF APPLIED PHARMACOLOGY

Applications are invited for this position at the School of Pharmacy, Hebrew University of Jerusalem, Israel.

The Chairman will be responsible for all administrative and academic activities of the department in its new facility, a part of the Hebrew University Hadassah Medical Center.

Applications giving a complete curriculum vitae should be sent by August 1 to A. Kreiser, Associate Dean, School of Pharmacy, Hebrew University, P.O.B. 12065, Jerusalem, Israel. (1879)

SHEFFIELD AREA
HEALTH AUTHORITY (TEACHING)
CENTRAL DISTRICT (TEACHING)
Group Department of Haematology

NON-MEDICAL SCIENTIFIC OFFICER (BASIC GRADE)

Applications are invited for the above post from candidates possessing an appropriate 1st or 2nd Class Honours Degree. The successful applicant will be required to work in the hospital's Blood Transfusion Laboratories. Previous experience in Blood Transfusion work is not required.

Candidates wishing to view the laboratories should telephone Mrs. M. S. Lepp, Principal Non-Medical Scientific Officer, Royal Infirmary, Sheffield S6 3DA (Telephone 0742-20977 Ext. 258).

Whitley Council Terms and Conditions of Service apply—Salary Scale: £1,953-£2,694 p.a.

Applications stating age, qualifications, experience and the names and addresses of two referees to the District Administrator, Sheffield Area Health Authority (Teaching), Central District (Teaching), 10 Beech Hill Road, Sheffield S10 2RZ by 25th May 1974. (1859)

UNIVERSITY OF SASKATCHEWAN
Saskatoon, Canada
DEPARTMENT OF MECHANICAL ENGINEERING
ASSISTANT OR ASSOCIATE PROFESSOR: MATERIALS AND METALLURGY

A vacancy exists for an Assistant or Associate Professor to teach and conduct research in the area of materials and metallurgy within the Department of Mechanical Engineering. The appointee would be expected to work as a member of the group headed by Professor I. Le May and involved in the study of aspects of mechanical metallurgy. The successful applicant should have specific interests in the mechanisms and mechanics of fracture, and in relationships between mechanical properties and microstructure. Experience in fractography and transmission electron microscopy is desirable.

Applicants, having a Ph.D. Degree and experience as outlined above, should submit applications together with the names of three referees to Dr. P. N. Nikiforuk, Dean, College of Engineering, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0.

The present salary scales, which are under review, are $12,000 to $15,600 for an Assistant Professor, and $15,750 to $20,300 for an Associate Professor. (1835)

TEXAS A & M UNIVERSITY
GRADUATE RESEARCH ASSISTANT

sought to assist in the study of the crystallographic phase problem through
BINARY INTEGER LINEAR PROGRAMMING TECHNIQUES

Applicants must intend to pursue graduate study in the fields of Computing Science or Operations Research. Interactive graphics display experience a plus. Stipend $350 to $375 monthly (half time)—resident rates on tuition available. Apply to Dr. Edgar F. Meyer, Dept. of Biochemistry & Biophysics, Texas A & M University, College Station, Texas 77843 USA or telephone (713) 845-1744. (1840)

UNIVERSITY OF GLASGOW
DEPARTMENTS OF BIOCHEMISTRY AND VIROLOGY
RESEARCH ASSISTANT

Applications are invited for a Research Assistant to work with Dr. C. A. Fewson and Dr. D. A. Ritchie on the genetic analysis of the bacterium **Acinetobacter calcoaceticus.** This study is part of an overall project on the regulation of metabolism in this organism.

Applicants must have an Honours degree with a suitable background in genetics, microbiology or molecular biology. The appointment, starting October 1, 1974, will be for one year initially and at a starting salary of up to £1,400 per annum. F.S.S.U.

Applications, with the names of two referees, should be sent to Dr. C. A. Fewson, Institute of Biochemistry, University of Glasgow, Glasgow G12 8QQ, as soon as possible. (1841)

MONASH UNIVERSITY
Melbourne, Australia
DEPARTMENT OF CHEMISTRY
SENIOR TEACHING FELLOW

Applications are invited from graduates whose previous experience and qualifications are appropriate to teaching organic chemistry and who should normally have completed a Ph.D. degree. The appointee will be expected to undertake research in addition to his teaching duties.

Salary Range: $A7,270 to $A8,698 per annum with superannuation based on an endowment assurance scheme, the employee and employer contributing 5% and 10% respectively.

Benefits: Travelling expenses for appointee and family, removal allowance, temporary housing for an initial period, repatriation after three years' service if desired.

Further general information and details of application procedure are available from the Academic Registrar, Monash University, Wellington Road, Clayton, Victoria 3168, Australia or the Secretary-General, Association of Commonwealth Universities (Apots), 36 Gordon Square, London WC1H 0PF. Enquiries about the Department to the Chairman, Professor R. D. Brown in the University.

Closing date: **June 11, 1974.**

The University reserves the right to make no appointment or to appoint by invitation. (1850)

TECHNICIAN
(QUALIFIED)

required for work in immunology in relation to renal disease. Experience in this field is not essential. Apply in writing, stating age and giving details of education and experience, to the Secretary, Guy's Hospital Medical School, London Bridge SE1 9RT, quoting Ref. R.U.2. (1854)

UNIVERSITY COLLEGE OF WALES
WELSH PLANT BREEDING STATION

APPOINTMENT OF BIOCHEMIST

Applications are invited for a Biochemist to join a team working on the regulation of photosynthesis and utilisation of photosynthetic products in forage plants with particular reference to their potential for genetic improvement.

Initially the post would be concerned with investigations on the pool sizes and metabolic fates of photosynthetic intermediates, but would also be linked to current studies on enzyme regulation in leaves.

Applicants should have post-graduate research experience in the general area of carbohydrate metabolism.

The appointment will be made at the Higher Scientific Officer/Senior Scientific Officer level in the salary range £2,221 to £3,895. The post is superannuable under F.S.S.U.

Applications should be made by letter, together with the names of two referees, and should be sent to the Secretary, Welsh Plant Breeding Station, Plas Gogerddan, Nr. Aberystwyth, Cards. SY23 3EB, on or before May 24, 1974. (1853)

UNIVERSITY OF NOTTINGHAM
DEPARTMENT OF CHEMISTRY

Post-doctoral Research Appointments

BIO-ORGANIC CHEMIST

Applications are invited for an S.R.C. Post-doctoral award to work with Dr. B. W. Bycroft on the Chemistry and bio-chemistry of Peptide Antibiotics.

Candidates should have an interest in, and preferably experience of, biological chemistry. The appointment is tenable for two years, commencing October 1, 1974 with a salary in the range of £2,118 to £2,412 with F.S.S.U.

Application forms and further details from the Staff Appointments Officer, University of Nottingham, University Park, Nottingham. Closing date: May 27, 1974. Ref. No. 368. (1857)

THE EDINBURGH SCHOOL
OF AGRICULTURE

RESEARCH ASSISTANT

Applications are invited for a research assistantship for work on animal behaviour under the direction of Dr. D. G. M. Wood-Gush. The work will be exclusively with pigs.

Salary: £1,500.

Further particulars and application form from the Secretary, The Edinburgh School of Agriculture, West Mains Road, Edinburgh EH9 3JG. (1786)

DEPARTMENT OF KINESIOLOGY
SIMON FRASER UNIVERSITY

ACADEMIC PHYSICIAN

An appointment of a physician with a well-developed research interest in any aspect of human physiology, bioengineering, prosthetics or human factors is offered in the Department of Kinesiology. The appointee will be required to work in an interdisciplinary environment with biochemists, engineers, work physiologists or biomechanics faculty, in a department which has growing graduate and undergraduate degree programmes.

Although the University itself does not have a medical faculty, the appointee will be encouraged to maintain and develop his clinical interests with the Faculty of Medicine at a nearby university and local hospitals. For suitably qualified applicants the possibility exists of a joint appointment with the Computing Science Department. The appointment will be made at either the assistant or associate professor level, depending upon qualifications. Salary negotiable.

Applications, together with three references, to:

Dr. E. W. Banister
Department of Kinesiology
Simon Fraser University.
BURNABY V5A1S6 British Columbia
(from whom further details can be obtained).
(1864)

CENTRAL PUBLIC HEALTH LABORATORY

TECHNICIAN

required by the Salmonella and Shigella Reference Laboratory. Suitable qualifications would be a relevant scientific degree; A.I.M.L.T. or H.N.C. in Medical Laboratory Sciences. This is the National Salmonella and Shigella Centre for Great Britain and the Commonwealth and offers training in the most advanced methods of Salmonella and Shigella identification.

Written applications with full details of age, experience and qualifications to the Personnel Officer, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT. (1730)

UNIVERSITY HOSPITAL OF WALES, HEATH PARK, CARDIFF
DEPARTMENT OF MEDICAL BIOCHEMISTRY

BIOCHEMIST OR SENIOR BIOCHEMIST

Applications are invited from suitably qualified candidates for this position in the Supra Regional Assay Laboratory for Polypeptide. The laboratory provides in association with other laboratories a diagnostic service using the technique of radioimmunoassay for centres throughout England and Wales.

The successful applicant will be expected to set up a computer based data handling and record system. He will also be responsible for the development and introduction of new assays. Opportunities exist for further training in hospital biochemistry leading to the qualification of Master of **Clinical Biochemistry**. Whitley Council conditions apply

Application forms available from the Personnel Department at the above hospital, to be returned by 7th June 1974, and further details may be obtained from Dr. G M Addison (0222 755944 Ext. 2811/2196) (1924)

Glaxo

HNC or HND in Chemistry

Ulverston

We have vacancies for Technical Officers or Laboratory Assistants with laboratory experience of organic chemistry at our plant at Ulverston, which is situated on the shores of Morecambe Bay and on the southern fringe of the Lake District National Park.

You will join an established group in the Chemical Process Development department working on a variety of problems connected with antibiotics manufacture. This work will include plant troubleshooting, pilot plant operations, process development, and applied research, and will be particularly suitable to practically-oriented people who have recently gained an HNC or HND qualification, or are engaged upon such courses. Every encouragement will be given to those wishing to continue their studies.

The starting salary will be according to experience, age and qualifications. The company offers good conditions of service including bonus, pension and sick pay schemes.

Please apply in writing, quoting reference U.530, to:
Personnel Officer (JE),
Glaxo Laboratories Limited,
North Lonsdale Road,
Ulverston. (1921)

SHEFFIELD AREA
HEALTH AUTHORITY (TEACHING)
CENTRAL DISTRICT (TEACHING)

SENIOR SCIENTIFIC OFFICER
SCIENTIFIC OFFICER

A vacancy exists within the Sheffield Supra-Regional Endocrine Investigation Centre for a Senior or Basic Grade non-medical Scientific Officer, to commence as soon as possible after 14th June 1974.

The post will be of great interest to graduates with a first or second class honours degree or higher qualification with a background of medical physics and/ or biochemistry.

The person appointed will be expected to aid the development of automated techniques and to help in the every-day maintenance of complex electronic equipment including a 16 K-store computer. The ability to write programmes and/or the will to learn would be advantageous.

Whitley Council Terms and Conditions of Service apply.

Salary Scales:
Basic Grade—£1,953 to £2,694 p.a.
Senior Scientific Officer—£2,964 to £3,843 p.a.

Candidates wishing to view the laboratories should telephone Mr. B. Bevan, Principal Non-Medical Biochemist, Jessop Hospital for Women, Sheffield. (Telephone 0742 29291 ext. 269)

Applications stating age, qualifications, experience and the names and addresses of 2 referees to the District Administrator, Central District (Teaching), Sheffield Area Health Authority (Teaching), 10 Beech Hill Road, Sheffield S10 2RZ, by 25th May 1974. (1860)

A.S.P. BIOLOGICAL AND MEDICAL PRESS (Elsevier division)

(Publishers of the International Biochemical Journal **Biochimica et Biophysica Acta**) has immediate openings in AMSTERDAM for full-time

editorial assistants

Description

- Preparation of manuscripts for printing
- Correction of proofs
- Language correction of contributions written by non-English speaking authors
- Supervision of free-lance workers
- Responsible and variable work

Requirements

- Degree, or equivalent, in chemistry, biochemistry or biomedicine preferred
- English mother tongue

A good salary, according to qualifications and experience, work in a congenial atmosphere and excellent social security arrangements are offered.

Handwritten applications, giving details of education and previous experience and an indication of salary expected, should be addressed to:

The Personnel Department
Associated Scientific Publishers
P.O. Box 2400, Amsterdam, The Netherlands.

(1944)

UWIST CARDIFF
University of Wales Institute of Science and Technology

RESEARCH ASSOCIATE APPLIED PHYSICS

with good Honours degree in relevant subject and Ph.D. or equivalent—required to work on the transport theory of tunnelling and its application to semiconductor devices problems.

Salary:
1st year, £1,929: 2nd year, £2,058 (under review), with FSSU

Requests (quoting Ref. N) for details and application form (returnable by June 7, 1974) to Personnel Section, UWIST, Cardiff CF1 3NU. (1855)

McGILL UNIVERSITY
MONTREAL, QUEBEC, CANADA

Applications are invited for a two year appointment at the Assistant or Associate Professor level for a biologist-physiologist with strong BIO-MATHEMATICAL interests in the Department of PHYSIOLOGY starting September 1, 1974. The department hopes to be able to extend the appointment. Responsibilities include teaching undergraduate and graduate level Science courses. Send applications with curriculum vitae and names of 3 references to:

Ms. Maria Olejar
Dept. of Physiology
3655 Drummond Street
McGill University
Montreal, Quebec, H3G 1Y6, Canada (1779)

UNIVERSITY OF BIRMINGHAM
BRITISH HEART FOUNDATION CHAIR OF CARDIOLOGY

Applications are invited for the British Heart Foundation Chair of Cardiology in the Department of Medicine. Appointment from a date to be arranged.

Salary in the clinical professorial range, maximum £7,599.

Further particulars obtainable from the Registrar, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, to whom applications (12 copies: one from overseas applicants) naming 3 referees, should be sent by June 17, 1974. (1742)

UNIVERSITY OF BIRMINGHAM
CHARLES HAYWARD CHAIR OF GERIATRIC MEDICINE

Applications are invited for the newly established Charles Hayward Chair of Geriatric Medicine in the Department of Medicine. Appointment from a date to be arranged.

Salary in the clinical professorial range, maximum £7,599.

Further particulars obtainable from the Registrar, University of Birmingham P.O. Box 363, Birmingham B15 2TT, to whom applications (12 copies: one from overseas applicants) naming 3 referees, should be sent by June 17, 1974. (1741)

UNIVERSITY OF SYDNEY
DEPARTMENT OF THEORETICAL CHEMISTRY

Professional Officer Grade I (Electron Spectroscopist)

Applications are invited from candidates with Ph.D. or equivalent qualification in Chemistry or Physics.

Salary up to $A7,857 per annum.

The appointment is initially for one year, renewable annually subject to mutual agreement for a period of three years.

Experience in experimental photoelectron spectroscopy or in a closely related field is essential. The appointee will take overall responsibility for maintaining and organizing the use of the equipment (at present, a Perkin-Elmer PS-18 spectrometer) and will take an active part in the design of new instrumentation. A suitable candidate will be encouraged to participate in the research programme of the Department.

Applications, including details of qualifications and experience and names of two referees, to the Registrar, University of Sydney, N.S.W., 2006, Australia. Further information from Professor N. S. Hush, School of Chemistry, University of Sydney. (1930)

university of wales

university college of swansea

Applications are invited for the following:

Postdoctoral Research Assistant

A Postdoctoral Research Assistant is required in the Department of Biochemistry to work on an S.R.C. sponsored research programme concerned with enzymic mechanisms involved in the formation of uracilyl amino acids. Preference will be given to applicants with experience in the separation and purification of enzymes, especially those of plants.

The commencing salary will be on the scale £2,118 to £2,412 per annum plus F.S.S.U. benefits, and the appointment will be for one year in the first instance from August 1, 1974, renewable annually up to December 31, 1976.

The closing date is **Friday, June 14, 1974.**

Department of Genetics

Research Studentship

The Medical Research Council is prepared this year to offer to a suitable candidate a research studentship tenable at the above University College. The Research will involve the study of mechanisms of genetic change and effects of environmental mutagens in yeast under the supervision of Dr. J. M. Parry. Applicants should have a first or upper second class degree in a biological subject.

The closing date is **Friday, June 7, 1974.**

Further particulars and application forms may be obtained from the Registrar/Secretary, University College of Swansea, Singleton Park, Swansea, SA2 8PP, to whom they should be returned by the appropriate date. (1884)

UNIVERSITY OF ABERDEEN
Senior Lectureship in Medical Physics

Applications are invited from experienced radioisotope hospital physicists to be responsible for team of physicists, technicians and ancillary staff engaged in wide range of research activities and providing nuclear medicine service to the Hospital Region. The successful candidate will also be responsible for team providing D.M.R.I physics course, and join in M.Sc. (Medical Physics) teaching. Salary on scale £4,368 to £5,496 rising to £4,707 to £5,844 at October 1, 1974, with initial placing according to qualifications and experience.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by May 31, 1974. (1880)

UNIVERSITY OF ABERDEEN
Research Assistant in Zoology

Applications are invited for above post for work on social behaviour and population ecology of shelduck at Culterty Field Station. Applicants should have a good degree in Zoology including courses in behaviour and ecology. Post is financed by N.E.R.C. for three years from October 1, 1974. Salary within range £1,305 by 87 to £1,659.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by May 25, 1974. (1877)

Microbiologist

A vacancy has arisen within our expanding organisation at Havant, Hants, for a Microbiologist to join a small team involved in the microbiological quality control of pharmaceutical nutritional products

The candidate should have a degree or equivalent qualifications in microbiology, age 25/45 and have at least two years experience.

A competitive salary will be paid depending upon age and experience.

The Company offers a non-contributory Pension and Life Assurance Scheme. Assistance with re-location expenses will be considered.

Please apply in writing to:-

Cameron Wolstenholme,
Director of Personnel & Industrial Relations,
John Wyeth & Brother Limited,
Huntercombe Lane South,
Taplow,
Maidenhead,
Berks, SL6 0PH. (1886)

Wyeth

ICI

CORPORATE LABORATORY-RUNCORN

The Corporate Laboratory at Runcorn has a vacancy for a

CELL PHYSIOLOGIST

with research experience in the culture **in vitro** of animal cells and in the study of the characteristic properties and functions of such cells. Applicants should be under 30 and hold a PhD in this field.

The successful applicant would join a multidisciplinary team engaged in long-term research in the Biosciences. While the team has a defined need for specialist skills in cell physiology, the successful applicant would be given every encouragement to initiate original research projects.

Runcorn, conveniently situated for Manchester, Liverpool and Chester, is within easy reach of the Lake District, North Wales and the Peak District, yet is only 2½ hours by train from London.

If you are interested, please write for an application form to:

Mr J. A. Hurst, Personnel Officer,
ICI Limited, Corporate Laboratory,
P.O. Box 11,
The Heath, Runcorn, Cheshire. WA7 4QE.

(1939)

GRASSLAND RESEARCH INSTITUTE
Hurley, Maidenhead, Berkshire

An opportunity exists for a HIGHER SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER to join a small group of scientists engaged in operational research on grassland farming enterprises with special reference to permanent grassland. Applicants should have a knowledge of farming systems and preference will be given to candidates with experience, or a special interest in developing methods of analysing the physical and socio-economic data being collected by the group.

Candidates should have a good honours degree in an appropriate subject, with at least two years post qualifying experience for appointment at H.S.O. (£2,221 to £2,854), and four years for S.S.O. (£2,798 to £3,895). Commencing salary according to qualifications and experience.

Superannuation under F.S.S.U. with a non-pensionable allowance to offset personal contributions.

Applications to the Secretary by June 7, 1974 giving curriculum vitae and the names of three referees and quoting Reference 10/G/16. (1934)

BIO-RESEARCH LABORATORIES LTD.
CANADA

Applications are invited from qualified Veterinary Pathologists interested in joining the Department of Toxicology in the capacity of Staff Pathologist. Responsibilities will include technical supervision of Autopsy Room and Histology Laboratory personnel and activities. Principally, however, the successful candidate will be responsible for the evaluation of histological specimen material arising out of toxicological research in a variety of laboratory animal species and for the preparation of reports appropriate to the research in question. Salary will be commensurate with qualifications and experience.

Bio-Research Laboratories Ltd is a Canadian research company operating in the life sciences field. Applications, curricula vitarum and requests for further details may be directed to Dr. C. I. Chappel, Director, Bio-Research Laboratories Ltd., 265 Hymus Blvd, Pointe Claire, Quebec, Canada H9R 1G6. (1799)

Theoretical Nuclear Physicists

are required to join a newly formed group at the Daresbury Laboratory. Theoreticians will be expected to collaborate with University Physicists in developing research programmes for the large tandem Van de Graaff accelerator (terminal potential 30 MV) being built at the Laboratory. Preference will be given to theoreticians interested in heavy ion reactions and the structure of heavy nuclei. Posts are normally available for a fixed term of three years.

The successful applicants will be appointed as Research Associates at salaries between £2,096 and £4,898. The level at which an Associate is appointed is dependent on age, ability and experience.
Applicants should possess a Ph.D or its equivalent, or expect to obtain their Ph.D during 1974. Superannuation is contributory under the Federated Superannuation System for Universities. Closing date: 24 May 1974.

Please write or telephone Warrington 65000, Ext 467, for an applicotion quoting reference DL/508/T to:

(1848)

SCIENCE RESEARCH COUNCIL — DARESBURY LABORATORY

**Personnel Officer,
Science Research Council
Daresbury Laboratory,
Daresbury,
Warrington WA4 4AD.**

WESSEX WATER AUTHORITY
Somerset Rivers Division

Assistant Biologist

With the agreement of the Water Services Staff Commission, applications are invited for the above post from persons at present employed in the Water Industry as defined by Circular 5/73 as amended by 8/73. First consideration will be given to applicants from within the Authority's Region.

This post is suitable for a young qualified limnologist wishing to gain experience.

The successful candidate will work under the direction of the Water Quality Officer. He will be involved in the sampling of Rivers, both chemically and biologically. An interest in instrumentation is desirable.

Commencing salary for the post will be within the range £1,353 to £1,926 per annum.

Job specification and application forms (which should be returned not later than 3rd June) may be obtained by writing to:-

Wessex Water

**Divisional Manager,
Somerset Rivers Division,
Wessex Water Authority,
The Watergate,
West Quay,
Bridgwater, Somerset TA6 3HN.**

(1918)

The Hatfield Polytechnic

DEPARTMENT OF BIOLOGICAL SCIENCES

Laboratory Superintendent

Applications are invited from suitably qualified and experienced technicians for this new post in the Department of Biological Sciences.

A high level of technical skills is required together with proven supervisory and organisational ability.

Salary scale T6 £2,235–£2,535 per annum.

Application forms may be obtained from the Staffing Officer, The Hatfield Polytechnic, PO Box 109, Hatfield, Herts. Telephone 68100, ext. 309. For further information please contact Mr K. Thomas, Head of Department, ext. 247. This advertisement appears with the approval of the Local Government Staff Commission. Please quote ref: 472.

(1920)

UNIVERSITY COLLEGE CARDIFF
DEPARTMENT OF BIOCHEMISTRY

Two 3-year research assistantships are available for stulies on:

(1) Control of protein catabolism (with Dr. J. Kay) starting from July 1 or as soon as possible thereafter. Experience in peptides and enzyme chemistry and in the operation of amino acid analysers would be advantageous but not essential.

(2) Lipid synthesis and its control in plants (with Dr. J. Harwood) starting on October 1. Experience in enzymology would be advantageous.

Applicants should possess a B.Sc. or similar qualification and have a good knowledge of basic biochemical techniques. Starting salaries in range £1,416 to £1,563 per annum (under review).

Further particulars available from Dr. Kay and Dr. Harwood, c/o Biochemistry Department.

Applications including a curriculum vitae and the names and addresses of 2 referees should be sent to: The Registrar, University College, P.O. Box 78, Cardiff CF1 1XL, no later than May 31, 1974. Please quote 0556. (1876)

ST. HELIER HOSPITAL
Carshalton, Surrey

A BASIC GRADE BIOCHEMIST

is required for a busy Clinical Chemistry department in the above hospital. The Department is highly automated with an SMA 12.60, etc., and undertakes a wide variety of work.

Previous experience in radioimmunoassay techniques desirable but not essential.

Applications, giving details of qualifications, experience and the names and addresses of two referees, should be sent to Dr. L. Chapman immediately. (1874)

LAURENTIAN UNIVERSITY
Sudbury, Ontario, Canada

Applications are invited for a faculty position at the rank of Assistant Professor in the Department of Biology. This is a temporary (sessional) appointment, for one year in the first instance.

Candidates should possess a doctorate and preferably be bilingual (English and French). A Zoologist is being sought to teach vertebrate biology-systematics and embryology to undergraduates.

Availability for appointment: September 1, 1974.

Closing date for receipt of applications: When a suitable candidate is selected.

Replies containing curriculum vitae, list of publications and the names of three referees should be forwarded to:

Chairman,
Department of Biology,
Laurentian University,
Sudbury, Ontario,
Canada P3E 2C6. (1878)

UNIVERSITY OF LIVERPOOL
Chair of Clinical Pharmacology

Applications are invited for the Chair of Clinical Pharmacology which will become vacant on September 30, 1974, following the retirement of Professor A. Wilson.

The salary of the Professor will be within the range approved for clinical Professorial salaries and initially will depend on the qualifications and experience of the successful candidate.

Applications (16 copies), together with the names of three referees, should be received not later than June 3, 1974 by the undersigned, from whom further particulars may be obtained. (Candidates overseas may send one copy only, by airmail). Quote ref. RV/N/276015.

H. H. Burchnall,
Registrar,
The University,
P.O. Box 147,
Liverpool L69 3BX. (1872)

WYE COLLEGE
(University of London)
DEPARTMENT OF PHYSICAL SCIENCES

POST-GRADUATE ASSISTANTSHIP

Applications are invited for an Agricultural Research Council three-year Post-graduate Assistantship to study the biophysical action of plant growth-regulating substances on roots and root membranes.

Applicants should be graduates with special interests in physical chemistry and those expecting to graduate this summer may apply. The Assistant may be allowed to register for a higher degree.

Applications with the names of two referees should be sent to the Secretary (N), Wye College, Wye, Ashford, Kent, from whom further particulars can be obtained. (1870)

UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF ELECTRONIC AND ELECTRICAL ENGINEERING

RESEARCH IN UNDERWATER ACOUSTICS

Applications are invited for the following posts:

Senior Research Associate

The Department has been awarded a three-year contract by the Ministry of Defence (Navy) to investigate various means of detecting acoustically objects buried in sediments. Applicants should be graduate electronic engineers with suitable post-graduate experience. An appointment can be made immediately.

Senior Research Associate/ Research Associate

The Science Research Council has awarded the Department a three-year research grant to study and optimise high frequency underwater acoustic transducer performance. Applicants should be graduates in either electronic engineering, electrical engineering, physics or mechanical engineering.

The appointment may be at the level of Research Associate or Senior Research Associate, depending upon qualifications and experience, and can commence on July 1, 1974, or as soon after as is possible.

Salary scales: (under review).

Senior Research Associate £2,223 to £3,048 (exceptionally £4,548)

Research Associate £1,569 to £2,223 (exceptionally £2,718)

Plus F.S.S.U.

Appointees wil join an active research team working in Underwater Acoustics and Underwater Technology. Previous experience in Underwater Acoustics is not essential.

Applications (3 copies) with names of 3 referees to Assistant Registrar (S), University of Birmingham, P.O. Box 363, Birmingham B15 2TT by May 31, 1974. Please quote reference: NR5. (1873)

ROYAL HOSPITAL FOR SICK CHILDREN, EDINBURGH

Graduate as Technician required for Cytogenetics Unit. Previous experience in cytogenetics and tissue culture an advantage but not essential as full training can be given.

Post would be suitable for new graduate wishing to make a career in cytogenetics. A suitable qualified appointee may be eligible for promotion to full graduate status should such a vacancy occur within the Unit.

Applications, giving the names of two referees, to the Sector Administrator, Royal Hospital for Sick Children, 1 Rillbank Terrace, Edinburgh EH9 1LN. (1867)

UNIVERSITY OF RHODESIA
DEPARTMENT OF GEOGRAPHY

SENIOR LECTURESHIPS/ LECTURESHIPS IN GEOGRAPHY
(TWO VACANCIES)

Following on recent additions to staff establishment, the Department will consider applications from geographers with a variety of topical interests, both human and physical. Applicants should indicate in their applications the full range of their interests and abilities as well as their special field of study.

Salary scales (approximate Sterling equivalents): Senior Lecturer £5,346 by £214 to £7,058; Lecturer Grade I £4,857 by £180 to £5,577; Lecturer Grade II £2,994 by £154 to £3,456 by £171 to £3,798 by £180 to £4,518 by £170 to £4,688.

Family passage and allowance for transport of effects on appointment. Assistance with accommodation for up to three years for persons recruited from outside Rhodesia. Sabbatical and triennial visits with travel allowance. Superannuation and medical aid scheme.

Applications (six copies) giving full personal particulars (including full names, place and date of birth, etc.), qualifications, experience and publications and naming three referees, should be submitted by **June 15, 1974** to the Registrar, University of Rhodesia, P.O. Box 2702, Salisbury, Rhodesia. An additional copy should be sent to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London, W.C.1, from whom further particulars may be obtained.

The Head of Dept. Professor G. Kay is at present in the United Kingdom and can be contacted through the Association of Commonwealth Universities. (1869)

Universiteit van Amsterdam

Applications are invited for a teaching and research post (lecturer) in the Department of Structural Geology. Duties will include instruction in structural geology and in fieldwork. Applicants should have considerable experience in structural geology. Experience in petrology or mineralisation problems would be favourable. Salary in accordance with background and experience.

Send curriculum vitae, precise research interests and names of at least two referees to: Dr. O. J. Simon, Geological Institute, 130 Nieuwe Prinsengracht, Amsterdam, The Netherlands.

(1871)

UNIVERSITY OF OXFORD
DEPARTMENT OF BIOCHEMISTRY

M.R.C. IMMUNOCHEMISTRY UNIT

A vacancy exists for a Junior Technical Officer/ Technical Officer in the above unit. Applicants should be qualified to Higher National Certificate standard and have several years' experience in biochemical/analytical techniques, including spectrophotometry, chromatography and radiochemistry. Some knowledge of protein chemistry and an ability to work with small animals is desirable, but not essential.

Salary, according to age and experience, on the scale £1,854 to £2,073 (bar) £2,184 to £3,057 (under review. Applications, including full details of education and career to date, and the names of two referees, to: The Administrator, Department of Biochemistry, South Parks Road, Oxford OX1 3QU. (1891)

SENIOR BIOCHEMIST
WESTERN INFIRMARY AND GARTNAVEL GENERAL HOSPITAL
Glasgow
(DEPARTMENT OF BIOCHEMISTRY)

The successful applicant for the above position will have the opportunity to gain extensive experience of automation or, alternatively, take part in specialist developments with this expanding department.

The Laboratories are a part of the Department of Pathological Biochemistry of the University of Glasgow, and therefore experience gained in an academic environment will be regarded as advantageous. The main Laboratories are situated in the recently opened Gartnavel General Hospital, and deal with the majority of the biochemical analyses for the Western District of Glasgow.

Potential applicants are invited to visit the Laboratories by appointment (Tel: 041-334 8122).

Applications stating age, experience, qualifications etc., together with the names of two referees should be sent to Dr. J. T. Robson, Department of Biochemistry, Gartnavel General Hospital, Great Western Road, Glasgow G12 0YN. (1916)

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE
(University of London)
KEPPEL STREET, WC1E 7HT

RESEARCH ASSISTANT experienced in Biochemistry is required for the Hepatitis Reesarch Unit (Professor A. J. Zuckerman). Salary up to £2,073 per annum plus £126 London Weighting. Written applications to Secretary (A1), London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT. (1889)

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF ORGANIC CHEMISTRY

Applications are invited from suitably qualified organic and biochemists for a Senior Research Assistantship. The person appointed would be expected to work on the biosynthesis of fungal metabolites from ^{13}C-precursors. The appointment will date from October 1, 1974 and the salary will be in the range £1,959 to £2,178 per annum (under review) plus F.S.S.U. Applications giving full academic qualifications, details of research experience and names of two referees should be sent as soon as possible to the Registrar, The University of Liverpool, P.O. Box 147, Liverpool L69 3BX. Quote ref. RV/N/276019. (1910)

UNIVERSITY OF READING

The Ministry of Agriculture, Fisheries and Food is prepared this year to offer to a suitable candidate a Studentship tenable in the Department of Physiology and Biochemistry in the University of Reading. The value of the MAFF award in 1974 will normally be £695 per annum. The subject for research leading to a Higher Degree will be either, protein and amino acids in the growing rabbit or citrate and malate metabolism in the pregnant ewe. Work should start in October 1974 and applicants should hold at least an Upper Second Class degree in a suitable subject or hope to qualify this summer. Further particulars may be obtained from Professor C. Tyler, Department of Physiology and Biochemistry, The University, Whiteknights, Reading RG6 2AJ. Ref. M.N.18. (1890)

DALHOUSIE UNIVERSITY
Nova Scotia, Canada
DEPARTMENT OF ANATOMY

TWO ASSOCIATE OR ASSISTANT PROFESSORS

Applications are invited from persons with a Ph.D. or a professional qualification for the above positions. Duties may include the teaching of Anatomy to Medical, Dental or Paramedical students. An ability to teach gross anatomy and a research interest in the nervous system would be an advantage. Salary (minimum $15,000) and rank commensurate with experience. Applications together with curriculum vitae and the names of two referees should be addressed to Dr. D. G. Gwyn, Department of Anatomy, Dalhousie University, Halifax, Nova Scotia, Canada by June 20, 1974 (1881)

COMMONWEALTH AGRICULTURAL BUREAUX
Vacancy for
SCIENTIFIC INFORMATION OFFICER
at the
COMMONWEALTH BUREAU OF SOILS
Rothamsted Experimental Station
Harpenden, Hertfordshire

Duties: Preparing abstracts for the abstracting journal "Soils and Fertilizers", which covers world scientific literature on soils and soil-plant relationships, and dealing with scientific enquiries within these fields.

Qualifications: A degree in soils or in agricultural science, or in sciences basic to these. Ability to write good and concise English essential. The successful candidate may be required to learn a foreign language. Experience or training in information work, including subject indexing, would be an advantage.

Salary: In scale £1,435 to £2,592 (bar) £2,798 to £3,478 (bar) £3,756 to £4,004, plus a compensatory allowance (taxable but not superannuable) of 4½% to offset personal contribution to F.S.S.U. Starting salary according to qualifications, experience and age.

Application forms: and full particulars from the Secretary, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Slough SL2 3BN

Closing date for applications: June 14, 1974. (1888)

CSIRO

DIVISION OF APPLIED ORGANIC CHEMISTRY

MELBOURNE VIC

RESEARCH FELLOWSHIP

The Commonwealth Scientific and Industrial Research Organization has a broad charter for research into primary and secondary industry areas. The Organization has approximately 6,500 employees—2,100 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

FIELD ORGANIC CHEMISTRY

GENERAL: The Division undertakes research into a wide spectrum of applied chemical problems including problems associated with the chemistry of natural problems.

DUTIES: Research study of biologically active compounds from plant or animal sources and possibly on the metabolism of biologically active compounds in animals. The research background of the appointee may be taken into account in selecting the research project.

QUALIFICATIONS: A Ph.D. in organic chemistry or equivalent qualifications, together with demonstrable research ability.

SALARY: Appointment will be made within the salary ranges of Research Scientist or Senior Research Scientist: $A9,380 — $A14,290 p.a.

TENURE: This position is available for a fixed term of three years.

Applications stating full personal and professional details, the names of at least two professional referees and quoting Reference Number 586/181 should reach:-

The Personnel Officer,
Australian Scientific Liaison Office,
64–78 Kingsway,
LONDON WC2B 6BD.

by the 14th June, 1974.

Applications in U.S.A. and Canada should be sent to
The Counsellor (Scientific),
Embassy of Australia, 1601 Massachusetts Avenue, N.W.,
WASHINGTON, D.C. 20036, U.S.A.

(1896)

SITUATION REQUIRED

Indian Agricultural Executive M.Sc. (Agriculture), forty years, with wide experience of Planning, Organising and Administration of Agricultural Projects, Sales and Sales promotion of Agricultural inputs, Farm Management, Agricultural Extension and research both in India and abroad seeks immediate assignment of two to four years residential tour. (1906)

RESEARCH UNIT requires an assistant who is interested in human genetics, to organise date and research projects relating to various aspects of the subject. Applicants should have experience in biomedical and/or secretarial work and be able to work on her own initiative. Please apply to the Administrative Secretary, Paediatric Research Unit, Guy's Hospital Medical School, London, SE1 9RT. (1927)

THE UNIVERSITY OF ASTON IN BIRMINGHAM

DEPARTMENT OF BIOLOGICAL SCIENCES

GENETICIST

Applications are invited from suitably qualified persons for a Lectureship. The responsibilities of the post will include the teaching of genetics and biometry to students of biological sciences and the biology of man and his environment. Appointment to commence from August 1, 1974.

Salary scale: Commencing salary in the range £1,929 to £2,388 per annum on a scale rising to £4,548 per annum (to be increased from October 1, 1974).

Requests for further details and application forms (which should be returned within 14 days of the appearance of this advertisement) should be sent, preferably on a postcard, quoting Ref. No. 972/6, to: Staff Officer, University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET. (1911)

COMMONWEALTH AGRICULTURAL BUREAUX

Vacancy for

SCIENTIFIC INFORMATION OFFICER

at the

COMMONWEALTH BUREAU OF ANIMAL BREEDING AND GENETICS, EDINBURGH

Duties include preparation and editing of abstracts for the journal "Animal Breeding Abstracts", indexing, information retrieval and dealing with enquiries.

Applicants must have a degree in the biological sciences or a degree in another subject plus experience of information work in a suitable animal science field, and a reading knowledge of one or more foreign languages.

Salary in scale £1,435 to £2,592 (bar) to £2,798 to £3,478 (bar) to £3,756 to £4,004 plus a compensatory allowance (taxable but not superannuable) of 4½% to offset personal contribution to F.S.S.U. Starting salary will be in the lower sector of the scale.

Application forms and full particulars from the Secretary, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Slough SL2 3BN. Closing date for applications: June 15, 1974. (1887)

GREENWICH & BEXLEY AREA HEALTH AUTHORITY

BROOK GENERAL HOSPITAL

Senior Technician—Neuropathology

A vacancy exists in a new department which will deal with routine material from Neurosurgical and Neurological Units and research projects.

The person appointed will be jointly responsible for implementing the services of the department.

Applications giving age, experience and 2 referees to:

Senior Chief Technician,
Brook General Hospital,
Shooters Hill Road,
Woolwich, London SE18 4LW. (1885)

POST-DOCTORAL RESEARCH

Two-year Post-doctoral Research position available immediately to study biochemical aspects of growth control in normal and leukemic lymphocytes. Experience in isolation and characterisation of nuclear proteins or general training in biochemistry preferred.

Further inquiries and a curriculum vitae with the names of two references should be sent to:

Dr. William I. Waithe
Service d'immunologie
Centre hospitalier de l'Université Laval
2705 boulevard Laurier
Québec G1V 4G2
Canada. (1883)

UNIVERSITY OF SURREY

Guildford, Surrey GU2 5XH

DEPARTMENT OF CHEMISTRY

Applications are invited for a Science Research Council Postdoctoral Assistantship for work with Professor J. A. Elvidge and Dr. J. R. Jones on a study of tritium exchange in heterocyclic compounds. The appointment is available from 1/10/74 and will be for either one or two years. Salary £2,058 by £165 plus F.S.S.U. Applications together with curriculum vitae and the names and addresses of two referees should be sent as soon as possible to one of the above-named persons. (1882)

CHELSEA COLLEGE
University of London

RESEARCH ASSISTANT—
ACTIVATION ANALYSIS—
GEOCHEMISTRY

Applications are invited from candidates with a first degree in Chemistry or Chemistry/Geology for a temporary RESEARCH ASSISTANTSHIP with a project (sponsored by N.E.R.C.) concerning the geochemistry of silicate melts. Work will entail activation analysis of rock and mineral samples and will be carried out at London University Reactor Centre, Ascot. Salary scale: £1,584 to £1,758 to £1,929 per annum. Superannuable. Further particulars and application forms from the Personnel Officer (N), Chelsea College, Manresa Road, London SW3 6LX. Closing date May 29, 1974.
(1907)

UNIVERSITY OF READING
Plant Science Laboratories
DEPARTMENT OF BOTANY

S.R.C./C.A.S.E. STUDENTSHIP
IN PHYTOCHEMISTRY

Applications are invited from Honours Graduates in Organic Chemistry, Biochemistry or Botany to work on "Comparative Aspects of Phytoalexin production in the family Leguminosae" in association with I.C.I. Plant Protection Ltd., Jealott's Hill Research Station, Bracknell, Berkshire.

The studentship is available from October 1, 1974 for a period of up to three years.

Application with a curriculum vitae and the names of two referees should be sent to:

Dr. J. B. Harborne,
Phytochemical Unit,
Plant Science Laboratories,
Whiteknights,
Reading RG6 2AS.

Closing date is June 15, 1974. (1897)

THEORISTS AT TRIUMF

Applications are invited for two positions to be filled on or after April 1, 1975. TRIUMF, a 500 MeV high intensity cyclotron, is operated on the University of British Columbia campus by a consortium of four universities.

Theoretical physicists will work closely with experimenters on problems of intermediate energy physics. Appointments initially for one year, will be renewable subject to availability of funds. Depending on experience, salary and rank will correspond to Assistant or Associate Professor.

Closing date is September 30, 1974. Send resume and references to:

Dr. J. R. Richardson, Director,
TRIUMF, University of British Columbia,
Vancouver V6T 1W5, B.C., Canada.
(1895)

UNIVERSITY OF KENTUCKY

ASSISTANT PROFESSOR
BIOCHEMISTRY

Several full-time faculty positions are available immediately for candidates committed to pursue fundamental research programmes in membrane biochemistry or molecular biology. Participation is expected in biochemistry courses for medical students, graduate students and undergraduates. Only candidates with post-doctoral experience will be considered. The University of Kentucky is an equal opportunity/affirmative action employer.

Send vita and three letters of reference to Chairman, Department of Biochemistry, College of Medicine, University of Kentucky, Lexington, Kentucky 40506. (1894)

UNIVERSITY OF BRISTOL
LONG ASHTON RESEARCH STATION

Applications are invited for a RESEARCH ASSISTANT to work on the promotion of flowering in Sitka Spruce. The work would be under the general direction of Dr. L. C. Luckwill and be carried out in collaboration with the Forestry Commission Northern Research Station at Roslin, near Edinburgh. Although based at Long Ashton the successful candidate would also be required to carry out field work in Scotland.

The assistantship is for a period of three years commencing in July 1974. Applicants should possess a good Honours degree in Botany, Applied Biology, Horticulture or Forestry and show a marked aptitude for applied research. Salary would be in the range £1,707 to £1,846 per annum. A current driving licence is essential.

Applications to: The Secretary, Research Station, Long Ashton, Bristol BS18 9AF, before June 14, from whom further particulars can be obtained.
(1893)

Scottish Health Service

COMMON SERVICES AGENCY

SCOTTISH BLOOD TRANSFUSION CENTRE

Applications are invited for the post of **PRINCIPAL SCIENTIFIC OFFICER** in the above Department. The successful applicant will be responsible to the Regional Director for the day to day management of the scientific, logistic and technical aspects of the Blood Bank and Blood Component Divisions in which 35 technical and scientific staff are employed.

The post is on the Principal Biochemist salary scale, and NHS terms and conditions of service will apply. Further particulars may be obtained from the National Administrator, Scottish Blood Transfusion Service, 17 Rothesay Terrace, Edinburgh EH3 7SF. Applications with the names of 2 referees should be forwarded to the Secretary, Common Services Agency for the Scottish Health Service, 17/18 Rothesay Terrace, Edinburgh EH3 7SF not later than 8th June, 1974.

(1935)

Reckitt & Colman

Dietician

To join the Medical Department of a well established and expanding Pharmaceutical Division of this major British Company.

The successful applicant will join a highly qualified team of experienced professionals and will provide his or her professional contribution on all subjects relating to nutrition. Initial work will be in the field of infant nutrition, particularly premature infants and neonates, and will involve liaison with clinical research workers in centres throughout the U.K.

The demands of this job will require the successful applicant to be well qualified and experienced, preferably with a research orientated career background which will enable him or her to function effectively in an atmosphere of new ideas and creative work.

The appointment is located in Hull and the salary will be attractive. Please write quoting ref U.75 for an application form to:

R. A. Sandow,
Divisional Personnel Manager,
Reckitt & Colman,
Pharmaceutical Division,
Dansom Lane, Hull HU8 7DS

(1940)

British Museum (Natural History)
Sub-department of Anthropology

Physical Anthropologist

■ **Develop and co-ordinate research on fossil and recent populations, in collaboration with Museum's Serological Unit ■ Curatorial responsibilities.**

☐ 1st/2nd hons degree in appropriate subject ☐ At least 2 years' postgraduate experience ☐ Age under 32 ☐ Appointment as Senior Scientific Officer (£2973-£4070) or Higher Scientific Officer (£2396-£3029) according to age and experience ☐ Salaries under review ☐ Ref: SB/23/DK.

☐ Application forms (for return by 10 June 1974) from Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, telephone Basingstoke 29222 ext. 500 or London 01-839 1992 (24 hour answering service).

Department of Agriculture and Fisheries for Scotland
Agricultural Scientific Services, East Craigs, Edinburgh

Analytical Chemist

■ **Join small team analysing residues of pesticides and heavy metals in relation to agriculture, food storage, wildlife and environment generally ■ Development of new analytical methods involved ■ Chemotaxonomy of plants using modern analytical techniques.**

☐ Hons degree, or equivalent, in Chemistry ☐ Experience of gas chromatography and other microanalytical methods an advantage ☐ Age under 30 ☐ Appointment as Higher Scientific Officer (£2221-£2854) or Scientific Officer (£1435-£2329) according to age and experience ☐ Salaries under review ☐ Ref: SA/4/DLA.

☐ Application forms (for return by 10 June 1974) from Scottish Office, Personnel Division, Room 214, 22/25 Queen Street, Edinburgh, EH2 1LY, telephone 031-556 9222 ext. 762.

Department of Agriculture and Fisheries for Scotland
Marine Laboratory, Aberdeen

Research Fellow – Mathematician

■ **To study theory of population structure in marine ecosystems ■ Problems include, interaction of physical processes with community dynamics, factors determining population stability and effects of pollution on marine life ■ Computer simulation modelling involved.**

☐ 1st/2nd hons degree, or equivalent, in Mathematics ☐ Experience in numerical analysis and interest in ecological problems an advantage ☐ At least 3 years' postgraduate experience ☐ Appointment for 3 years as Senior Research Fellow (£2794-£3480 under review) ☐ Ref: SC/1/DLC.

Biologist

■ **Join team engaged on study of pollutants in marine ecosystems ■ Investigate effect of pollutants on food chains, leading to particular species of commercial fish ■ Design and operate field experiments to determine distribution of selected pollutants ■ Field and laboratory work involved.**

☐ Degree, or equivalent, in Biology – preferably good hons ☐ At least 2 years' postgraduate experience ☐ Interest in chemistry desirable ☐ Experience of experimental work, particularly with large-scale tank systems an advantage ☐ Age under 32 ☐ Appointment as Senior Scientific Officer (£2798-£3895) or Higher Scientific Officer (£2221-£2854) according to age, qualifications and experience ☐ Salaries under review ☐ Ref: SA/6/DLC.

☐ Application forms (for return by 12 June 1974) from Scottish Office, Personnel Management (PTS) 1, Room 214, 22/25 Queen Street, Edinburgh EH2 1LY, telephone 031-556 9222 ext. 762.

Science group
CIVIL SERVICE

(1900)

THE UNIVERSITY OF ASTON IN BIRMINGHAM
DEPARTMENT OF MATHEMATICS
LECTURERS

Vacancies exist for well qualified mathematicians/Statisticians in the Department of Mathematics. The Department is directly responsible for a first degree in Mathematics/Statistics, an M.Sc. course in Industrial Mathematics and Statistics and for research. In addition it offers a service to the University in the teaching of Mathematics and Statistics. The duties of those appointed will be in this service area. Preference will be given to those applicants whose research interests are in the application of mathematics/statistics to science and technology.

Commencing salary within the range £2,118 to £2,580 per annum on a scale rising to £4,896 per annum (payable with effect from October 1, 1974).

Requests for further details and application forms (which should be returned not later than June 3) should be sent, preferably on a postcard, quoting Ref. No. 974/6, to the Staff Officer, The University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET. (1912)

M.R.C. CLINICAL RESEARCH CENTRE
(Northwick Park Hospital)
Watford Road, Harrow, Middlesex HA1 3UJ
RESEARCH ON THE FUNCTIONAL PSYCHOSES

Applications are invited from suitably qualified candidates for a full-time research post in the recently established Division of Psychiatry at this Centre. The Division has been established to study physiological and biochemical correlates of mental illness and the successful candidate wil contribute to the psychological and behavioural aspect of these investigations.

The appointee will work on the psychological deficit in schizophrenia and some knowledge of biochemical or pharmacological aspects of psychiatric disease would be an advantage but is not essential.

Salary according to age and experience.

Further details and application forms may be obtained from Mrs. J. Tucker-Bull.

Please quote ref. 125/1. Closing date: Jun 29. (1914)

EAST BIRMINGHAM HOSPITAL
DEPARTMENT OF CLINICAL CHEMISTRY
BASIC GRADE BIOCHEMISTS

Due to expansion of the department and extended facilities vacancies have arisen for Basic Grade Biochemists. One post is available now, the other will be available in September. These posts are most suitable for those in, or about to qualify for, the post-probationary grade, for example with an M.Sc. in Clinical Chemistry, but other Honours Graduates would be considered.

The laboratories are well equipped and offer a wide range of activities giving full training for M.C.B. Automation is by single channel systems and reaction rate analysers, thus offering ample opportunities for development. Off line computer facilities are about to be installed. Several research programmes is collaboration with clinical staff are also being pursued.

Further details may be obtained from D. H. G. Sammons, Department of Clinical Chemistry, East Birmingham Hospital, Bordesley Green East, Birmingham B9 5ST, to whom all applications naming two referees should be made. (1915)

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY
DEPARTMENT OF BOTANY AND PLANT TECHNOLOGY
LECTURESHIP IN TIMBER TECHNOLOGY

A Lecturer is required from October 1, 1974, to undertake teaching and research in association with the Timber Technology section of the Botany Department, and to assist also in general departmental teaching preferably in the field of plant anatomy and morphology. The work of the section is concerned with the anatomy and fine structure, the processes of fungal decay, and the methods of preservation of timber. Postdoctoral or equivalent experience is desirable, and, given a knowledge of plant anatomy, could be in fungal physiology, enzymology, or plant biochemistry.

Initial salary in the range £2,118 to £3,636 plus £162 London Allowance, according to experience.

Further information can be obtained from Professor A. J. Rutter, Department of Botany, Imperial College of Science, Prince Consort Road, London, S.W.7, to whom applications, giving details of education and experience, and the names of two referees, should be made by June 14. (1919)

GUY'S HOSPITAL MEDICAL SCHOOL

JUNIOR TECHNICIAN OR TECHNICIAN

required for Gastroenterology Research Unit. For Junior Technician O.N.C. or Science A levels essential; for Technician H.N.C. Junior Technician salary scale rises to £1,395 plus £126 London Weighting; Technician scale £1,440 to £2,292 plus London Weighting.

Apply in writing to the Secretary, Guy's Hospital Medical School, London Bridge SE1 9RT, quoting Ref. G.R. (1917)

The Open University

Research Assistant in Earth Sciences

Applications are invited for a Research Assistantship in Earth Sciences tenable from September 1, 1974, or as soon as possible thereafter. The person appointed will work with Professor I. G. Gass on Petrochemistry and genesis of volcanic rocks. Applicants should possess a good honours degree in Earth Sciences and post-graduate qualifications/experience in geochemistry. Commencing salary based on age, qualifications and experience, will be within the scale: £1,323 to £2,058 per annum (under review).

Details are obtainable from the Personnel Manager (RE1), The Open University, P.O. Box 75, Walton Hall, Milton Keynes MK7 6AL. (Telephone Milton Keynes 74066, extension 3068).

Closing date: Monday June 3, 1974.

(1931)

MASSEY UNIVERSITY
Palmerston North, New Zealand

LECTURER/SENIOR LECTURER IN FOOD PROCESSING/PROCESS DEVELOPMENT

Applications are invited for the abovementioned position in the Department of Food Technology. Applicants should be suitably qualified in Food Technology, Chemical or Biochemical Engineering and have subsequently gained experience in food processing or applied food research. It is essential that the applicant have a strong quantitative approach to processing.

The major commitment of the appointee will be in teaching Food Processing and Process Development to undergraduate and postgraduate students taking the Food Technology option of the Technology Degree. He will be encouraged to develop liaison with one or more sectors of the food processing industry, and to develop an appropriate research area.

Appointment will be on the Lecturer-Senior Lecturer scale according to qualifications and experience.

Salary: Senior Lecturer NZ$8,718 to NZ$10,232 (bar) NZ$11,139; Lecturer NZ$6,753 to NZ$8,568. NZ$1=62p (approx.).

Further details of the position and of the University together with general conditions of appointment, may be obtained from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University.

Applications close on **June 28, 1974.** (1953)

UNIVERSITY OF ABERDEEN
RESEARCH ASSISTANT IN BIOCHEMISTRY

Applications are invited for above post to work with Dr. S. B. Wilson, on aspects of ion uptake by potato mitochondria. The post is tenable for up to 3 years from October 1, 1974. Candidates should have an honours degree in biochemistry or a related subject or be graduating this summer. Suitably qualified candidates may be permitted to register for a higher degree. Salary within scale £1,305 by £87 to £1,479.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by June 22, 1974.

(1948)

Haematologist

This vacancy is within our new Toxicology and Drug Safety Unit and involves responsibility for the section studying the effects of drugs on the Blood Picture during the assessment of drug safety.

Applicants should possess a degree in Physiology or Biochemistry and have a sound knowledge of haematological techniques. An interest in, or experience of, Immunology would be advantageous but is not essential.

The Medicinal Research Centre is located close to Harlow New Town and combines easy access to London with proximity to pleasant countryside. Development Corporation housing may be available to suitable applicants at economic rent.

A competitive starting salary will be paid and there are the usual fringe benefits which one associates with a major British-based company.

If you would like to be considered for this post, please write, outlining your career to date, to:

BRL

John Atkinson,
Site Personnel Officer,
Beecham Research Laboratories,
The Pinnacles.
Harlow, Essex

1972

(1899)

The Hatfield Polytechnic
Principal Lecturer in Psychology
and
Senior Lecturer in Psychology

Immediate application is invited for these two appointments to begin in September, 1974.

Applicants must be specially well qualified either in experimental or physiological psychology. Both posts imply taking a significant role in teaching on an existing Honours BSc degree and on an MSc degree which is being planned. Staff are expected to develop their own research interests for which there are good facilities.

Salary scale: Principal Lecturer, £3,831 to £4,269 bar £4,815 per annum; Senior Lecturer, £3,522 to £3,912 bar £4,209 per annum.

Further details and application form may be obtained from The Staffing Officer. The Hatfield Polytechnic, PO Box 109, Hatfield, Herts AL10 9AB, or ring Hatfield 68100, Ext 309. Completed applications should be returned by 31 May, 1974.

Please quote reference 498. (1947)

UNIVERSITY OF EDINBURGH
FACULTY OF MEDICINE

Department of Biochemistry
University Demonstrator

The University Court invites applications for the post of Demonstrator which is tenable from 1st October, 1974. The appointment will be for one year in the first instance and subject to renewal annually up to a maximum of five years.

The major duties of the Demonstrator will be to participate in teaching programmes for medical, dental or science students. The initial salary will be at a suitable point on the scale £1659 - £2346 (under review).

Further particulars may be obtained from the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh, EH8 9YL, with whom applications (5 copies) together with the names of two referees should be lodged not later than 10th June, 1974. Please quote reference 1023.

(1941)

DUNSTAFFNAGE MARINE RESEARCH LABORATORY
Oban, Argyll

PHYSICAL OCEANGRAPHER

A Physical Oceanographer is required at the above laboratory to join a small group studying the circulation and mixing processes in the waters between the west coast of Scotland and Rockall Bank. The work will involve both hydrographic surveys from ships and measurements from moored buoys. Applicants should have at least a good honours degree; the appointment being at a level commensurate with age and experience. Application forms may be obtained from the Secretary, Scottish Marine Biological Association, P.O. Box 3, Oban, Argyll. (1951)

THE EDINBURGH SCHOOL OF AGRICULTURE
RESEARCH ASSISTANT

Applications are invited for a research assistantship tenable for 3-4 years to work on **Plasmodiophora brassicae** under the direction of Professor N. F. Robertson.

Applicants should possess a good honours degree and post-graduate experience in plant pathology.

Salary according to qualifications and experience: Grade III £2233 - £3895, or Grade IVa £1616 - £2060.

Further details and application form from the Secretary, The Edinburgh School of Agriculture, West Mains Road, Edinburgh, EH9 3JG. (1942)

UNIVERSITY OF LIVERPOOL

Department of Biochemistry

Applications are invited for the post of Research Assistant, financed by the Medical Research Council, to work on the role and regulation of bacterial ethanolamine kinase. The post is open to honours graduates in biochemistry. The appointment, which will be for three years, could be taken up as soon as possible. The post is available under the normal conditions for M.R.C. postgraduate awards. Applicants should indicate whether or not they would wish to work for a higher degree.

Applications, giving details of age, qualifications and experience and the names of two academic referees, should be received as soon as possible by the Registrar, The University, P.O. Box 147, Liverpool, L69 3BX, from whom further particulars may be obtained. Quote ref RV/N/276022.

(1937)

NATIONAL INSTITUTE FOR MEDICAL RESEARCH
MILL HILL, LONDON

JUNIOR TECHNICIAN/TECHNICIAN required to assist in research on the metabolism of plasma proteins in the Division of Biophysics. Candidates should have a minimum of 4 GCE 'O' levels including biology and chemistry and preference will be given to those with GCE 'A' levels which should include chemistry. Experience of immunological methods would be an advantage. Those with GCE qualifications will be appointed as Junior Technicians on scale £834 (at 16) to £1,531 p.a. while HNC holders will be considered for appointment as Technicians £1,566 to £2,418 p.a. Please apply quoting ref: JT/BP.

JUNIOR TECHNICIANS/TECHNICIANS required to work in the Laboratory for Leprosy and Mycobacterial Research. Candidates with 'O' levels, 'A' levels or ONC will be considered for appointment as Junior Technicians £834 (at 16) to £1,461 p.a., while HNC holders will be appointed to the Technician grade £1,566 to £2,418 p.a. Two posts are available:

(a) To work using tissue culture techniques. Experience of sterile techniques would be a distinct advantage. (Quote ref: Lep/A).

(b) To work using bacteriological techniques particularly involving clinical trial material from overseas research units. Experience in bacteriology would be an advantage. (Quote ref: Lep/B).

ELECTRON MICROSCOPY TECHNICIAN familiar with thin tissue sectioning is required to assist in the examination of cells and sub-cellular components during animal development. Experience in electron microscopy would be an advantage. Initial salary, according to age, qualifications and experience, will be on scales £1,566 to £2,418 p.a. or £2,202 to £2,949 p.a. Please quote ref: EM/DB.

Applicants for these positions should write to J. H. Woodcock, N.I.M.R., The Ridgeway, Mill Hill, NW7 1AA. Tel: 01-959 3666 with details of age, qualifications and experience. (1952)

SCIENCE RESEARCH COUNCIL

DIRECTOR

ROYAL GREENWICH OBSERVATORY

Herstmonceux Castle, Hailsham, Sussex

The Science Research Council wish to appoint a successor to the present Director, Dr. A. Hunter, who is retiring on December 31, 1975.

The Observatory has a staff of about 220 and a budget of approximately one million pounds a year. Its programme of work is in three main areas: astronomical research; astronomical instrumentation; and the provision of national and international services for astronomy and navigation, including the National Time Service.

The Council intend that the Observatory shall become increasingly involved in the provision of national ground-based astronomy facilities for use by astronomers in universities and polytechnics as well as in the Royal Observatories. It will be a major task of the new Director to complete the restructuring of the Observatory that will be necessary to effect this change of work.

The post is pensionable either under the Council's own non-contributory Superannuation Scheme or under the F.S.S.U. Scheme. The Salary of £8,500 a year is under review.

Applications should be sent to Professor S. F. Edwards, F.R.S., Chairman, Science Research Council, State House, High Holborn, London WC1, to arrive before June 30, 1974.

Further information about the work and conditions of service may be obtained from the Chairman's Office. (1955)

University of London

CHAIR OF PHYSIOLOGY AT QUEEN ELIZABETH COLLEGE

The Senate invite applications for the above Chair which includes Headship of the Department of Physiology at the College. Initial salary to be agreed but not less than £5973 a year plus £162 London Allowance. Applications (10 copies) should be received not later than 20 June 1974 by the Academic Registrar, (N) University of London, Senate House, WC1E 7HU, from whom further particulars should first be obtained. (1945)

THE INSTITUTE OF PHYSICS

requires an

EDITORIAL ASSISTANT

to join its permanent staff in Bristol.

The Publishing Division of the Institute has a staff of about 60, many of whom are professionally qualified. The main activity is the publication of a range of international physics journals, and there are plans for expansion in this and related areas.

Applicants should be prepared to work steadily in a small editorial team. Working conditions are pleasant, in a modern office.

Candidates should have an honours degree or equivalent in physics, mathematics or chemistry. Starting salary (under review) between £1440 p.a. (minimum for a recent graduate) and £1800 p.a. with regular increases to £2340 p.a. and promotion prospects to £2940 p.a. No previous editorial experience is required. Training will be given in the basic techniques of sub-editing and proof reading, with the possibility of further training in wider aspects of publishing.

Further information and application form from: Managing Editor, Institute of Physics, Techno House, Redcliffe Way, Bristol BS1 6NX. (1936)

TEMPORARY RESEARCH OFFICER
(for nine months)

Psychophysiologist/Physiologist required as Temporary Research Officer on an EEG and Language Project in the Department of Linguistic and Regional Studies, University of Surrey. Candidates should be psychology or physiology graduates with research experience and should have knowledge of signal averaging and frequency analysis.

Salary: £166 per month.

Applications should be sent to: Professor H. P. Walz, Department of Linguistic & Regional Studies, University of Surrey, Guildford, Surrey GU2 5XH. (1925)

NATIONAL VEGETABLE RESEARCH STATION

SCIENTIFIC OFFICER required by Chemistry Section to take charge of a programme of fertiliser experiments on commercial vegetable farms. A degree, HND, HNC, or equiv. in horticulture or agriculture is required, and some training in field experimentation desirable. Driving licence essential. Starting salary in scale £1,435 - £2,329 p.a. according to qualifications and experience. Superannuation with allowance to offset contributions. Full particulars and application form (to be returned by 13th June 1974) from the Secretary, NVRS, Wellesbourne, Warwick CV35 9EF. (1928)

UNIVERSITY OF SYDNEY

The Henry Bertie and Florence Mabel Gritton Post-Graduate Research Scholarship

Applications are invited for the above-mentioned Scholarship from graduates of any university within the Commonwealth, or from graduates of any university who are British subjects. The Scholarship is tenable in the University of Sydney for up to three years.

The scholar will be expected to undertake post-graduate research in Chemistry as it is related to Industry or Agriculture.

Depending on the applicant's qualifications, the Scholarship will be awarded in either the senior grade (in the range of $A7,270 to $A8,413 per annum) or the junior grade (stipend $A3,550 per annum), plus other allowances, where appropriate, including a travel allowance.

Applications, which should include a certified copy of the applicant's academic record and the names of two referees, close with the Registrar, University of Sydney, N.S.W. 2006, Australia on **17 June 1974.**

Further information and application forms may be obtained from the Registrar's (1929)

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY
ROYAL SCHOOL OF MINES

LECTURESHIP IN MINERAL PROCESSING CHEMISTRY

Applications are invited for the post of Lecturer in Mineral Processing Chemistry in the Department of Mining and Mineral Technology. Applicants should have a good first degree, preferably in Chemistry (Physical), and several years of research or industrial experience in a field relevant to mineral processing, e.g. hydrometallurgy.

The successful candidate will be required to teach basic physical chemistry and its applications to mineral processing to undergraduate and postgraduate students, to take laboratory and tutorial classes, and to develop research work within the Department's area of activity.

The appointment will date from 1st October, 1974, or as soon as possible thereafter.

Initial salary, depending on age, qualifications and experience, will be within the Lecturer scale £2,118-£4,896 plus £162 p.a. London Allowance. F.S.S.U. superannuation benefits apply.

Further information and an application form can be obtained from Professor M. G. Fleming, Department of Mining and Mineral Technology, Royal School of Mines, Imperial College of Science and Technology, London SW7 2BP.

(1922)

The University College of Wales
Aberystwyth

DEPARTMENT OF BIOCHEMISTRY

Applications invited for a postdoctoral appointment as RESEARCH ASSOCIATE to work with a group studying the microbial metabolism of alicyclic compounds.

Experience of microbial biochemistry, the metabolism of cyclic structures or of mixed function oxygenase systems and enzyme purification an advantage.

Two years from 1 October. Initial salary: £2,058 per annum.

Enquiries to Dr. P. W. Trudgill, Department of Biochemistry, U.C.W. Aberystwyth. (Tel. 0970 3111 Ext. 100). Application forms available from the Registrar. (1926)

THE QUEEN'S UNIVERSITY OF BELFAST

Department of Pure and Applied Physics

Research Associate

Applications are invited for the post of Research Associate tenable from 1st October 1974. The successful applicant will be expected to carry out research within large well equipped research groups in one of the following fields:

(a) Atomic and molecular collision physics.

(b) Laser physics, non-linear optics and opto-electronics.

(c) Thin film optics.

Candidates should possess a Ph.D. degree and relevant research experience. Salary wil be dependent on qualifications and experience within the range £2,118 to £2,412, normally plus F.S.S.U.

The appointment will initially be for one year but is renewable annually thereafter. Applications giving full details of academic record and experience together with the names of two referees should be sent to the Personnel Department, The Queen's University of Belfast BT7 1NN, Northern Ireland, before May 29th.

(1923)

FELLOWSHIPS AND STUDENTSHIPS

MINISTRY OF AGRICULTURE, FISHERIES AND FOOD

Research Fellowship Myopathy in Farm Animals

Applications are invited for a Research Fellow to work on morphological aspects of muscle disorders in farm animals. The Fellow will be attached to the Department of Pathology of the Central Veterinary Laboratory, Weybridge, and will join a multidisciplinary group concerned mainly with developmental myopathies in pigs, cattle and sheep.

QUALIFICATIONS. Candidates should have a basic degree in veterinary science or in biology with PhD (or equivalent) and considerable post graduate experience in either muscle biology or pathology.

Appointment would be at the level of Junior or Senior Research Fellow, though appointment at Principal Research Fellow level might be considered in the case of an exceptionally well qualified candidate.

REMUNERATION. The value of the Fellowship will be determined by the interview but will lie within the following ranges:-

Principal Research Fellowship	£3,713 - 4,898
Senior Research Fellowship	£2,794 - 3,480
Junior Research Fellowship	£1,914 - 2,498

Application forms may be obtained from Mr. J. S. Barnes, Ministry of Agriculture, Fisheries and Food, Room 6/11, Victory House, 30-34 Kingsway, London WC2B 6TU. Telephone 01-405 4310, Ext 308.

Closing date for completed forms 14th June 1974.

(1858)

Post Doctoral Research Fellow

required in Department of Applied Biology and Food Science. (Head: Dr. P. Wix).
to work in one of the following fields:—

Invertebrate toxicology or physiology
Helminthology
Fresh water microbiology or ecology
Physiology of fresh water plants
Microbial biochemistry
Fermentation technology
Food volatiles
Food additives
Food product development

The successful applicant will be expected to initiate and develop research and may be required to arrange an annual programme of Seminars.

Salary: £2,151—£2,421

Apply by letter, stating full personal and career details, to the Clerk to the Council (Room 665), Polytechnic of the South Bank, Borough Road, London SE1 0AA.

Polytechnic of the South Bank

(1956)

AUSTRALIAN NATIONAL UNIVERSITY

RESEARCH SCHOOL OF EARTH SCIENCES

PROFESSOR/PROFESSORIAL FELLOWSHIP (2 Posts)

The Research School of Earth Sciences, under the direction of Professor A. L. Hales, proposes to develop research in three areas, Economic Geology, Environmental Geochemistry and Geophysical Fluid Dynamics. It is proposed to make senior appointments in two of these three fields initially.

Economic Geology: The successful appointee will be expected to lead a group or Department conducting basic research directed towards the scientific study of mineral deposits, including their petrology, geochemistry, geological environment and genesis. Fundamental investigations of the chemistry of ore-forming solutions and the entire range of physico-chemical processes occurring between the ultimate source and depositional locale are also envisaged. Experimental and laboratory investigations devoted to these topics will constitute an important aspect of the activities of the Department or group and applicants should have appropriate backgrounds in these areas.

Environmental Geochemistry: The successful applicant will be expected to lead the researchers of a group or of a department working in broadly-based and fundamental aspects of the field of environmental geochemistry. It is intended that researches in this area will be directed towards the large-scale chemical interactions between the atmosphere, oceans, solid earth and biosphere. The geochemical cycling of elements between these reservoirs (including specialised interface studies) and the chemistry of these reservoirs (particularly the atmosphere and oceans) will also be investigated. The use of physicochemical tracer techniques to study the dynamical circulations of the oceans and atmosphere is also envisaged. Another field of concern to the research group or department will be palaeoclimatology. The approach to be adopted towards these problems will have a strong experimental bias with intensive application of sophisticated isotopic, radiometric and trace element analytical techniques.

Geophysical Fluid Dynamics: The successful appointee will be expected to lead a group or Department conducting basic research in fluid dynamics as applied to geophysical processes. Studies of the large scale motions of the sea and/or atmosphere will be emphasised in the initial stages of the development. Although the group may not be involved initially in data collection it is envisaged that the analysis of data, both physical and chemical, relevant to the problems of the large scale motions of the sea and atmosphere and their interactions will form an important part of its activities.

Salaries: Salary for a Professor is $A19,102 per annum and for a Professorial Fellow $A17,648 per annum.
Other Conditions: Tenure—To retiring age (at present 65).

Reasonable travel expenses are paid and assistance with housing is provided. Superannuation is on the F.S.S.U. pattern with supplementary benefits.

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Candidates are urged to write to Professor A. L. Hales, Director, Research School of Earth Sciences, Australian National University, P.O. Box 4, Canberra, ACT 2600, Australia, for further information.

G. E. Dicker, Acting Academic Registrar. (1933)

UNIVERSITY OF ABERDEEN
UNIT FOR RESEARCH ON ADDICTIVE DRUGS

A S.R.C. Studentship (Co-operative Award in Science and Engineering, C.A.S.E.), will become available on October 1, 1974. It is tenable for 3 years in co-operation with the Pharmaceutical Division of Reckitt and Colman Ltd., Hull, and is open to First and Upper Second Class Honours Graduates in Pharmacology, Biochemistry or Physiology. The research project is concerned with the action of newly-synthesized morphine-like compounds on neurotransmitter release and on behavioural patterns in normal and morphine-dependent animals.

Applications (3 copies) to be submitted to Professor H. Kosterlitz, from whom further particulars may be obtained, University of Aberdeen, Marischal College, Aberdeen AB9 1AS, not later than May 31, 1974. (1844)

UNIVERSITY OF WARWICK
Postdoctoral Fellowship in Biological Sciences

Applications are invited for the above post to work on the biochemistry of virus RNA replication. The post would be suitable for a biochemist with an interest in animal virology or a virologist with a good knowledge of biochemistry. Experience of working with nucleic acids or their polymerases would be an advantage. The appointment is for twelve months commencing October 1, 1974. Salary in the range £2,118 to £2,247 to £2,412 p.a. with F.S.S.U. Further details and application forms may be obtained from the Academic Registrar, University of Warwick, Coventry CV4 7AL, to whom completed forms should be returned by May 27, 1974, quoting Ref. No. 40/Q/74. (1852)

UNIVERSITY OF SOUTHAMPTON
DEPARTMENTS OF BIOLOGY AND CHEMISTRY

Applications are invited for an S.R.C. Studentship, starting in October 1974, for work on predator-prey interactions of tropical ants and termites. In particular, defensive secretions will be studied with a view to the development of integrated control methods. This will involve collaboration with the Centre for Overseas Pest Research and fieldwork in Nigeria.

Further particulars may be obtained from the Academic Registrar's Department, The University, Southampton SO9 5NH, to whom applications should also be sent. (1812)

CHELSEA COLLEGE
University of London

Applications are invited for the post of POST-DOCTORAL RESEARCH FELLOW as member of an S.R.C. supported team investigating THE INTERACTION OF THE HUMAN THERMO-REGULATORY AND CARDIOVASCULAR CONTROL SYSTEMS in collaboration with the Engineering in Medicine Laboratory at Imperial College.

The successful candidate will probably be a Physiologist with an interest in human experimentation and data analysis, but applications from candidates with other backgrounds but a real interest in the project will be welcomed.

The appointment will be for three years commencing as soon as possible. Salary: £1,746 to £1,920 to £2,091 per annum. Superannuable. Application forms from the Personnel Office, Chelsea College, Manresa Road, London SW3 6LX. (1845)

RESEARCH STUDENTSHIP

Applications are invited for a research studentship to study the photodegradation and photostabilisation of acrylonitrile-butadienestyrene copolymers. This will involve the study of a novel photo-stabilisation mechanism and will be carried out in collaboration with an industrial research laboratory. Candidates with an interest in organic and/or polymer chemistry will be preferred. The studentship will be at current S.R.C. rates and will be renewable annually up to a maximum of three years.

Applications to:

Professor G. Scott,
Department of Chemistry,
University of Aston in Birmingham,
Gosta Green,
Birmingham B4 7ET. (1821)

UNIVERSITY OF DURHAM
DEPARTMENT OF BIOLOGY

Applications are invited for studentships tenable from October 1, 1974, from chemists, biochemists or botanists, to work on the biochemistry of grain legume improvement. Successful candidates may register for a higher degree.

Applications to Professor D. Boulter, Department of Botany, Science Laboratories, South Road, Durham DH1 3LE. (1813)

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL
DEPARTMENT OF PHYSIOLOGY
Cardiovascular Research

Applications are invited from honours graduates or prospective honours graduates in physiology, pharmacology or a related discipline, for a studentship for training in research methods tenable from October 1974. The holder will be eligible for registration as Ph.D. student of the University. The value of the award and conditions of appointment will be those of M.R.C. Studentships.

Facilities are available for the postgraduate student to join a group working on several aspects of autonomic nervous control of the cardiovascular system in animals and in man, under the supervision of Dr. P. H. Fentem. Applicants should write to Dr. Fentem giving a brief curriculum vitae and the names and addresses of two referees, at the Department of Physiology, The Medical School, University of Nottingham, Nottingham NG7 2RD. (1820)

Dunstaffnage Marine Research Laboratory

Applications are invited for a research studentship funded by the Natural Environment Research Council to work on the breakdown of organic material in near shore marine sediments. The studentship would be tenable for a period of three years and the applicant would be required to register for a Ph.D. under the regulations of the University of Dundee. The student would be part of a small multidisciplinary team working on the long term effects of particulate effluents on sea lochs in the West of Scotland. Application forms and further information can be obtained from the Director, Dunstaffnage Marine Research Laboratory, P.O. Box 3, Oban, Argyll PA34 4AD. (1863)

POSTGRADUATE STUDENTSHIP

Applications are invited from graduates with first or upper second class honours degree. A general knowledge of biochemistry is required, preferably with specialisation in mitochondrial or muscle biochemistry.

The successful candidate will be expected to study the general biochemical properties of red and white, and fast and slow muscles. The project would involve assay of the myoglobin haemoglobin and mitochondrial contents of the muscle, together with a study of their myofibrillar ATP-ase, mitochondrial and Ca-pump activities.

The work is expected to form the basis of an M.Sc. or Ph.D. thesis.

Further particulars of award, allowance, together with a form of application from:

Secretary, Meat Research Institute, Langford, Bristol BS18 7DY. (1831)

UNIVERSITY OF LEICESTER

Applications are invited for a C.A.S.E. Studentship. The successful applicant will work with Professor M. C. R. Symons on a research project entitled "Quantitative Study of Autoxidation Reactions with the aid of E.S.R. and Kinetic Techniques". The research will be conducted in co-operation with Dr. N. Uri and the student will spend a proportion of his/her time at the Research Laboratories at Waltham Abbey during the 3-year Ph.D. training.

Applications should be made to Professor M. C. R Symons, Department of Chemistry, University of Leicester LE1 7RH, from whom further details may be obtained. (1815)

THE UNIVERSITY OF MANCHESTER
DEPARTMENT OF LIBERAL STUDIES IN SCIENCE

Applications invited for Research Studentships supported by the S.R.C. tenable from October 1. The Department is engaged in teaching and research on economic, social, political and historical aspects of science and technology. Research topics can be chosen from the role of science and technology in public policy, sociology of science and technology, economics and innovation, technology assessment, or related areas. Applicants should have, or expect shortly to obtain, a first or upper second class honours degree, preferably incorporating elements from both the natural and social sciences. Applicants with specialist science or social science degrees may be accepted subject to the completion of appropriate coursework. Applications, giving full details of qualifications and experience and names of two referees, and indicating a preference regarding research area, should be sent to Dr. R. D. Johnston, Department of Liberal Studies in Science, The University, Manchester M13 9PL by June 7. (1819)

QUEEN MARY COLLEGE
University of London
KINETIC SPECTROSCOPY

Two S.R.C. supported Postdoctoral Fellowships are available from September/October 1974 for 1 or 2 years, for work with Dr. M. A. A. Clyne. One is for a new project on laser-induced fluorescence of diatomic and triatomic free radicals in the gas phase. A background in spectroscopy of small molecules, vacuum techniques, gas kinetics or lasers an advantage.

The other post is for studies of elementary reactions of ground state atoms, including halogen atoms, using vacuum ultraviolet atomic resonance spectrometry linked to a discharge-flow system. A background in spectroscopy of atoms or small molecules, gas kinetics, vacuum techniques or fluorine inorganic chemistry of small molecules an advantage.

Salary (under review) in range £1,929 to £2,223 p.a. plus £162 London Allowance. Applications (giving age, qualifications, experience and names and addresses of two referees) as soon as possible to The Registrar, Queen Mary College, Mile End Road, London E1 4NS. (1824)

UNIVERSITY OF SOUTHAMPTON
RESEARCH FELLOW IN GAMMA RAY ASTRONOMY

Application is invited for the post of Research Fellow to work on the development of a telescope for Low Energy Gamma Ray Astronomy using high altitude balloons. Applicants should be familiar with particle counting techniques and associated electronic circuitry. The post is initially for two years. Candidates should possess a Ph.D. degree or equivalent qualification. Salary will be in the range £1,929 to £2,388 plus F.S.S.U. Applications including curriculum vitae and the names and addresses of two referees should be sent to the Deputy Secretary's Section, The University, Southampton SO9 5NH, quoting reference number 223/R/Na. (1834)

University of Strathclyde
Department of Metallurgy

RESEARCH FELLOWSHIP: ELECTRONIC STRUCTURE OF ALLOYS

Applications are invited for a Research Fellowship, tenable in the first instance for 1 year and renewable for a further year, from candidates with interests in MATERIALS PHYSICS. The salary will be within the range £1929 — £2553, with placing according to qualifications and experience. F.S.S.U. benefits.

The successful applicant will work with an established group, supported by the Science Research Council, in the field of ELECTRONIC STRUCTURE OF METALS AND ALLOYS studied by soft X-RAY SPECTROSCOPY and X-RAY PHOTOELECTRON SPECTROSCOPY. A PhD. in physics, metallurgy or chemistry, and relevant research experience is desirable.

Applications (quoting R 14/74) should be addressed to Dr. L. M. Watson, Department of Metallurgy, Colville Building, 48 Portland Street, Glasgow, G1 1XM. (1902)

HERIOT-WATT UNIVERSITY - EDINBURGH
Postgraduate Research Studentships in Department of Physics

Prof. S. D. Smith **Tel. 031-449 5111**

Applications are invited for SRC, NERC and University Research Studentships tenable for 3 years from September 1974. The principal research interests of the Department are in solid state and laser physics, non-linear optics, tunable laser spectroscopy and satellite meteorology. Active and substantial projects are current on applications of the spin-flip Raman laser, opto-acoustic spectroscopy with tunable dye lasers and the remote measurement of atmospheric temperature and pressure from satellites. (1851)

MEDICAL RESEARCH COUNCIL OF NEW ZEALAND
University of Otago Dental School
SENIOR RESEARCH FELLOWSHIP

Applications are invited from suitably qualified graduates for the above-mentioned position. Proven experience in research and the ability to initiate and direct projects is essential, although applicants need not necessarily be dentally qualified. The principal task of the appointee will be to carry out research in connective tissue biology. It is hoped that the appointee will also be prepared to act in a consultant capacity to other research workers.

There are, at the appointee's disposal, facilities for histology, histochemistry and some biochemical analytical procedures. The acquisition of a new transmission electron microscope is currently being negotiated. It is expected that the appointee will apply eventually to the Council for other equipment and technical assistance necessary for his own research. Travel grants will also be made to allow attendance at approved scientific conferences.

The initial salary will depend on the qualifications and experience of the appointee but will be comparable with what he would be offered in the university at the level of senior lecturer but in the case of an exceptionally well qualified applicant the Council will consider appointment at a level equivalent to that of Associate Professor. The Council will also pay the employer's contribution to superannuation and in the case of an appointment from overseas, financial assistance will be given for travel at rates and under conditions determined by the Council.

Further information and form of application may be obtained from J. A. Borrows, Technical Secretary, at P.O. Box 6063. Dunedin. New Zealand, with whom applications close on **July 31, 1974.** (1826)

RESEARCH STUDENTSHIPS IN PHARMACOLOGY

Applications are invited from graduates in pharmacology, biochemistry or physiology for Research Scholarships, to work on projects in neuropharmacology and immunopharmacology. Successful applicants will be expected to register for a higher degree.

Applicants should send their c.v. and names and addresses of two referees to Professor J. L. Mongar, Department of Pharmacology, UNIVERSITY COLLEGE LONDON, Gower Street, London WC1E 6BT. (1913)

IMPERIAL COLLEGE LONDON
DEPARTMENT OF ZOOLOGY AND APPLIED ENTOMOLOGY
RESEARCH STUDENTSHIP IN PARASITOLOGY

Applications are invited for an S.R.C. Research Studentship (under the co-operative award science and engineering scheme) to study the biology of the sheep tapeworm MONIEZIA EXPANSA.

The project will involve a close collaboration between the Department of Zoology and the Pharmaceutical Division of Imperial Chemical Industries. The award holder will be expected to carry out part of the work each year at the I.C.I. Laboratories.

Candidates should have a 1st or upper 2nd class Honours Degree or an M.Sc. in a biological science.

The studentship will be at the current S.R.C. rate (£695 p.a.).

Applications, giving the names of 2 referees, should be sent to Professor J. D. Smyth, Department of Zoology, Imperial College, London SW7 2BE (Phone 01-589 5111, Ext. 2264).

Closing date: May 25, 1974. (1838)

UNIVERSITY OF EXETER
DEPARTMENT OF PHYSICS
RESEARCH STUDENTSHIP

The Science Reesarch Council has allocated a C.A.S.E. (Cooperative Award in Science and Engineering) studentship for three years (starting October 1974) to suitably qualified graduates interested in carrying out at the University of Exeter a "Study of frequency and time dispersion of high frequency radio waves".

The cooperating body is the Royal Aircraft Establishment, Farnborough. The value of the award is not less than £695 or £540 (for students living in the parental home).

Applicants should possess, or expect to obtain in the Summer of 1974, a first or upper second class honours degree.

Further details and application forms can be obtained from:

Professor G. K. T. Conn,
Department of Physics,
University of Exeter,
Stocker Road,
Exeter EX4 4QL.

Applications should be sent in as soon as possible and in any case not later than June 15, 1974. (1903)

UNIVERSITY OF RHODESIA
FACULTY OF SCIENCE
SENIOR LECTURESHIP OR LECTURESHIP IN MATHEMATICS

Applications are invited for a Senior Lectureship or Lectureship in the Department of Mathematics.

Salary Scales and Conditions (approximate conversions from Rhodesian $): Senior Lecturer £5,346 by £214 to £7,058; Lecturer Grade I £4,857 by £180 to £5,577; Lecturer Grade II £2,994 by £154 to £3,456 by £171 to £3,798 by £180 to £4,518 by £170 to £4,688.

Family passages and allowance for transport of effects on appointment. Installation loan of up to half of one year's salary if required. Unfurnished University accommodation guaranteed for a period of at least three years for persons recruited from outside Rhodesia. Sabbatical and triennial visits with travel alllowance. Superannuation and medical aid schemes.

Applications: (6 copies) giving full personal particulars (including full names, place and date of birth, etc.), qualifications, experience and publications and naming three referees, should be submitted by **June 15, 1974** to the Assistant Registrar (Science), University of Rhodesia, P.O. Box MP 167, Mount Pleasant, Salisbury, Rhodesia, from whom further particulars may be obtained. An additional copy should be sent to the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF, from whom further particulars may also be obtained. (1954)

ROYAL HOLLOWAY COLLEGE
(University of London)
Egham Hill, Egham, Surrey
A TUTORIAL RESEARCH STUDENTSHIP

is offered in DEPARTMENT OF ZOOLOGY (Comparative Physiology) from October 1, 1974 at an award of £800 a year. Further details may be obtained from the Personnel Officer to whom applications should be sent as soon as possible. (1904)

UNIVERSITY OF SUSSEX
RESEARCH FELLOWSHIP IN BIOCHEMISTRY

Applications are invited for a post-doctoral fellowship in the Biochemistry group, tenable for 2 years from August 1, 1974. The successful candidate will join a group studying the control of protein synthesis in mammalian cells, and prior experience in a related area would be an advantage.

Salary will be on the Research Fellow scale, with initial placement in the range £2,118 to £2,931.

Applications including a curriculum vitae and the names of two referees should be sent to Dr. J. E. Kay, School of Biological Sciences, University of Sussex, Brighton BN1 9QG. (1901)

LASER-INDUCED TRANSITIONS

Applications are invited for a Postdoctoral Research Fellowship tenable in the Davy Faraday Research Laboratory of the Royal Institution (Director: Professor Sir George Porter, F.R.S.). The successful candidate will investigate transient chemical and physical phenomena occurring in the nanosecond and picosecond time regions. Applicants should be experienced in the use of pulsed lasers and general electro-optic techniques. The appointment is tenable for one year, and subject to satisfactory performance, renewable up to three years. The salary scale is £2,400 to £3,000 p.a.

A curriculum vitae and the names of two referees should be sent to Dr. M. A. West, The Royal Institution, 21 Albemarle Street, London W1X 4BS, U.K., by **June 28, 1974.** (1898)

UNIVERSITY OF READING
DEPARTMENT OF PHYSIOLOGY AND BIOCHEMISTRY

The Ministry of Agriculture, Fisheries and Food is prepared this year to offer to a suitable candidate a Studentship tenable in the Department of Physiology and Biochemistry in the University of Reading. The value of the MAFF award in 1974 will normally be £695 per annum. This studentship is for a place on the taught course for an M.Sc. in Physiology and Biochemistry of Farm Animals to start in October 1974 and lasting nine months. Applicant should hold at least a Lower Second Class degree in a suitable subject or hope to qualify this summer. Further particulars may be obtained from: Professor C. Tyler, Department of Physiology and Biochemistry, The University, Whiteknights, Reading RG6 2AJ. Ref. M.N.16. (1892)

UNIVERSITY OF DUNDEE
DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS
RESEARCH STUDENTSHIP

Applications are invited for an M.R.C. Research Studentship for training in research methods available from October 1, 1974, for up to three years. The training programme available is entitled "Methods of Studying Plasma and Tissue Binding of Drugs" and the work will be carried out in the Department of Pharmacology and Therapeutics in the new Ninewells Hospital and Medical School complex.

The successful applicant may register for a Ph.D. degree and will receive a stipend in accordance with the rates for M.R.C. scholars.

Candidates should have, or expect to obtain this year, a first or upper second class honours degree or equivalent in pharmacy, pharmacology, biochemistry or related subject. Applicants should write to Professor J. Crooks, Department of Pharmacology and Therapeutics, University of Dundee DD1 4HN, giving their qualifications and the names of two referees. Applications should be submitted by May 31. (1866)

UNIVERSITY OF NEW SOUTH WALES
SCHOOL OF BOTANY
Teaching Fellow

Good honours degree in botany or agriculture required. Initial appointment is for one year with prospects of annual renewal for a further two years; appointee will undertake research for a higher degree and will have some teaching duties.

Salary: between $A5,740 and $A7,015 per annum. Commencing salary according to qualifications and experience

Details of appointment, including superannuation, study leave and housing scheme, may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close in Australia and London on **7 June 1974.** (1943)

PORTSMOUTH POLYTECHNIC
Department of Biological Sciences
S.R.C. Case Research Studentship

The S.R.C. has awarded a CASE research studentship for a neurophysiological and toxicological study of the mode of action of pyrethroid insecticides in locusts. The work will be done in collaboration with Rothamsted Experimental Station, and the student will be expected to spend a significant period of time in the Insecticides and Fungicides Department at Rothamsted.

Applicants should have, or expect to obtain, a First or Upper Second class degree in a biological science, or its equivalent. The student will be expected to register for a Ph.D.

Application forms and further particulars may be obtained from the Staff Officer, Portsmouth Polytechnic, Alexandra House, Museum Road, Portsmouth, PO1 2QQ, to whom completed applications should be returned by 3rd June 1974. Please quote ref. B.10. (1946)

THE QUEEN'S UNIVERSITY OF BELFAST
DEPARTMENT OF BIOCHEMISTRY
POSTDOCTORAL RESEARCH FELLOW

Applications are invited for the above post, which involves the investigation of biochemical aspects of animal viruses. Applicants should have experience in some aspects of protein chemistry, enzymology, immunology or virology. The position will be financed by the Agricultural Research Council at a salary from £1,929 by £131 per annum plus F.S.U. according to age and qualifications.

Applications, including the names of two persons to whom reference may be made, should be sent to Dr. S. J. Martin, Department of Biochemistry, Medical Biology Centre, Queen's University of Belfast, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland. (1875)

UNIVERSITY OF WARWICK
RESEARCH FELLOWSHIP IN THEORETICAL PHYSICS

Applications are invited for a Postdoctoral Research Fellowship in the Department of Physics. The field of study will be low mobility, narrow band materials containing transition metals. Of particular interest are metal-insulator and metal superconductor phase transitions associated with lattice instabilities in such materials. The fellowship is tenable for a period of up to two years in the salary range £2,118, £2,247, £2,412 p.a. (exceptionally £2,580 p.a.), with FSSU. Letters of application (no forms) giving details of previous experience should be sent to the Academic Registrar, University of Warwick, Coventry CV4 7AL, quoting Ref. No: 41/2Q/74. Closing date for receipt of applications is 31st May, 1974. (1938)

THE UNIVERSITY OF SHEFFIELD
RESEARCH STUDENTSHIPS IN SOLID STATE PHYSICS

Applications are invited for two C.A.S.E. Research Studentships, tenable from October 1, 1974, leading to the degree of Ph.D., on the following: (1) Radiation damage effects on the electrical and optical properties of transparent semi-conductors, in collaboration with the British Aircraft Corporation. (2) High field and photo-effects in semiconducting glasses, in collaboration with S.T.L., Harlow. The awards will be at the appropriate rate for S.R.C. studentships. Candidates should have or expect to gain a first or upper second class honours degree in physics or a related subject. Applications giving full curriculum vitae and names of two referees should be sent as soon as possible to either Dr. I. G. Austin or Dr. T. M. Searle, Department of Physics, The University, Sheffield S3 7RH. (1950)

THE UNIVERSITY OF SHEFFIELD
DEPARTMENT OF CHEMISTRY

Applications are invited for the following:

1. POSTDOCTORAL RESEARCH FELLOWSHIP for studies on chemical carcinogenesis. The appointee will join a team working on chemical and biochemical aspects of polycyclic aromatic hydrocarbon binding to DNA, ranging from synthetic and structural organic chemistry to tissue culture. Candidates should hold (or expect to gain) a Ph.D. in organic or biological chemistry and experience in nucleic acid chemistry is desirable but not essential. The appointment is from October 1, 1974 or a date to be arranged until September 30, 1975 at a salary not less than £1,923 per annum plus F.S.S.U.

2. AN S.R.C. (C.A.S.E.) STUDENTSHIP for a project involving the synthesis of a novel group of heterocyclic compounds. These are designed to possess controlled chemical reactivity and potential herbicidal character. The work will be carried out in conjunction with I.C.I. Plant Protection Limited and part of the programme will be performed at their Jealott's Hill Research Station. Applicants should have (or expect to obtain this summer) a good Honours degree or G.R.I.C. in chemistry and the successful candidate will be expected to register for a Ph.D.

Applications for either of the above, including a curriculum vitae and names and addresses of two referees to: Dr. G. M. Blackburn, Department of Chemistry, The University, Sheffield S3 7HF (from whom further information is available in respect of the Studentship). (1949)

LECTURES AND COURSES

UNIVERSITY OF OTAGO
Dunedin, New Zealand
LECTURER OR SENIOR LECTURER IN MINERAL TECHNOLOGY

Applications are invited for a position within the grade of Lecturer/Senior Lecturer in the Department of Mineral Technology. Applicants should have recognised qualifications in the field of mineral processing and experience in the application of mathematical methods to problems in the mineral industry. A knowledge of physical and applied chemistry would be an advantage.

Salary Scales: Lecturer: $NZ6,753 to $NZ8,568 per annum; Senior Lecturer: $NZ8,718 to $NZ11,139 per annum with a bar at $NZ10,232 per annum.

Academic salary scales are subject to both triennial review and regular cost-of-living adjustments.

Further particulars are available from the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF or from the Registrar of the University.

Applications close in New Zealand and London on **August 15, 1974.** (1829)

FEBS Advanced Course No. 32

Ion Transport and Mitochondrial Function

Budapest 31 August—1 Sept. 1974

This Advanced Course will take the form of a colloquium in which a panel of experts will lead discussions on (1) Transport of Substrate Anions: Mechanisms and Physiological Implications; (2) Relationship between Proton Transport and Mitochondrial Function; and (3) Transport of Cations: Mechanisms and Physiological Implications.

Participants may be able to present 10-minute communications on relevant results.

Fee: Swiss Fr. 30.00.

Enquiries as soon as possible to Dr. A. Fonyo, H-1082 Budapest, Ülloi ut 78/a, Hungary. (1842)

University of Bradford

POSTGRADUATE SCHOOL OF STUDIES IN PHARMACOLOGY

MSc COURSE IN EXPERIMENTAL PHARMACOLOGY

Applications are invited from graduates in pharmacology, pharmacy or other appropriate subjects, for admission to the course starting in October, 1974. The course is of one year in duration and consists of four parts concerned with quantitative pharmacology, toxicology, psychopharmacology and a research project. The aim is to provide instruction and experience of research methodology as appropriate to the investigation of mechanisms of action of drugs.

Further information and application forms are obtainable from the Secretary of the School of Studies in Pharmacology, University of Bradford, Yorkshire BD7 1DP. (1865)

JAMES COOK UNIVERSITY OF NORTH QUEENSLAND

Applications are invited for the following appointments:

LECTURER IN GEOGRAPHY: Applicants should have a good honours degree in Geography or related field and, preferably, should also have a higher degree or be well on the way to the completion of the relevant research. An ability to teach and research in the physical or human aspects of resource management and evaluation is required.

LECTURER IN ZOOLOGY: Applicants should have an initial degree in Zoology or Biology, a Ph.D. and some experience in university teaching and research. The successful applicant will be required to continue teaching the existing courses in Entomology and to contribute to the general teaching programme in Zoology.

Salary range: $A8,698 - $A11,982 per annum, plus locality allowance of $A142 p.a. for a married male or $A71 p.a. for a single appointee. Conditions of appointment include F.S.S.U. superannuation, invalid pension scheme, housing assistance, study leave and allowance for travel and removal expenses on appointment.

Closing dates: Lectureship in Geography **14 June;** other post **7 June 1974.**

Further details and application forms obtainable from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. (1932)

UNIVERSITY OF SURREY LECTURER IN HUMAN BIOLOGY
DEPARTMENT OF BIOLOGICAL SCIENCES

Applications are invited for the post of Lecturer in Human Biology, tenable from September 1, 1974. The salary will be on the Lecturer scale £2,118 to £4,896 (1.10.74), and the post is superannuable under F.S.S.U.

Further particulars may be obtained from the Academic Registrar (LFG), University of Surrey, Guildford GU2 5XH. Telephone: Guildford 71281, extension 452. Applications, in the form of a curriculum vitae, including the names and addresses of two referees and present salary, should be sent to the same address by Friday, June 14, 1974. (1833)

UNIVERSITY OF DURHAM
DEPARTMENT OF PSYCHOLOGY

Applications are invited for the post of LECTURER IN PSYCHOLOGY from October 1, 1974, or such later date as may be arranged.

Salary on the scale £1,929 to £4,548 per annum (under review), plus F.S.S.U. benefits.

Applications (three copies) naming three referees should be sent by May 31, 1974 to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained. (1856)

THE UNIVERSITY OF SHEFFIELD
DEPARTMENT OF GEOGRAPHY

Applications are invited for the following posts in the above Department, both tenable from October 1, 1974:

1. LECTURER, in the field of PHYSICAL GEOGRAPHY; preference will be given to persons with an interest and research experience in soils and/or hydrology. Initial salary in the range £1,929 to £2,388 on the scale £1,929 to £4,548, with F.S.S.U. provision.
2. JUNIOR RESEARCH FELLOW; candidates should be geographers. Salary £1,473 a year.

Further particulars from the Registrar and Secretary, The University, Sheffield S10 2TN, to whom applications (5 copies) should be sent by May 25, 1974 Quote Ref. R.69/G. (1849)

LA TROBE UNIVERSITY
VICTORIA, AUSTRALIA

LECTURER/SENIOR LECTURER IN PURE MATHEMATICS

Applicants are invited for the above-mentioned position. The successful applicant will be expected to carry out normal teaching duties while continuing with mathematical research.

Salary: Senior Lecturer $A12,268 to 5 by $A408 to $A14,308; Lecturer $A8,698 to 1 by $A470 to 6 by $A469 to $11,982.

Further information and application forms are available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar, La Trobe University, Bundoora, Victoria, Australia 3083.

Aplications close on **June 30, 1974.** (1827)

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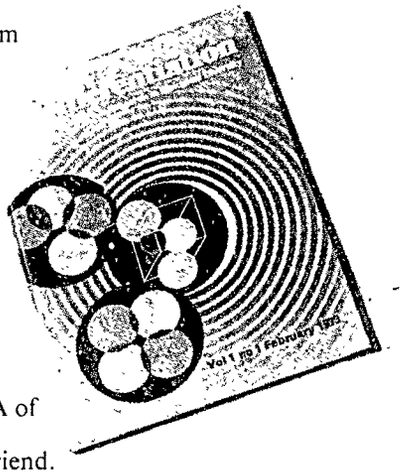
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Studies on cytology and differentiation in Sciaridae. 5. Electron microscopic studies on the salivary glands of *Bradysia elegans* Morgante (Diptera, Sciaridae). A. B. Da Cunha, R. W. Reiss, C. Pavan, J. J. Biesele, J. S. Morgante and M. C. Garrido.
The principle of sequential dependence in cellular differentiation. P. A. Riley.
Time lapse filming of cellular interactions in organ culture. 2. The behaviour of malignant cells. E. J. Ambrose and Dorothy M. Easty.
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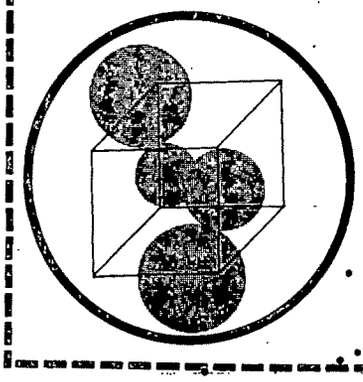
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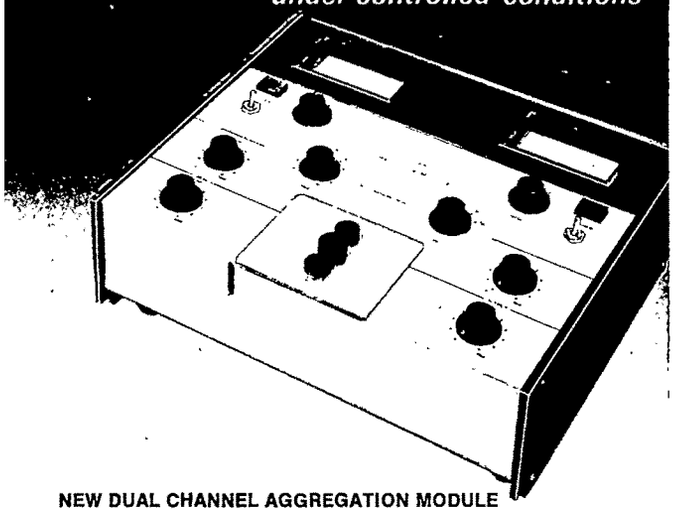
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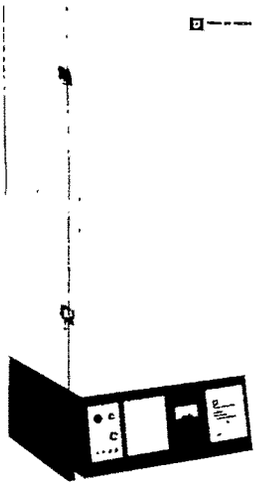
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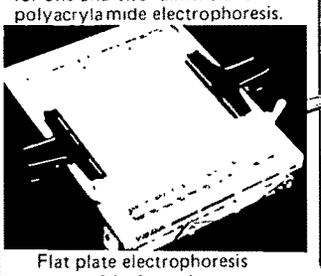
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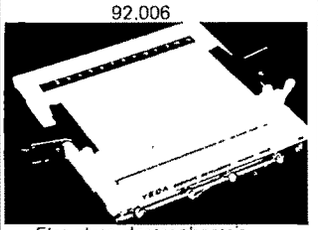
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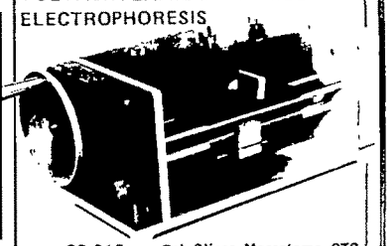


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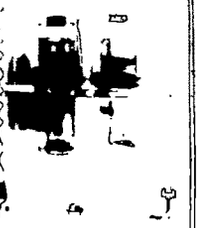


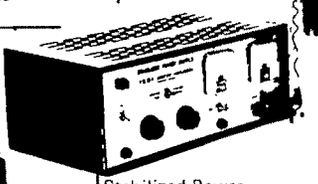
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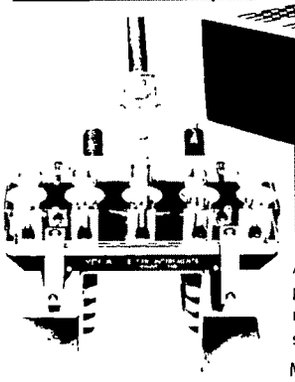
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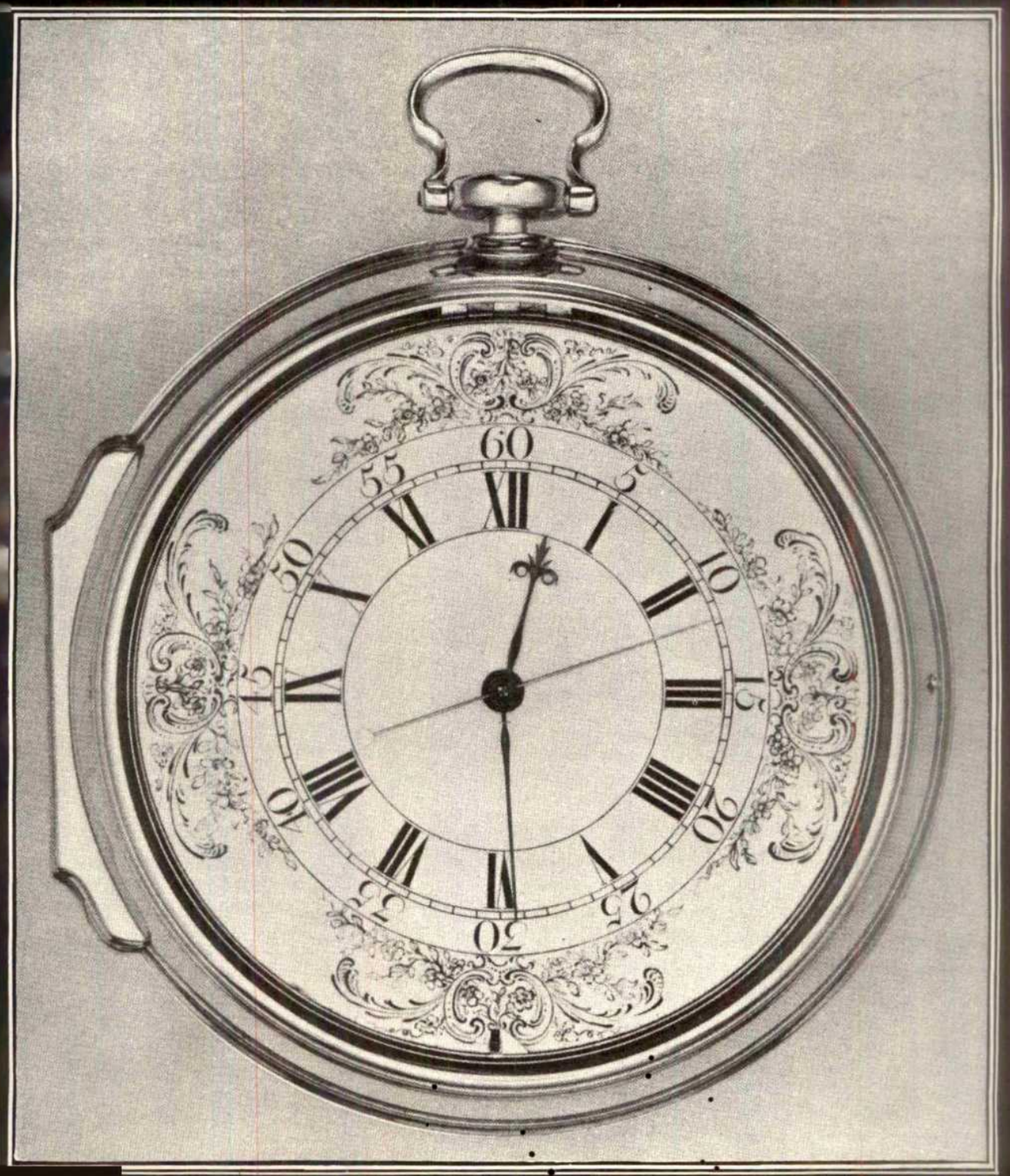


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John Harrison's masterpiece—the watch H4 that won him £18,750 in 1773 in the longitude competition. See page 297.

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Portland problems . . .

BRITISH defence research has always been conducted in conditions of obsessive secrecy, and so the news that the Admiralty Underwater Weapons Establishment (AUWE) at Portland was to let selected guests in was a pleasant surprise. In 1968, the Select Committee on Science and Technology had urged wider dissemination of Portland's problems and achievements, and this was their belated response. Dr G. L. Hutchinson, the Director, indicated that he hoped that industry and universities would come into closer relationship with the establishment; he must also have had an eye on the defence review now being conducted. His budget is £9 million which he regarded as inadequate for some of his tasks.

The establishment is responsible for research and development for offensive and defensive naval requirements beneath the ocean surface. Within AUWE's orbit is torpedo homing, data collection in submarines, hydrodynamics, and all manner of other problems not all of which, doubtless, were on view; the greatest problem as far as one can tell is the antisubmarine warfare (ASW) requirement of finding submarines—in particular nuclear submarines—in the complex environment of the oceans. Sound in the kilocycle frequency range is the tool used but it is severely blunted by the sound velocity structure of the oceans which is neither laterally homogeneous, simple to parametrise, nor time invariant. The medium is barely conducive to propagation of sound over distances of more than a few tens of kilometres.

A first look at a new institution may be deceptive. Certainly security still poses enormous problems; one guesses from the unease with which some questions were answered or deflected that many would be glad when the whole thing was over and they could get back to work. There was an indication that open days would not become a regular feature of Portland's life; this, one hopes, is a sentiment which will be strongly resisted by the government, for the establishment has a lot to lose and nothing to gain by returning to obscurity.

The overall impression is somewhat sad. Amongst the exhibits were no surprises of the sort which mark out some laboratories as vigorous. Maybe there was more in what was not shown, but that is unlikely. The argument that the real stuff cannot be unveiled for reasons of security has always rung somewhat false and as likely as not means there is no 'real stuff'.

But there was more to the sadness than that. Many military laboratories all over the world were created in something like their present form in the ten years following the Second World War and recruitment was at its most vigorous at that time. They have all encountered the problems that Portland seems to have—of a now middle-aged professional staff with much experience (in this case of the hostile marine environment) but a diminishing capability for aggressive scientific thinking and yet insufficient other outlets. Universities offer teaching, committee work and administration. Industry simply discards the nonproducers. Government scientific establishments can do none of this.

The results are two-fold. First, the creative ability declines as old solutions to problems are carefully refined whilst new solutions are not perceived rapidly enough. Second, research establishments become steadily less attractive to the brightest young graduates.

How far Portland conforms to this pattern is difficult to tell but all the symptoms are there. In addition, there is the resigned spirit so often manifested in government laboratories—the seeing of decisions as descending from 'our elders and betters' with the unambiguous indication that although elders not betters. There is also the horizontal compartmentalisation of problems—workers are not expected to know nor to take steps to know what is going on down the corridor. The compact rule of security, that the enquirer must not only be cleared but must also have a 'need to know', may cut down on spying but it reduces research creativity alarmingly. It is doubtful that many in Portland have access to enough pieces of, say, ASW research to put together a coherent up-to-date story of present abilities and future prospects. And yet any competent research student constrained from acquiring a global view of his subject would rightly walk out.

The problem of ASW is perhaps insoluble and it may be that this is our greatest guarantee of a measure of strategic stability for the next twenty years. On the other hand, there are many in the academic community who would like to become more involved in matters of defence —more, maybe, than the Ministry of Defence appreciates.

. . . Greenwich solutions

CHARLES II is said to have informed the Royal Society that he was "graciously pleased" with their work on useful problems such as finding the longitude at sea but not with their "childish diversions" such as weighing air. In the next hundred years, with the stimulus of a prize of £20,000, the art of navigation moved from complete ignorance about longitude to precise determination. It is a marvellous tale, always worth another hearing, and Eric Forbes' recent monograph (*The Birth of Navigational Science*, National Maritime Museum, Greenwich) is a good retelling because the role of Tobias Mayer and the method of lunar distances are fully explored.

Latitude is a relatively easy quantity to measure (so few look at the motions of celestial bodies these days that we make no apologies for an elementary exposition). The elevation of the Pole Star above the horizon at night is almost constant. During the day the maximum elevation of the sun also rapidly leads to the latitude. So with rudimentary devices latitude had been roughly determined since the sixteenth century. Longitude is altogether different, as some means of knowing both local time and some standard time, such as Greenwich, is needed. Local time is relatively easy to obtain from midday, and even quite inaccurate clocks can be used with daily corrections. Greenwich time is an altogether different proposition. So serious was the problem to mariners that large cash prizes were offered for its solution by the governments of France, Holland, Spain and Venice in the sixteenth and seventeenth centuries. As early as 1530 two distinct methods had been proposed: one that a high quality clock be carried on a ship, to take Greenwich time along, as it were; the

other that a celestial clock be used such as the steady progression of the Moon amongst the stars.

The two most promising methods—superior clocks and so-called lunar distances—were still far from accurate enough when the British government set up the Board of Longitude in 1714. Standard practice on long voyages was to run down lines of latitude into one's destination, a potentially catastrophic procedure if sailing to an island and unsure whether east or west of it. As late as 1741 Anson in the *Centurion* lost eighty men of scurvy in the twelve days he spent hunting for Juan Fernandez (of course not only were ships often misplaced, so, occasionally, were islands). The Board offered a results-related award—£20,000 for an accuracy of half a degree in longitude at the end of a six-week voyage to the West Indies, £10,000 for an accuracy of a degree. Sir Isaac Newton summarised the problems in making an accurate enough timekeeper—"the Motion of a Ship, the Variation of Heat and Cold, Wet and Dry, and the Difference of Gravity in different Latitudes". An accuracy of about two minutes would be needed at the end of the prescribed sea voyage for a half-degree accuracy, and clearly no pendulum clock would be up to that. Spring-driven clocks had been made as early as 1500 but by 1714 had never been compensated properly for temperature. Lunar distances might take the prize.

On the practical side one had to measure the angular distance between the moon and some prominent zodiacal star. This changes 13° in every 24 hours, so the invention in the 1720s of the reflecting octant with an angular accuracy of 1′ implied that if the lunar motion were perfectly tabulated, time by the Moon could be accurate to about two minutes once atmospheric refraction and geocentric parallax were allowed for. Two minutes of time is half a degree of longitude so the prize was in sight, at least if the tables could be perfected. It was to this that Tobias Mayer of Nuremberg and Göttingen most successfully devoted fifteen years from 1747.

Mayer was a cartographer and his interest in astronomical determinations of longitude came not from any nautical experience (he never even saw the sea) but from a desire to improve land surveying in Germany. He realised as he searched for higher accuracy that existing lunar data was unreliable and even that some of the stellar positions were unsatisfactory. Having read Euler on analysis and mechanics he ventured into the theory of the Moon's motion around a spheroidal Earth.

His first tables of the motions of the Moon in 1752 were criticised for discrepancies and for a certain inscrutability but by 1754 he had shown that the discrepancies were in contemporary star tables, and the usefulness of the tables for longitude determination was demonstrated. Euler now persuaded him to go for the prize money which he did in a Memorial to the Lords of the Admiralty beginning: "Having by indefatigable application and a great deal of trouble found out the longitude at sea . . . "

His work was well received in London, but the Board of Longitude delayed and Mayer died before a decision was made. Nevil Maskelyne, Astronomer Royal, used the tables with success on a voyage to St Helena in 1761 to observe a transit of Venus, and there was general agreement that Mayer's widow merited the £10,000 prize. The Board thought otherwise (they were probably somewhat embarrassed by their simultaneous encouragement of Harrison and his chronometers) and eventually awarded her only £3,000. This grudging acknowledgement did not inhibit the Commissioners of Longitude from using Tobias Mayer's tables to generate the first Nautical Almanac for 1767. But now the potentially more accurate chronometer, untied to problems of the Earth's rotation and the Moon's complex orbit, began to come to the fore.

John Harrison (1693–1776) stands out as a clock maker head and shoulders above his contemporaries. He came to London in 1728 with two inventions which were to start him on a lifetime of battling with temperature fluctuations and poor lubrication—the 'gridiron' pendulum of brass and steel rods and the 'grasshopper' escapement which required no oiling. His first clock H1, was tested so effectively on a voyage home from Lisbon in 1736 that Harrison, having estimated the position one and a half degrees west of the calculation by dead reckoning was proved right on landfall.

The money was not that easily won. The Board helped Harrison financially, but the Spanish wars and his own self criticism prevented a direct assault being made on the prize until 1761 by which time Harrison had built two other chronometers with which he was not totally satisfied, and now produced the watch H4, a mere five inches across. H4, surely the finest watch ever, was taken to Jamaica in an inconclusive trial, but in 1763 was tested again and showed an error of less than one tenth of a second per day. This should have brought Harrison his £20,000. The Board paid half but demanded that he secure his second £10,000 by showing that H4 could be made by others. Eventually George III exerted pressure and in 1773 Harrison received another £8,750.

Go to Greenwich to see Harrison's chronometers. They are displayed in a serene room almost inaccessible to the casual visitor like the Lady Chapel of some cathedral. The display is superb, as the big clanking clocks come on you one by one down the room. And then suddenly there is a small ticking watch, H4. The atmosphere is almost religious, and the visual surprise will live with you for a long time.

100 years ago



Colonel Gordon left Khartoum on March 21, and in his last letter from Fashoda, 10° N., he touches on some of the scenes on the banks of the river—the storks, which he was in the habit of seeing arrive on the Danube in April, laying back their heads between their wings and clapping their backs in joy at their return to their old nests on the houses, now wild and amongst the crocodiles 2,000 miles away from Turkey; the monkeys coming down to drink at the edge of the river, with their long tails, like swords, standing stiff up over their backs; the hippos and the crocodiles. Such scenes to a lover of nature, as Col. Gordon is, doubtless would serve to make up in some measure for the loss of civilised society and comforts.

Does cancer research have realistic aims?

The Imperial Cancer Research Fund tries with its 1973 report, recently published, to appeal to a wider audience. Brian Ford takes a look at the report and at broader issues in cancer research.

CANCER research is a blend, not altogether a happy one, of the assured with the uncertain, the esoteric with the mundane; yet in recent years some remarkable improvements in survival rates have been achieved in fields that include breast cancer, leukaemia and the lymphomas. That alone is sufficient to refute allegations that this speciality is uniquely able to consume public funding for little tangible benefit. Successes in specific areas are, however, not themselves a guarantee that priorities are correct or that courses of action have been chosen for valid criteria. The latest report of the Imperial Cancer Research Fund (Lincoln's Inn Fields, London WC2) throws an interesting light on the advancing front of progress, and mirrors some deficiencies in the philosophy that underlies it.

Past reports have been issued by the fund in a different form. As the Foreword acknowledges, they have been an unsatisfactory mixture of facts, figures and data presented (particularly where clinical findings and research are concerned) in the specialist form least likely to attract interest or even understanding from the outside reader. The survey for 1973 is therefore novel in approach, for it embraces three specific and distinct areas of interest: the accounts (which are bound as a separate volume), the research reports as such and the Director's assessment. There has been a deliberate attempt to word this introductory section carefully so that it may be assimilable to the intelligent outsider (who is described as a 'non-scientist' by the Foreword, but in this era of isolationist specialisation is as likely to be a psychologist or physicist) and Michael Stoker has taken the brave step of using his easy manner and flow of commonplace English to accomplish the task. For so long has it been widely assumed that understandable terminology and uncomplicated syntax are indicators of superficiality in approach, that this step is perhaps doubly notable. Yet in some respects, by casting a more direct light on the state of the art, it is a less than satisfactory exercise.

He draws a close parallel between the immune response that may be invoked against malignant cells and that seem to act "against measles". Similarly far-fetched analogies are becoming popular (indeed the Nature-Times News Service report dated April 22, 1974, draws a similar parallel) yet it is arguably unrealistic. Surely the infective invasion of susceptible tissues by a transmissable viral genome is operationally distinct from neoplastic transformation, and the quest for a genotypically polyvalent vaccine, as it were, must be a different kettle of fish from the development of measles immunisation. One looks back to the trial-and-error attempts of Jenner to experiment with vaccinia and Pasteur with rabies, both by good fortune working with material which responded uniquely to their techniques though neither had much reason to suppose it would do so; and one assumes there is something more predictable, less metaphysical, about modern medical methodology. Yet here we are, in the 1970s, with electroshock in the psychiatric hospitals and vaccine-shock in oncology, hopefully injecting BCG or irradiated leukaemic blast cells, in the hope of gingering up the immunological sensitivities of a cancer patient.

In Stoker's words it looks sound enough: "The vaccine is made from leukaemic blood from another patient, which is slightly different from the recipient's own blood and may, therefore, give an additional boost to the defences". Yet it is this very clarity which reveals the extent to which our knowledge is clouded and incomplete.

ICRF headquarters in London



There are many other fields of investigation in which the dearth of detailed knowledge, and the inadequacy of many orthodox beliefs, is demonstrated. The report mentions that Rubens and Dulbecco have investigated the use of cytotoxic compounds adsorbed onto tumour-specific antibodies, the principle being to utilise the antibody as a vehicle for the drug, only to find that the loose covalent association that was assumed did not, apparently, occur in their trials. It now seems that the increase in susceptibility of the transformed cells is coincidentally induced by the presence of antibody; how remains uncertain.

Though some of the practical problems of cancer management remain poorly understood, the study of what we might in the broad sense term oncogenetics is inspiring. So too is the interest in conceptualising about cell growth. I believe it is helpful to construct greater models by which to account for the lesser datum; holistic concepts through which to marry reductionist dogmas. In this respect it is heartening to see the work which Martin and his colleagues have under way, which construct novel interpretations of the dynamic status of mature cell communities; models that are not profound and whose principal benefit lies in their being far from complex, but which provide an intellectual synthesis of observed phenomena.

Growth-rate regulatory mechanisms are what cancer is all about, and it is fitting that one of the leading ICRF groups should be the Department of Cell Regulation. Yet, when our understanding of these mechanisms is so profoundly complex in some respects, how surprising it is that Stoker can demonstrate the simple fact that boundary layer gradients may be as important as cell-to-cell contact in regulating cell growth rates. This contrast between the detail of our understanding in some respects, and our ignorance of far more fundamental, practical issues in others, can be illustrated by other reports in the document. Though it is now beginning to seem possible that we may be able to screen urine samples as a means of detecting those patients peculiarly susceptible to breast cancer, it is still not known whether radical mastectomy or wide excision are preferable in the early stages of the disease. Here too we see progressive developments in one aspect of a discipline



Research in progress at St Bartholomew's Hospital, London, where the Imperial Cancer Research Fund established a Professorial Chair in Medical Oncology in 1971.

contrasting with ignorance of a seemingly elementary nature in another.

In certain respects the sense of imbalance that parts of the ICRF report engender are no more than a mirror of the greater problems faced by modern science. To rely, in some primitive Baconian sense, on observing phenomena and then adopting the correctly self-critical mode advocated by the followers of Professor Popper, seems to be the only true path to objectivity and insight. Too often it tends, instead, to blind one to the true relationships between the larger fragments of science, and it conveniently ignores the weaknesses and foibles of human nature. I believe we should now recognise the inability of modern scientific research to fulfil its function adequately by relying solely on what one might term inspirational research. Perhaps we need something of what the Americans would term the 'project' approach, in which teams or individuals are asked to prosecute a given line of investigation which fitted into the whole.

The argument against this is that in this way we tend towards the dictatorial direction of research. What we seem to forget is that research is being surely directed at the moment, but directed by whim, by fancy, by inclination; it is directed by current trends, by the concensus paradigm subconsciously obeyed by an introspective discipline of science; and it is directed by the distribution of funds and support. Though there is no need to dictate science policy, or to set the sights of science at some invisible target, there is certainly room for a little calculated realism somewhere in the mix.

Even though we pay little attention to the criteria by which our research priorities are selected, they certainly exist. To utilise a framework of agreed values to supplement—though in no sense replace—'personal fulfilment' guidelines is more likely to bring us nearer the truth than shots in the dark, no matter how inspired the marksman. Though it is undeniably true that free-wheeling chance gave us *Penicillium notatum* and the earlier discovery of antibiosis, it was the directed research of wartime that gave the world penicillin and the antibiotic age. In an area such as cancer research, where the main concern of all is to bring about an increase in knowledge, unless they or their relatives are suffering from the disease, when the prime requirement is seen as the saving of life; here more than anywhere we need guidance by concensus and objective-setting as a supplement to inspiration.

To speak of cost-effectiveness in research is irksome—until we realise how costly it is, and how badly civilisation needs the effects.

If we have to pick out one example of an individual revelation from the ICRF report, there can be no better example than the discovery of a demonstrable difference between the cell surface in normal and transformed tissues, namely the absence of a normally-present protein from the surface of malignant cells. This has been the work of Richard Hynes in the Department of Tumour Virology, and is a sound and hopeful advance. Indeed it is in virological research that the ICRF has perhaps its greatest claim to fame, and around which at least four of the groups base their work. Yet man seems to show few signs of suffering from virus-induced tumours, and current estimates suggest that 60–90% of all malignancies in man are caused by chemical carcinogens. Should one suggest that—if resources are limited—more attention should be paid to the practical problems of patient care in hospitals and at home?

We still know little about cells in communities. A model of histological or developmental normality—cell sociology, one might say—is at least as important in understanding malignancy as would be a comprehensive understanding of a single cell. If we are to promulgate pure research, ought it not to concern itself with relatively unexplored avenues such as these?

It is often said that in cancer one cannot experiment on humans. But one does, of course, perpetually in a manner which (though entirely justifiable and ethical) must surely seem incongruous alongside specialised research in other fields. How is it there is still the scent of alchemy in the air where much chemotherapy is concerned, utilising drugs of high potency but uncertain action in an empirical and almost haphazard manner? Should the arm be immobilised after breast surgery or allowed freedom of movement as an aid to fluid drainage? Are steroids helpful in the therapy of malignant disease? These questions (which seem fundamental indeed alongside specialised virus research) are only now being resolved, by experimental trial.

When there are many short term, immediate questions to be answered, is it entirely justifiable to press ahead in such detail on animal research based on cancer models that are not apparent in mankind? Perhaps we should take heed of the comments in last year's report from the International Agency for Research on Cancer in Lyon concerning the near impossibility of extrapolating the mass of data from animals to man. Indeed, is not the problem of cancer research that we know too much: we have too many data and too few conceptual pegs on which to hang them? And is the balance of cancer research entirely fair to the patient?

international news

INSTITUTE OF ... Date ...

US at chemical war crossroads

Colin Norman, Washington

THE prospects for international control of chemical weapons could well hang on the results of an intensive review of the United States chemical warfare policy now being conducted by the Nixon administration. It is the first such review for nearly five years.

Begun quietly by the National Security Council (NSC) in December last year, the review is a two-pronged study, first of an impasse which for four years has prevented the United States from ratifying the most important chemical weapons treaty ever negotiated—the so-called Geneva Protocol of 1925, which bans the first use of chemical weapons in war. And, at the same time, the NSC is taking a close look at the Pentagon's plans to modernise its existing nerve gas stockpiles by replacing them with a new generation of chemical weapons called binaries. According to Adminstration spokesmen who testified on nerve gas policies before a congressional subcommittee earlier this month, the review should be completed in the next few months.

Meanwhile, Congress is also faced with an important decision in the chemical warfare area, for the Pentagon has already requested approval of funds to begin producing binary weapons at the Pine Bluff arsenal in Arkansas. A decision must be made this summer on whether or not those funds will be approved.

These reviews and decisions are taking place against a backdrop of international negotiations being conducted in Geneva under the auspices of the 26-nation conference of the Committee on Disarmament (CCD), the ultimate aim of which is to negotiate a treaty banning not only the use of chemical weapons in war but also their production and stockpiling. In other words, the goal is complete worldwide disarmament. What happens in Washington in the next few months will be crucial to the outcome of those negotiations.

At present, the official Administration policy on chemical warfare, as outlined by President Nixon in a statement in November 1969, is that the United States will never be the first to use chemical weapons in a war, and that it has renounced entirely the use, production and stockpiling of bacteriological weapons. Coupled with the no-first-use policy, however, is the understanding that in the absence of worldwide chemical disarmament, the United States army needs to maintain extensive stockpiles of nerve gases to deter other countries from initiating chemical warfare or, if deterrence fails, so that the United States can retaliate in kind. For those reasons, some 45 million pounds of nerve gases are now stockpiled in bases throughout the United States, in Johnston Island in the Pacific, and at one location in Germany.

Unfortunately, however, since the United States has never ratified the Geneva Protocol the policy of no first use of chemical weapons is nothing more than a promise, and until the protocol is ratified, United States intentions will never have the force of international law. At present, ratification is bogged down in a dispute between the Administration and the Senate Foreign Relations Committee over the Administration's interpretation of the terms of the protocol. It will be crucial to the outcome of the CCD talks that this impasse be broken.

The nub of the matter is that when President Nixon submitted the protocol to the Senate for approval in 1970, he did so with the express understanding that riot control agents and herbicides are not included in its provisions. In other words, even if the United States government formally ratifies the protocol it would still consider itself free to use those agents in warfare—in fact the United States army was doing just that in Vietnam at the time Nixon submitted the protocol to the Senate. The Senate Foreign Relations Committee argued, however, that the protocol should include all chemical weapons and it is refusing to put the matter to a vote in the full Senate until the Adminstration backs down from its interpretation.

There the issue has rested for the past four years, but at least the NSC is now taking a close look at the Administration's position. Whether a complete change of mind will ensue from the review is, however, doubtful to judge by statements made by Administration officials who testified before a subcommittee of the House Foreign Affairs Committee earlier this month. Mr Amos A. Jordan, Assistant Secretary for Defense for International Security, argued that herbicides and riot control agents played a valuable and "humane" role in the Vietnam war, for example, and he said that the Administration's position has not changed.

Failure to ratify the Geneva Protocol is understandably viewed within the CCD talks as a severe stumbling block in the path of efforts to secure a more extensive treaty which would outlaw chemical weapons entirely. Furthermore, a treaty outlawing the production, use and stockpiling of biological weapons, which was negotiated at the CCD talks a couple of years ago, is also held up in the Senate Foreign Relations Committee awaiting a final resolution of the Geneva Protocol dispute.

That is bad enough, but according to a number of observers, the Pentagon's plans to develop binary weapons could eventually kill the CCD talks entirely. Binary weapons have been under development by the United States army for the past ten years or more, but they are now coming up for a number of crucial decisions. In short, the weapons consist of two "relatively non-toxic" chemical components which form a lethal nerve gas when they are mixed together. The idea is that they would be kept apart until they are required for use, and they would then be fed into separate compartments in a shell and allowed to mix only when the shell is safely on its way to the target.

The Pentagon's argument for binaries is simple enough. Since the weapons are safe to store, produce and transport, they form ideal replacements for the existing ageing stockpiles of nerve gases. According to official army statements, nerve gas munitions have shelf life of only 15 years, and thus existing stock of filled weapons will have to be replaced by about 1985. Consequently, in September last year, the army began to tout the advantages of binary weapons in public statements and in congressional testimony, and it has now asked Congress to approve a request for $5.8 million to begin production of 155-mm binary-equipped artillery shells. That request is now under consideration—in secret—by a Congressional Appropriations Committee: it is being hinted that the request is running into some trouble.

The binary programme is being opposed by a small number of congressmen concerned chiefly over the possibly damaging effects of a massive new chemical weapons programme on the Geneva CCD talks, and they seem to be picking up some influential support from a few conservatives connected with military affairs—a sign, perhaps, of the way the wind is blowing, is that the House Armed Services Committee recently voted to cut about $1.9 million from the Pentagon's request for research and development related to binaries (this, it should be noted, would be an entirely separate demand from the demand for production funds).

One particularly effective argument, which surfaced during the House Foreign Affairs Committee hearing earlier this month, is that the Administration is now conducting an "urgent review" of the binary programme itself, and that Congress should therefore delay approving the Pentagon's request for production funding, at least until the National Security Council has decided whether or not the Administration is actually in favour of the programme. At present the Congress is placed in the position of deciding whether or not to provide money for a programme which may be killed off by executive fiat at some future date—a situation that would arise only in the highly sensitive region of defence spending, and which gives substance, to the view that there is little executive scrutiny of the Pentagon's annual demands before their submission to the Congress.

Although that argument may ultimately carry most weight in the Congress, it became clear during the House hearings that the binary programme could well be in trouble for a variety of other reasons, and that there is a division of opinion actually within the Administration on the merits of pushing ahead with a chemical weapons programme of any variety at the current stage of the Geneva talks. The NSC review of the binary programme will inevitably be subjected to some critical 'input' from the Arms Control and Disarmament Agency, which has admitted serious disquiet over the possibility of a pro-binary decision damaging—perhaps fatally—the CCD discussions. Dr Fred Ikle, Director of ACDA for the past year, said, under heavy questioning from the commitee chairman last week that: "It is my judgment, based on arms control considerations—and this is a personal view—that at this time the pros and cons come out in favour of not going into production (of binaries)".

The military view, as expressed by Mr Jordan, is that present nerve gas stockpiles "do not fully provide the capability we believe is necessary to adequately support all United States forces in case chemical warfare is used against us. Binaries," he said, "provide significant operational and safety advantages over any other known approach which could have been selected for modernisation."

The situation is therefore the familiar one of the official United States negotiating position being in favour of disarmament, yet at the same time the Pentagon is pushing for a new weapons programme. According to Representative Patricia Schroeder, who attended the CCD talks last year as an official United States observer, "Where many nations last summer simply regarded the United States as having assigned a rather low priority to CCD activities, by mid-March, as the 26 reconvened, one of the more influential western delegates, Dr Alfonso Garcia Robles, leader of the Mexican delegation, went so far as to suggest that we plan to trigger a chemical arms race."

Another factor which has recently been injected into the binary debate—and one which could ultimately prove decisive—is the potential opposition of Western European Governments to the programme. In fact, Mr Leon Sloss, Assistant Secretary of State for Political-military Affairs, admitted under questioning by the House Foreign Affairs Committee that the binary programme is running into some diplomatic difficulties.

The reason is simple. Binary weapons make sense only if they are deployed in forward positions, ready to retaliate against an aggressor who initiates chemical warfare. In other words, nerve gases stored in depots in the United States would be of little use as quick response weapons against the Soviet Union, and the only place it makes sense to keep the weapons is in Europe. Sloss refused to discuss the subject in open session, but admitted that any attempt to expand nerve gas stocks in Western Europe would run into difficulty.

Some British observers feel that the outcome of all this will be that the Adminstration will decide that the programme is not worth the diplomatic storm that it may cause and that it will eventually be stopped. But sources on Capitol Hill, while remaining moderately optimistic, believe that the issue is far from decided.

Another refusal to join the NAS

For most American scientists, election to membership of the National Academy of Sciences (NAS) is regarded as an accolade second only, perhaps, to being awarded the Nobel Prize. But during the past four years, two scientists have resigned from the august body, and two others have refused to accept membership when it was offered to them. The chief complaint all four have raised is the academy's close involvement with the research programmes of the Department of Defense.

The latest to decline association with the academy is Richard Levins, a mathematical biologist from the University of Chicago, who was elected to the Academy at the general meeting in April. Levins turned down the offer in a letter dated May 10, stating that his "first and most urgent concern is the continuing participation of the academy in military matters".

Levins also sharply criticised the policies of the academy's President, Philip Handler, but he argued that the organisation's connections with the military are "not the result of a perversion of the academy's nature by Phillip [*sic*] Handler or his predecessors, but of a faithful interpretation of its charter and traditions".

The academy's charter, received from President Abraham Lincoln, specifically gives it the role of advising the government, and Levins therefore argues that it would be self defeating to accept membership in the academy and then try to reform from the inside. His letter states: "I cannot hope to remedy this situation by planning with other colleagues to replace Mr Handler with a more liberal president, or by manoeuvring to restore the NAS to its true mission: it is performing its true mission, and I find that mission repugnant."

Levins specifically attacks Handler for trying to "weaken any criticisms of the military, as he did in his covering letter (to the academy's report on herbicide damage in Vietnam), where among other things he dismisses the evidence of damage to human health and of death caused by herbicides."

For his part, Handler claims that the amount of classified work undertaken by the academy has declined during the past few years. He stated in his annual report to the membership that about 5% of the funds funnelled through the academy this year were concerned with that resulted in the printing of classified reports.

Be that as it may, Levins and others who have attacked the academy for its association with the military would like to see the organisation completely sever its ties with the Department of Defense. In refusing to accept membership of the academy, Levins is following a precedent set by Dr George Field, a Harvard astronomer.

State of the world: IISS reports

"ALMOST medieval turbulence under a nuclear umbrella", is how the International Institute of Strategic Studies' (IISS) annual report for 1973 describes the military and political scene last year (IISS, 18 Adam Street, London WC2). In a year marked by the withdrawal of the United States from Vietnam, a commitment between President Nixon and Secretary Brezhnev to negotiate a SALT II agreement, the downthrow of President Allende, the Middle East War, the appearance of the oil weapon and ominous creakings in unity not only within Europe but with the United States, the epithet is well deserved.

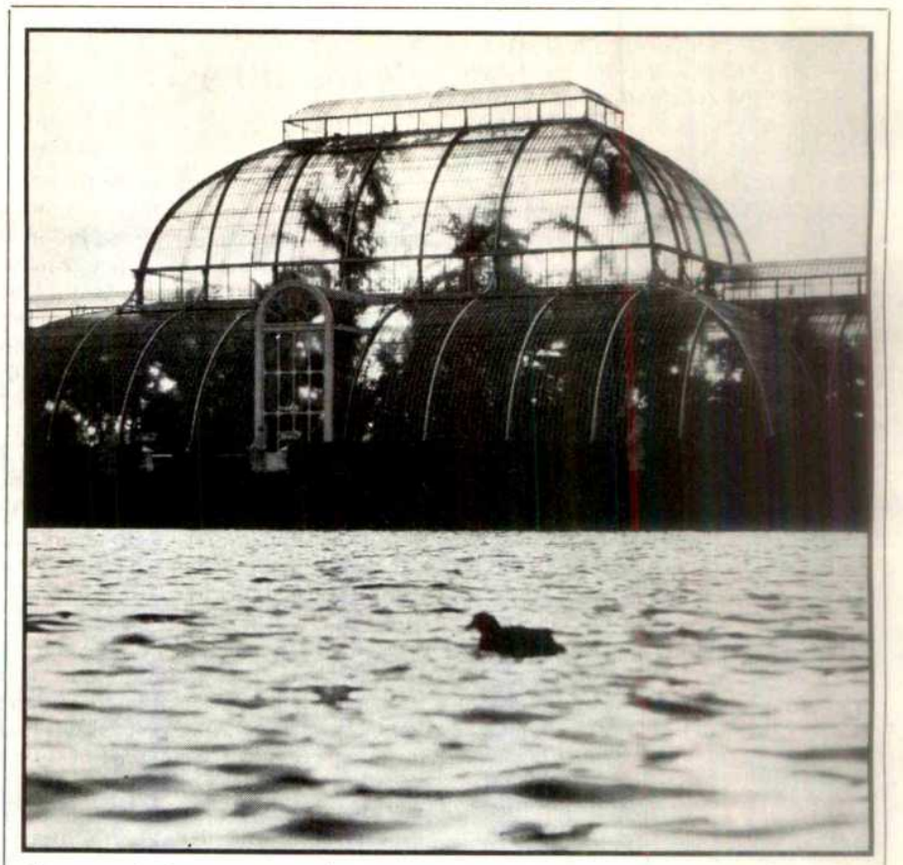
The greatest strategic development was undoubtedly the use of oil as a weapon which "opened up prospects of entirely new political balances". The quadrupling within a year of oil prices led to Dr Kissinger's proposal for an Energy Action Group, but there were many defections from the united front that was hoped for as western Europe and Japan pursued bilateral deals with oil producers; the ingredients of many of these included arms sales. Already in the eighteen months prior to the October war nearly $5,000 million worth of arms deals had been made with Persian Gulf states—mainly Iran and Saudi Arabia. Iran, in particular, sees threats both internal and external—indeed there are potential dangers on all sides except from Pakistan. The IISS finds it difficult to predict the military future in the Gulf, so complex is the scene. On the one hand armed-to-the-teeth means bloody conflict; on the other, the strength of Iran and Saudi Arabia means stability. A familiar problem for strategists.

The prospects for SALT II are gloomy, as United States–Soviet tensions grew in 1973. There are some sticky problems for negotiators. In August the United States announced MIRV tests had begun in the Soviet Union thus starting the process by which American qualitative superiority though with numerical inferiority in missiles is eroded. And ultimately the accuracies of land-based missiles are so great that if the number of such missiles possessed by one side exceeds that of the other, first strike by the bigger force against the other side's launchers as good as ensures missile victory. The United States is believed to be aiming in SALT II for some sort of numerical equality with limited freedom to mix missiles. This, of course, leaves the American bomber superiority untouched. The Soviet Union is said to have responded that numerical equality would require the dismantling of American Forward-based Systems such as European submarine bases. She also proposed a prohibition on further research and development of bombers, cruise missiles, penetration aids and air-to-surface nuclear missiles. "Outrageous", said the United States.

The problem for the 1990s is the submarines, the institute believes. Much will hang on breakthroughs, if any, in anti-submarine warfare technology, and there are various actions that could be taken to ensure submarine invulnerability. Continuous tracking of submarines could be prohibited, as could large scale sonar installations. Sanctuaries could be provided for submarines. This is the stuff of arms control in the late 1970s and the sooner it is widely discussed the better.

Will the Soviet Union attack China in 1974? The IISS sees this as a serious option for Soviet policy, but concludes that the days are over when a surgical strike against Chinese nuclear facilities would have destroyed the country's weapons programme. Further, China now has more allies, and is capable of inflicting some damage on Soviet cities.

The troubles of Angola, Mozambique, Rhodesia, Namibia and South Africa with insurgents are given four pages, the Northern Ireland troubles not a word. A pity; a brief analysis in the IISS's lucid style would have helped.



Kew: the Palm House

VISITORS had an opportunity to see the other side of Kew when the Royal Botanic Gardens held its open days recently, and the laboratories, library and herbarium were open to the public. The Director, Professor J. Heslop-Harrison, stressed the importance of the gardens as a repository for living wild plant species. The plant kingdom is in retreat, he said, and about a tenth of known angiosperm species are in grave danger. By the end of the century half of these would almost certainly be extinct. As part of a worldwide effort to conserve this fast disappearing genetic resource, a seed bank has already been set up which he hopes will eventually form the core of a national seed bank comparable with those in Leningrad and Colorado. Equally as disastrous as the disappearance of individual species is the destruction of large areas of natural habitat by man's expansion. Professor Heslop-Harrison said that although the general public is now sympathetic to the idea of wildlife conservation the less glamorous concept of vegetation conservation is difficult to communicate, although of prime importance. If you want to save the tiger, he said, first save the teak forests in which it lives. In the gardens themselves, the Temperate House, designed by Decimus Burton and built between 1860 and the 1890s is now being completely restored and renovated as was the Palm House some years ago.

CIA comes clean, almost

John Hall

EUROPEAN manufacturers of PVC are to spend at least £4 million on major plant modification where workers are liable to come into contact with vinyl chloride monomer (VCM). The move follows reports earlier this year that exposure to VCM had caused the deaths of plastics workers in the UK and the United States. Subsequent enquiries have revealed 18 cases of liver cancer among PVC workers—12 in the United States, one in Britain and possibly five in Europe. Inronically, the very rarity of the angiosarcoma involved has allowed researchers to say so concretely that it was caused in the occupational environment. Since no other chemical agent is known to produce angiosarcoma so directly it is difficult for the industry's scientists to look for outside causes, whereas a common or garden lung cancer might easily have been attributed to any number of external factors.

The Chemical Industries Association (CIA), which represents the big four British plastics producers involved in the affair, is at great pains to point out the highly moral way in which the companies have responded to the news that VCM is a bad smell in a production process which looks like a bed of roses profit-wise. Not only did the manufacturers sponsor Maltoni's Bologna programme (which put the finger on VCM) but having found themselves on the spot when the unpleasant tidings broke earlier this year they have in the space of three months reduced airborne VCM concentrations from 150ppm to 50ppm in polymerisation plants, and to about 5ppm on the actual VCM production line. And this when the legal limit stands at 200ppm. It should be noted on the other hand that the 200ppm limit existed simply as an anti fire and explosion safeguard before anybody had any inkling that the stuff was no good for the liver.

In those days, ladies were spraying their curls with lacquer carried on pressurised jets of VCM from candy-coloured aerosols, nobody turned a hair at the thought of orange pop coming home in PVC bottles and an American liquor company was even planning to market the hard stuff in PVC packaged doses. They ran a programme just to see if alcohol was affected by contact with the plastic, and the answer was that it was OK except that it seemed to leach out the VCM. Nobody knew VCM was bad news then, but the liquor people ditched the idea to be on the safe side.

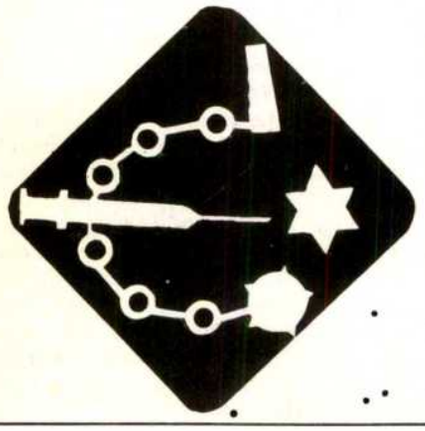
VCM hair lacquer is now a thing of the past, but the pop bottling and food packaging business is still booming, and the CIA's men recently pointed out that this was because the amount of VCM absorbed from PVC packaging in the diet of your average man was in order of 0.001ppm, and that at this meagre rate of absorption your average man would need to be around for 50,000 years if he wanted to absorb an ounce of VCM. What's more, they remarked, there was no evidence that absorption through the gut rather than through the lungs was in any way harmful. They did not give any indication of what your heavy pop-swagger might expect by way of a daily dosage, or indeed any indication of what dose might be considered lethal, and what safe.

On this last question—the point at which it is safe to absorb a noxious substance and the degree to which an essential industry can divert its energies to eliminating that substance from the air which its workforce breathes—the CIA has struck a philosophical note. Quite correctly, they point out that PVC is not a luxury product, but a major industrial material which is an integral part of modern life. The car industry, coal mining, domestic and industrial building, water distribution, gramophone record production and various other blessings of civilisation depend upon a ready supply of PVC.

If society demands an absolute zero risk for the 2,000-odd men working on PVC production, then this could be met only by ensuring absolute zero exposures, and since this is not a practical proposition, then the producers would have to stop producing, and life as we know and love it would grind to a halt. With improvements in valves and pipe joints carying the pressurised gas concentrations as low as 10ppm may be achieved in existing plants, and in entirely new plants, using the most modern technology, 5ppm might be achieved. This, says the CIA, is probably safe, and in any case achievements beyond this point are not conceivable, and so it's up to society to say its piece. If perfection is demanded, the wheels of industry stop turning; if something a little less will do while research proceeds, then we can have our drain pipes and cable covering. And waxing ever so slightly metaphysical, the chairman of the CIA's VCM Committee, Dr A. W. Barnes even volunteered the opinion that five parts per million was arguably closer to nothing than to something, which is stretching public relations to breaking point, even if you are speaking for the combined interests of ICI, BP, Vinatex and British Industrial Plastics.

Red badge of protest

from our Soviet Correspondent



ON the occasion of the great international celebration-that-never-was of the 250th anniversary of the Soviet Academy of Sciences (or, more correctly, of the old Russian Imperial Academy which it replaced), a small, but significant, "celebration" of the event was held in London by the Medical and Scientific Committee for Soviet Jewry.

The symbol of the meeting, distributed to the participants in the form of a lapel badge, was a redrawing of the official emblem of the Soviet Union—in which the sickle has become a ball and chain, and the hammer the new, scientific and sophisticated weapon of restraining dissidents, the hypodermic. This theme of the repression of the scientific and intellectual community, especially of those members of it who wish to emigrate from the Soviet Union, formed the basis of the address by Heinz Woolf, who compared the current Soviet policy to that of Ivan the Terrible, who, after St Basil's Cathedral had been completed, had the architect blinded so that no else might commission a similar masterpiece. The current Soviet measures, dismissal and harassment of those scientists who wish to emigrate to Israel, merely continues the same tradition of blocking a brain drain.

A lively discussion followed, on the role which scientific societies and individual scientists outside the Soviet Union can and should play in countering this policy, and the sensitivity of the Soviet authorities to criticism from bodies like the "Royal" Societies and Colleges or publications like *The Times* and *Nature* which they feel to have a certain official or quasi-official status, was particularly stressed as a potential means of counter-attack.

news and views

Recurrent Mach

A STUDENT of the science of science might find it entertaining to try to discover what it is that reawakens interest in Mach's principle every three or four years, why each ingenious suggestion on the subject has almost no effect upon any subsequent ingenious suggestion—except possibly to stimulate the attempt to be more ingenious—and above all why nobody ever actually states Mach's principle. All of which points seem to be instructively illustrated by the article "Relative-distance Machian Theories" by Barbour in this issue of *Nature*. It does seem to be a few years since at any rate *Nature* carried a contribution on the subject: Barbour's suggestion is ingenious and it does not make use of any previous work; he starts from an assertion of something that "Mach's principle, in essence, requires", and he proceeds to treat this requirement itself as a "postulate". But, once again, no statement of Mach's principle! And there never can be a statement of Mach's principle until somebody discloses that Mach actually stated a principle, and in fact nobody has done this. Mach discussed this problem of inertia in a general way, and he seems to have had some idea that inertia depends upon matter in the Universe in the large. But anybody who wants to go further does have to state his own "postulate" because Mach does not help him.

Inertia in the technical sense is a notion of Newtonian physics. Before Newton, the systems of Ptolemy, Copernicus and Kepler each treated a whole Universe—in a very simplified way, of course. Wonderful as they were, they were arid when it came to solving any new problem. Newton came along and said, in effect, that we can break the Universe up—divide and conquer; we may take any small part of the Universe and assert that a frame of reference exists such that the part obeys laws of motion which take certain forms (Newton's laws as usually stated) when this frame is used. The frame of reference—any inertial frame will serve— is the rest of the Universe in the model. Incidentally, it can be seen at once why the Newtonian treatment cannot apply strictly to the whole Universe, because the treatment depends upon this partition of the Universe. Then Mach came along and, so to say working from the opposite end, redetected what Newton had done. From one point of view, it was no great achievement to suggest that an inertial frame might have something to do with the Universe in the large, when all the time the inertial frame is the Universe in the large. From another point of view, Mach did something great in making people wonder why the Newtonian treatment is so successful. People are still wondering about this, and Barbour's paper is an obviously interesting endeavour to push it back to more rudimentary postulates. It might be inappropriate here to discuss details, since he remarks that he is preparing a paper on a different model with more interesting properties.

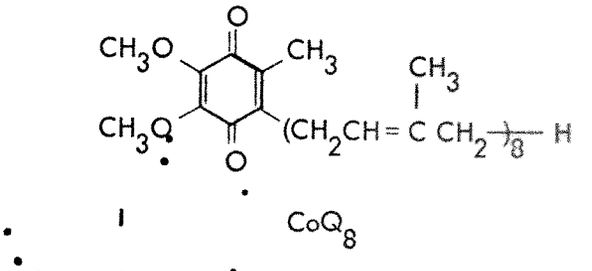
There is still the general question as to whether such ideas carry over into relativistic models. Barbour evidently believes that his treatment will extend to special relativity; this would be natural, for special relativity also is founded upon the use of inertial frames. It is outside his present scope to say much about general relativity. In general relativity, however, every problem produces an entire universe (in general, not an interesting one); so there is no Newtonian partition and therefore Mach's problem does not arise in the same way. Some writers have indeed, produced sophisticated criteria as to whether any particular general relativity model is "Machian" or not, but this seems to be somewhat far from Barbour's interpretation of "Machian".

W. H. McCREA

Possible new antimalarial drugs

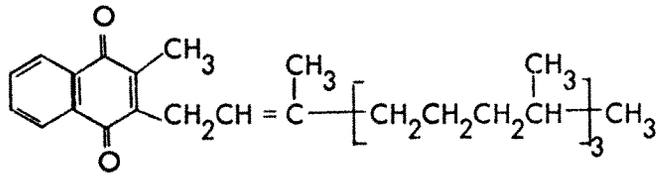
ALTHOUGH it is well established that malaria parasites inside host red blood cells utilise glucose, the extent to which this process provides the parasites with energy has been little explored. One of the technical problems hindering such an investigation was the difficulty of distinguishing between the biochemical processes of the intracellular parasite and those of the host cell. Until recently much effort was directed towards separating the parasite from host erythrocytes and from other cellular blood elements, particularly white cells and platelets, the activities of which could invalidate biochemical assays of, for example, oxygen consumption and the action of classical metabolic inhibitors. Homewood and her colleagues (*Proc. helminth. Soc. Wash.*, **39** (special issue), 382; 1972), have utilised a technique based on the observation that the pigment of erythrocytic stages of the rodent malaria parasite *Plasmodium berghei*, clumps when exposed to a low concentration of chloroquine, and that this process is dependent on energy (Warhurst and Robinson, *Trans. R. Soc. trop. Med. Hyg.*, **65**, 11; 1971). From a study of the action of various respiratory inhibitors on pigment clumping induced by chloroquine, Homewood and her colleagues postulated that these parasites possess a very unusual, branched electron transport chain. The major branch, they suggested, terminates in an unidentified acceptor of parasite origin, and they believe that the minor branch passes electrons finally to oxygen by way of a cyanide-sensitive cytochrome oxidase system.

There is now good evidence to show that the erythrocytic stages of a variety of *Plasmodium* species, including *P. berghei*, do indeed contain cytochrome oxidase (mainly in the mitochondria), and that the enzyme occurs also in the mosquito and pre-erythrocytic liver stages (Howells *et al.*, in *Comparative Biochemistry of Parasites*, Academic, London and New York, 1972). The idea that electron transport mechanisms may play a vital part in the malaria parasite has now been exploited by Wan, Porter and Folkers (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 952; 1974) who have synthesised a series of structural analogues of coenzyme Q_8 (I).



During the Second World War antimalarial drug screening

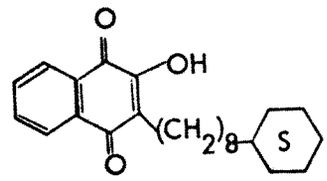
programme Fieser and his associates (*J. Am. chem. Soc.*, **70**, 3151; 1948) synthesised several 2-hydroxy,1,4-naphthoquinones that proved to be highly inhibitory to growth of the duck malaria parasite *P. lophurae* and two of these were eventually tested in man. They produced, however, some side reactions—apparently due to an effect on blood clotting mechanisms—that were, fortunately, antagonised by the administration of vitamin K1 (II). Subsequent investigations



II vitamin K_1

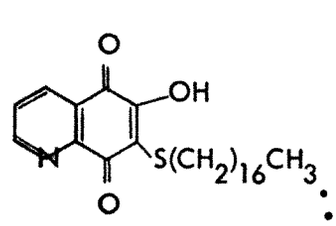
showed that these antimalarials inhibit mammalian and parasite respiration, an effect that could be reversed in the former by the addition, *in vitro*, not of vitamin K1 but of ubiquinone 10 (also referred to as coenzyme Q_{10}, or CoQ_{10}).

It is now known that *P. lophurae* synthesises ubiquinone 8 and 9 (Rietz *et al.*, *Int. Z. VitamForsch.*, **37**, 405; 1967) and that ubiquinone 7 and 8 are synthesised by *P. berghei* and two simian malaria species which do not contain vitamin K_1 (Skelton *et al.*, *J. med. Chem.*, **13**, 602; 1970). These observations have opened a new line of thought as to the mode of action of the napthoquinones, as well as providing a unique target for selective toxicity, since the host tissues of both birds and mammals synthesise a different coenzyme, namely ubiquinone 10. It was soon demonstrated that both the older 2-hydroxy, 1,4-napthoquinones as well as some newer members of the series, including menoctone (III), inhibit DPNH



III menoctone

oxidase and succinoxidase systems *in vitro*, both systems being ones in which CoQ plays an essential part. These observations encouraged the synthesis of further compounds structurally related to ubiquinone 8, the most recent of which are 7-alkylmercapto-6-hydroxy-5,8-quinolinequinones (Wan *et al.*, *loc. cit.*). One of them, the heptadecyl derivative (IV), combines a high level of activity both against the blood stages of *P. berghei* and especially against the tissue stages of the avian parasite *P. gallinaceum* with a very low level of toxicity to the host. There are thus two chemical series, one characterised by menoctone (III) with its 8 carbon alkyl, 3 cyclohexyl sidechain, the other by compound IV with 16+1 carbons in the saturated straight alkyl chain, both of which show a high degree of antimalarial activity and low host toxicity. It seems quite possible that they function as antimetabolites by blocking one or more sites concerned with the biosynthesis of CoQ_8 within the malaria parasite. In the menoctone series activity against *P. berghei* is lost as the chain length is either decreased or increased, both in relation to the blood (Berberian and Slighter, *J. Parasit.*, **54**, 999; 1968) and tissue stages (Berberian *et al.*, *ibid.*, 1181).



IV 7-heptadecylmercapto-6-hydroxy-5,8-quinolinequinone

Menoctone has been shown to cause selective structural damage to the well developed mitochondria of the tissue stages of avian malaria (Beaudoin *et al.*, *Milit. Med.*, **134**, 979; 1969). Although in the blood stages of *P. berghei* the mitchondria appear to be relatively primitive, it now seems clear that they do play an important part in the production of energy through the electron transport chain. It is therefore interesting to note that menoctone also induces ultrastructural changes in these mitochondria (Howells *et al.*, *Ann. trop. Med. Parasit.*, **64**, 203; 1970).

These compounds thus approximate to the definition of the ideal antimalarial which has been stated to be one that would (among other virtues) combine action against all stages of the parasites with minimal toxicity to the host. Although preliminary studies with menoctone against *P. falciparum* in man (*Tech. Rep. Ser. Wld Hlth Org.*, No. 529, 70; 1973) and the 7-octadecylmercapto analogue of IV against *P. cynomolgi* in monkeys (Wan *et. at., loc. cit.*) have been disappointing, the basic experimental data and theoretical considerations indicate that the further exploration of these potential antagonists of CoQ_8 may well provide a rare opportunity for the rational synthesis of an antiparasitic agent with highly selective toxicity, the dream of every experimental chemotherapist since Paul Ehrlich. A word of caution, however, is necessary. The observations of Howells *et al.* (*loc. cit.*; 1970) indicate, perhaps not surprisingly, that the malaria parasite may be equipped already with a means of overcoming such interference with its vital respiratory processes.

W. PETERS

Another bursal equivalent

STUDY of the lymphoid system in birds has revealed a thymus at the anterior and bursa (of Fabricius) at the posterior end. Both organs are thought to play a primary part in the development of the lymphoid system and immunological responsiveness. Stated in its simplest form the notion is that the thymus produces T cells which are responsible for cell-mediated immunity (that is against foreign grafts) and the bursa emits B cells which produce humoral antibody.

Initially it seemed that these two modes of immunity, like the antithetic East and West of Rudyard Kipling, should never meet. In mammals, particularly mice, the thymus is present and it produces T cells and these cells are responsible (at least in part) for cell-mediated immunity. The bursa in mice is less obvious and murine T cells collaborate with murine B cells (B stands for bursal equivalent here) to facilitate the production of antibody by the B cells. All this looks sensible and seems to indicate that birds and mammals differ in their lymphoid systems and manner of immunological responsiveness. (It would be more accurate to say that chickens and mice differ.) But immunologists are spending much time and energy in trying to reconcile these apparent differences, and the definition of a mammalian bursal equivalent in particular has become a sort of crusade. Owen, Cooper and Raff raise the banner once again on page 361 of this issue of *Nature*. Owen *et al.* first assem-

bled a series of fluorescein-conjugated, class specific, anti-mouse immunoglobulin antibodies in order that they could identify B lymphocytes by direct absorption of the fluorescent antibody and subsequent counting of stained cells in an appropriate microscope. They make the assumption that surface immunoglobulin on a morphologically indentifiable lymphocyte is sufficient definition of the cell as a B cell.

These methods were then applied to liver and spleen of foetal mice of various gestational ages. It was found that until 17 days of foetal life, no B (or T) lymphocytes were detectable. They then took 14-day foetal liver and cultured it *in vitro* for up to 7 days. Within 3 days 0.1% of all cells were stained and within 7 days as many as 3%. Over the same time course there was found in the explanted livers an increase in the proportion of small lymphocytes from 0% to 3%. In these circumstances Owen *et al.* very reasonably conclude that B lymphocytes develop in relatively large numbers in foetal liver in tissue culture. They also point out that, whether or not their deduction that the foetal liver is a bursal equivalent proves to be validated, the system of experimentation they have adopted provides an opportunity for the study of the putative class switch in immunoglobulin synthesis, and the generation of antibody diversity in the absence of T cells.

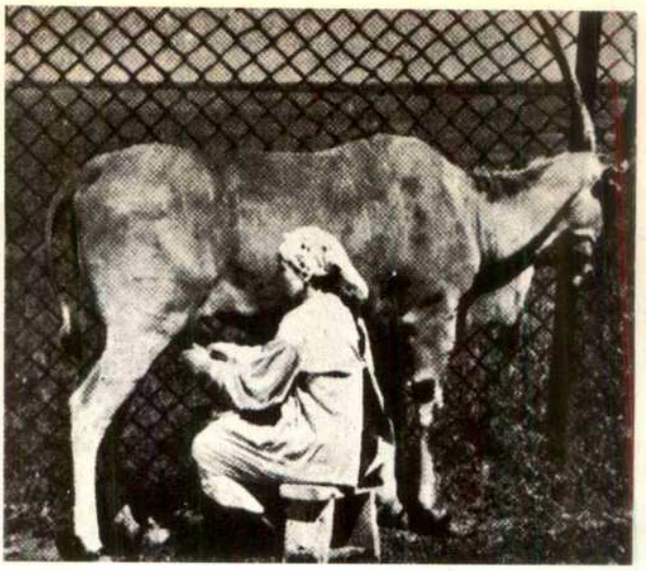
It is being found with increasing frequency that non-lymphoid cells can demonstrate some of the best recognitional characteristics of T and B lymphocytes (see, for example, Elliott, Kerbel and Phillips, *Nature*, **248**, 514; 1974). It is to be hoped that this does not prove true of surface-bound immunoglobulin, because if it did Owen *et al.* would have to show that their fluorescent cells were morphologically small lymphocytes.

A. J. S. Davies

Protein for population

from our Animal Ecology Correspondent

Of the many problems facing policy makers of sub-Saharan Africa today, the increasing protein deficiency for the spiralling human population is about the greatest. It is of historical interest to note that protein production has traditionally rested on the performance of domestic livestock. In some African countries the problem has been tackled by the importation of protein-rich foods. But the high price of these foods renders them unavailable to all but 25% of the population. The other 75% rely on caterpillars, maggots, snails, fish and wildlife for their protein.



African eland being milked at Askanyia Nova in the Crimea, USSR, where there is a domesticated herd. The milk has more fat and protein and less water than that of domestic cattle and is more resistant to some bacteria (from Crawford, *Oryx*, **12**, 321; 1974).

Asibey has pointed out (*Biol. Cons.*, **6**, 32; 1974) that natural meat, or bushmeat as it is called, represents big money. In one market in Accra species such as hare, giant rat, grasscutter, duiker, green monkey and bushbuck fetched US $0.46 per pound. During 18 months the cash turnover from 348,000 pounds (158,000 kg) of meat was US $160,000. If planners need to see the future in terms of money they should take a look at their own back garden. This, above all, may convince them that the true solution rests with the effective and efficient exploitation and management of naturally occurring wildlife.

There are other, more important, reasons why the bushmeat industry should be encouraged and given the protection of careful management. These concern conversion and growth efficiencies of wild game compared with domestic stock. Eland show a liveweight gain of 0.73 pounds (0.33 kg) per day; domestic cattle on reasonably managed rangeland in East Africa gain about 0.3 pounds (0.14 kg) per day. Crawford (*Oryx*, **12**, 351; 1974) uses this example to highlight the need for field studies. When eland and cattle are kept in the same laboratory their conversion efficiencies are similar. But on the arid plains the eland's performance is characterised by lability. By allowing its body temperature to rise with the ambient temperature—and it can even exceed the air temperatures and radiate heat—it has no requirement to sweat or pant. The saving of body water, critical in many parts of Africa, is considerable. Of course it is only able to allow its body temperature to rise because of certain adaptations to its heat balance system. These include a *rete mirabila* overlying the brain which functions as a heat sink. Furthermore, bushmeat species are remarkably resistant to infections that kill domestic stock, and they seldom destroy the phytosociological structure of their habitats.

Both Asibey and Crawford independently make a plea for rational and scientific management of natural stocks. Clearly, if the resource is to withstand fairly heavy exploitation then close scrutiny must be made of population processes in the wild. Crawford sees two possible ways in which management could be directed. One is towards the establishment of monocultures of eland, wildebeest, gazelle or kob. The other is towards polyculture establishment using a range of game animals occupying different feeding niches and maintaining a great complexity and diversity of vegetation. Ecologically speaking the polyculture system is the more viable since overgrazing and overbrowsing are less likely to occur than in monoculture.

With the standing crop of bushmeat being around 70,000–100,000 pounds per square mile (12,000–17,400 kg km^{-2}) and of domestic stock around 11,000–16,000 pounds per square mile (1,900–2,800 kg km^{-2}), it is inconceivable that this valuable resource will have to wait long before heavy exploitation begins. And neither it should, if the people of Africa are to have meat to eat. Asibey's plea for intensive and detailed management studies must not go unheeded by the architects of the new Africa.



African buffalo. (Photo.: M. Crawford). Their long gestation period (compared with domestic cattle) ensures that calves are born in the rainy season.

Galapagos seismic gap filled

from Peter J. Smith
Geomagnetism Correspondent

ALTHOUGH all active oceanic ridges are marked by chains of shallow focus earthquakes, the density of epicentres is far from uniform throughout the ridge system. There are, for example, far fewer events along fast-spreading ridges than along slow-spreading ridges. Macdonald and Mudie (*Geophys. J.*, **36,** 245; 1974) have examined earthquakes of magnitude 5.0+ along spreading ridge sections (that is, excluding those events associated with known transform faults), as recorded during the years 1961-1969 by the World Wide Standardised Seismograph Network (WWSSN), and find that on average there were 8 shocks per 1,000 km of ridge for the northern mid-Atlantic Ridge, where the spreading rate is 12 mm yr^{-1}, but less than one shock per 1,000 km along the East Pacific Rise, where the spreading rate is 35–50 mm yr^{-1}. Moreover, fast-spreading ridges are particularly prone to apparent gaps in seismic activity of up to hundreds of kilometres in length. Along the East Pacific Rise between 36° S and 49° S, for example, there are seismic gaps (as revealed by WWSSN records) as long as 1,400 km.

Another area in which there seems to be a seismic hiatus, according to the WWSSN, is the 600-km long central Galapagos spreading centre, a ridge section spreading at a half-rate of 32.5 mm yr^{-1} between the Cocos plate to the north and the Nazca plate to the south. But is earthquake activity truly absent from this region, or is the gap in seismicity merely a reflection of the low sensitivity of land based stations? To find out, Macdonald and Mudie used sonobuoys in an attempt to detect seismicity at close range along the Galapagos spreading centre—in other words, to see if, notwithstanding the lack of large earthquakes, they could detect microearthquake (magnitude less than 4.0) or earthquake swarm activity. The attempt succeeded. During 45 h of recording time (not continuous) Macdonald and Mudie detected an average of 15 events an hour near the ridge axis and up to 80 shocks an hour at the peak of a swarm which occurred a few kilometres from the ridge. Magnitudes ranged from $m_b = -0.4$ to $m_b = +0.8$. This is the first time that the microseismicity of a fast-spreading ridge with no activity detectable by the WWSSN has been measured.

The association with an active ridge leaves little doubt that the recorded microearthquake activity is caused by seafloor spreading processes, although the specific origin is more difficult to determine. Sykes (*J. geophys. Res.*, **75,** 6598; 1970), who investigated ridge swarms elsewhere recorded by the WWSSN, attributed such swarms to volcanic, hydrothermal or magmatic processes on the ridge crest; and it seems probable that one or more of these processes are also responsible for the Galapagos events. Williams *et al.* (*Eos*, **54,** 244; 1973) suggested that hydrothermal circulation may be an important method of heat transfer at the Galapagos ridge crest, for heat flow there can be greater than 30 heat flux units and narrow, near-bottom, positive water temperature anomalies of several hundredths of a degree have been found over the crest near, as it now turns out, the source of the microearthquake activity. But what is probably the most convincing evidence comes from the Macdonald-Mudie study itself. The b value for the Galapagos events (the frequency-magnitude parameter in the relation $\log N = a - bm_b$, where N is the number of events of magnitude m_b or greater) is very high at 3.0, which is consistent with a volcanic or magmatic source but not with the generally much lower b values for fracture zone events.

Flowing superfluid films

from Peter V. E. McClintock
Condensed Matter Correspondent

THE thickness of a superfluid helium film below 1 K is reduced by an increment which varies quadratically with the velocity at which the film is flowing, according to an experiment by Williams and Packard of the University of California at Berkeley (*Phys. Rev. Lett.*, **32,** 587; 1974).

Some of the most dramatic demonstrations of superfluidity in liquid ^{4}He below its superfluid transition temperature at 2.18 K are found in the remarkable phenomena associated with helium films. Any surface dipping into the liquid rapidly becomes covered with a film whose thickness depends on the nature of the surface, and the height above the 'bulk liquid, but which is usually a few hundred Å.

Up to a certain critical velocity the film encounters no resistance to its motion and, being thus enabled to flow freely at several tens of cm s^{-1}, can act as a channel through which relatively large volumes of the liquid can be transferred: in fact the film can act as a syphon through which a small vessel of helium will empty itself in a matter of minutes, the helium flowing up the inner wall and then down the outside.

The film is held in position by the attractive Van der Waals force between the wall and the helium atoms, and measurements of the thickness of static films are found to be in reasonable agreement with theoretical predictions based on the assumption that the film always adjusts itself so as to minimise its total potential energy in the combined Van der Waals and gravitational potentials.

In the case of a flowing film, however, it may not be possible for the thickness to reach this equilibrium value since the velocity of flow is limited to the critical velocity, above which dissipative processes set in very rapidly. Although the situation is very complicated, Kontorovich was able to show (*Zh. eksp. teor. Fiz.*, **30,** 805; 1956) that, under these conditions, the thickness should be reduced by an amount depending on the square of the flow velocity.

Subsequent experiments above 1 K in a number of laboratories, however, demonstrated conclusively that the thickness was actually independent of the velocity. A possible explanation of this apparent conflict between theory and experiment was given by de Bruyn Ouboter (*J. low Temp. Phys.*, **12,** 3; 1973) who pointed out that Kontorovich's analysis had considered equilibrium conditions only within the film itself and had ignored the influence of the surrounding helium vapour: a flowing film in internal dynamic equilibrium would clearly be in disequilibrium with the vapour which, by condensing, would tend to bring the thickness back to its value for a static film. One obvious experiment to test this hypothesis was to repeat the measurements at temperatures below 1 K where the saturated vapour pressure is so small that condensation on the moving film would be a negligible effect. This is the experiment which has now been carried out by Williams and Packard.

Their experimental cell was cooled by means of a dilution refrigerator to temperatures well below 1 K, and was designed to measure the transfer rate of helium between two reservoirs, while at the same time monitoring the thickness of the flowing film. The latter measurement was performed by making the film flow between a pair of capacitor plates so that a change in its thickness resulted in a small change in their capacitance, which could be measured externally. By this means, changes as small as 2 Å could be detected. By using a second pair of capacitor plates to form the walls of one of the reservoirs, the volume of liquid present could be measured as a function of time, thus enabling the film flow rate to be deduced. The authors found that the film became considerably thinner when flowing, than when it was static. At 0.26 K with a

critical velocity of 28 cm s^{-1} the film thickness was reduced by 56 Å.

By investigating transient effects which occurred as the flow started and stopped, they were able to measure the film thickness for velocities less than the critical velocity and they found that the thinning was proportional to the square of the velocity as had been predicted by Kontorovich.

These measurements provide a satisfying demonstration of the general validity of Kontorovich's analysis. They also, however, demonstrate the corrections of de Bruyn Ouboter's suggestion of the importance of vapour condensation for films at temperatures above 1 K, which has implications for a whole range of other experiments and proposed experiments. Williams and Packard point out, in particular, that by providing a dissipative mechanism, such condensation processes may be responsible for the experimental non-appearance of persistent currents in helium films above 1 K. So that this latter hypothesis may be put to the test, it is to be hoped that further experiments seeking persistent currents in helium films will soon be carried out at temperatures well below 1 K, where the influence of the vapour is negligible.

Complexities of the Afar triple junction

from Ian Gass

DURING the past five years the Afar has been the site of intensive investigations by French, German and Italian research teams, as well as by individual scientists from the United Kingdom and United States. A meeting on the Afar region, held at Bad Bergzabern, West Germany, from April 1-6, was anticipated with mixed feelings. Undoubtedly much new information would be available, but would it in any way make the processes at this unique terrestial triple junction any more explicable? These misgivings were unfounded: the symposium was an outstanding success. The lasting impression is of the excellence and abundance of the German geophysical data and of the French and Italian field, structural and petrochemical studies.

The following points were repeatedly emphasised; that (1) the continental crust beneath Afar has been markedly attenuated; (2) the underlying upper mantle is anomalous to depths of 300 km with maximum recorded P wave velocities in the range 7.3–7.4 km s^{-1}; (3) movement, both vertical and horizontal, volcanic activity and deformation are episodic with periods of activity interspersed with intervals of quiescence; (4) the major volcanic activity commenced some 34 Myr ago and that older dates on trap basalts are open to considerable doubt; (5) the disposition of volcanic rock types is far more complex than originally thought; (6) the precise demarcation line between the Nubian, Somali and Arabian plates can be identified more closely than before within the Afar depression but that its position on or around the Danakil Alps is still a matter of personal prejudice.

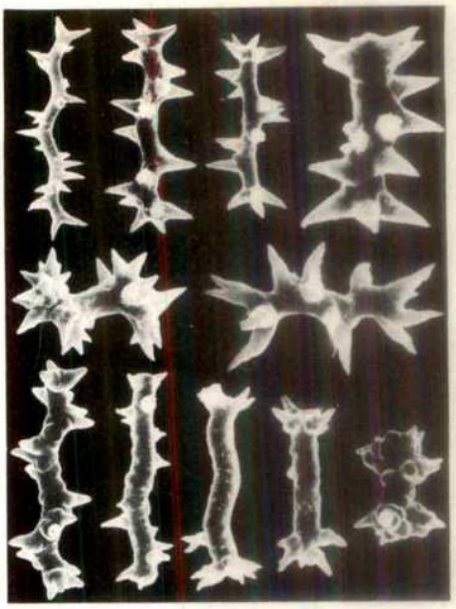
Both R. Black (University of Montpellier) and W. H. Morton (University of Addis Ababa) convincingly demonstrated that crustal attenuation had taken place by a process of rotating fault blocks. Morton proposed that the angle of faulting was a direct reflection of the degree of attenuation in the upper brittle crust while the lower crust deformed plastically. The German seismic data, reported by H. Berckhemer (University of Frankfurt), and the gravity information (J. Makris *et al.*, University of Hamburg) proposed anomalous mantle under Afar though that below the highland is normal. The gravity model presented showed a mantle of 3.15 g cm^{-3} within 20 km of the surface and thinnest beneath the rift zones; a space form similar to that proposed by Gass in 1969 on petrological criteria is indicated. Magnetotelluric measurements (A. Berktold, University of Munich), deep electrical conductivity (H. Porath *et al.*, University of Dallas) and surface wave dispersion (R. C. Searle, National Institute of Oceanographic Sciences, Wormley) all support the presence of low density mantle at high level beneath Afar. Farther to the south a similar space form was proposed for the Kenya rift by R. E. Long (University of Durham), J. D. Fairhead (University of Leeds) and N. Logatchev (Institute of the Earth's Crust, Irkutsk) on teleseismic, gravity and petrological data respectively, although the depth to anomalous mantle varies somewhat.

Time and time again speakers emphasised the periodicity of activity. H. Faure (Centre National de la Recherche Scientifique, Bellevue) demonstrated spasmodic vertical uplift in the order of 0.3 mm yr^{-1} around the Gulf of Tadjura. F. Berberi *et al.* (University of Pisa) and J. Varet (University of Paris) also emphasised the spasmodic nature of uplift which they dated as occurring mainly between 45-40, 25-20 and 5-2 Myr with volcanic maxima between. Although the generally spasmodic nature of events was acknowledged, the longest pause—that proposed by R. W. Girdler and P. Styles (University of Newcastle-upon-Tyne) for a cessation of spreading in the Red Sea between 34-5 Myr—was questioned. Many found it difficult to accept that all was quiet in the Red Sea while volcanism, uplift, crustal attenuation and deformation were going on all around.

Petrologically the picture has altered from the symplistic views of the late 1960s and this is mainly due to the recognition of tholeiitic basalts within the Ethiopian Trap series as well as within the depression. Although generations of basaltic liquids within an area of high heat flow (the use of the term mantle plume being deliberately avoided) is generally accepted, it is quite apparent that factors other than depth of equilibration are relevant and more correlated field, age, petrographic and geochemical data are required before a realistic petrogenetic model can be erected.

Lastly, and perhaps appropriately, the position of the dividing line between the three plates, although becoming more precisely defined, is still masked by the

Inside a sponge



To anybody whose familiarity with sponges goes no further than the bathroom, it may come as a surprise that the bath sponge *Euspongia* is just one of many examples of the sponge phylum Porifera. The strange-looking structures in the figure shown here make up the skeleton of another form of sponge, *Cliona dioryssa*, which burrows into calcareous rocks. This species is one of eight burrowing sponges which Dr Klaud Rützler has been studying in Bermuda (*Smithson. Contrib. Zool.*, No. 165; 1974). Although *Cliona* is sufficiently similar to *Euspongia* for both to be included in the same broad class, the Desmospongia, the skeletons are different; *Cliona*'s skeleton is made up of the siliceous spicules shown here whereas that of *Euspongia* is composed of a horny substance called spongin.

complexity of surface deformation and the super-abundance of Recent volcanic products. Over large areas it is, within limits, a matter of choice as to where it is put—and that alone will keep the arguments going.

Support for deep mantle convection

from Peter J. Smith
Geomagnetism Correspondent

ASSUMING that the engine driving the Earth's lithospheric plates involves some sort of convection in the mantle, do the convection cells reach down to the core-mantle boundary or are they relatively shallow? Views on this point have changed significantly since the idea of motions in the mantle was first proposed. Put briefly and simply, convection as originally envisaged was regarded as mantle wide, with the currents moving the passive lithosphere by viscous coupling. As the new global tectonics developed during the 1960s, however, the nature of the asthenosphere became clearer and the lithospheric plates themselves came to be seen as possible driving forces (acting under gravity, for example). The result was that convection cells became conceptually shallower, being limited to the upper mantle or even to the lithosphere–asthenosphere. More recently, sporadic attempts have been made to reverse this trend by reviving mantle-wide convection but have generally received little support. The latest such attempt is by Robinson (*Earth planet. Sci. Lett.*, **21**, 190; 1974) who has managed to refute the two serious criticisms of mantle-wide convection put forward some years ago by Ringwood (*ibid.*, **14**, 233; 1972).

Robinson's two-dimensional model involves steady cellular convection driven by heat from below. The cells are 3,000 km deep (the thickness of the mantle) and 1,400 km wide (which is within observed trench-ridge separations), but their most important characteristic is that their limbs are narrow. In other words, the convection of heat between the core-mantle boundary and the Earth's surface layer takes place within a narrow (100–200 km) boundary layer, the vertical segments of which form narrow ascending and descending 'plumes'. This immediately avoids Ringwood's first problem, which is that the necessary 200° C mean temperature difference between the ascending and descending currents in the conventional wide-limbed mantle-wide convection model would imply gravity anomalies at the Earth's surface more than 100 times greater than those observed. In Robinson's model the corresponding temperature difference is of the order of 1,000° C but is confined to the narrow plumes. The resulting gravity anomalies are thus comparable to those actually observed.

The second problem that Ringwood pointed to was that of "re-establishing a superadiabatic gradient by thermal conduction after completion of half a turn of the cycle", the point being that superadiabatic gradients are necessary to drive the convection currents. According to Ringwood, the time required for conduction through 3,000 km of mantle would be 2.5×10^{10} yr, so that "only one convective overturn could have occurred throughout the history of the Earth". But according to Robinson, this problem does not arise in his model because the time scale for conduction through a narrow boundary layer is much shorter than that of the convective cycle. In any case, the superadiabatic gradient in the plumes (the temperature gradient inside the cells is adiabatic) could be maintained by energy stored in the core. Ringwood rejected this notion on the grounds that unacceptable radioactive abundances would be required in the core, and that even if the necessary deep heat could be found it would lead to unacceptably high surface heat flow. Robinson argues, however, that the narrow plumes of his model avoid the need for high surface heat flow and that one can envisage heat sources in the core which do not involve radioactivity.

Advances in lichenology

from a Correspondent

PROGRESS and problems in lichenology were considered at a meeting at the University of Bristol on April 8-10. It was the first international symposium held in Britain devoted solely to lichens and was organised jointly by the Systematics Association and the British Lichen Society.

Some fundamental questions as to the nature of lichens were raised by P. W. James (British Museum (Natural History)) and A. Henssen (Botanical Institute, Marburg) who reported convincing evidence that a single fungal partner with different types of algae (green and blue green) can produce morphologically very different individuals traditionally placed in different genera. The production of some chemical components used in lichen taxonomy may also be affected by the algae present. The use of chemical characters in lichen taxonomy was reviewed by D. L. Hawksworth (Commonwealth Mycological Institute, Kew) who suggested guidelines for the taxonomic treatment of chemical races in lichens, and R. W. A. Oliver (University of Salford) demonstrated the value of mass spectrometry and high pressure liquid chromatography for the accurat determination of substances in smal amounts of herbarium specimens Lichen taxonomy is also being clarifie by studies of ontogeny (Henssen algal components (E. Tschermak Woess, Botanical Institute, Vienna and by scanning electron microscop (M. E. Hale, Smithsonian Institution

On the ecological side F. Ros (King's College, London) showed th value of lichens as indicators of ancien woodlands in relatively unpollute parts of Britain, M. R. D. Seawar (Trinity and All Saints' Colleges, Leeds reported on the performance o *Lecanora muralis* in urban areas an A. Fletcher (University College o North Wales, Anglesey) described th ecophysiology of marine and maritim lichens. Distributional studies on world scale (P. W. James) and i Britain (B. J. Coppins, Royal Botani Garden, Edinburgh) were also reported A novel technique for measurin lichen growth rates was proposed b R. A. Armstrong (University o Oxford) and R. H. Bailey (Departmen of Extra-Mural Studies, London pointed to gaps in knowledge of th establishment and dispersal of lichens

Physiological studies formed the basi of several contributions. Sulphur up take and metabolism using radio actively-labelled sulphur — subject relevant to the use of lichens as ai pollution indicators—were reported b B. W. Ferry (Bedford College, Lon don) and the uptake of heavy metal in lichens was discussed by D. H Brown (University of Bristol). Nitroge fixation and transfer in lichens con tinue to provide an interesting field fo research as was shown by J. W. Mill bank (Imperial College, London). Th physiology of symbiosis itself and th transfer of carbohydrates were dis cussed by J. F. Farrar (Imperial Colleg Field Station, Silwood Park) and D. J Hill (University of Newcastle upo Tyne).

D. C. Smith (University of Oxford compared the lichen symbiosis wit other symbiotic systems; he pointe to many similarities and stresse that from the laboratory stand point lichens were ideal organisms i which to study symbiosis itself. Th concept of controlled parasitism of th algal partner by the fungus may, how ever, be preferable to one of symbiosi in lichens.

In conclusion R. Santesson (Univer sity of Stockholm) pointed to the re surgence of interest in lichens and th stimulus which this meeting provide for further work. It is clear that lichen ology is a field in which many prob lems, both old and new, require fur ther research, but also one in which i is still relatively easy to make import ant original contributions.

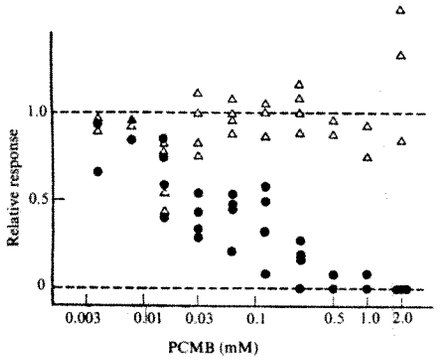
Sugar receptor sites in the fly's tongue

from our
Insect Physiology Correspondent

SINCE the isolation of a 'sweet-sensitive protein' from the bovine tongue there has been increasing interest in the properties of sensory receptor substances and receptor sites. Von Frisch studying the honeybee in 1935 and Dethier studying the blowfly in 1955 failed to discover configurations that were common to the stimulating carbohydrates and were absent in the tasteless ones. But their results underlined the extreme specificity of insect receptors as compared with those of mammals. Dethier observed, however, that the weakly stimulating sugar, mannose, markedly reduced stimulation by fructose but had no effect on glucose sensitivity. Evans (1961) inferred that there must be at least two different sites in the sugar receptor of the blowfly tongue, a 'glucose site' and a 'fructose site'.

Shimada *et al.* (*J. Insect Physiol.*, **20**, 605; 1974) have now re-investigated this problem in the large labellar hairs of the flesh fly *Boettcherisca peregrina*. In earlier work these authors had found that the sugar receptor in this fly with respect to sucrose could be modified by treatment with the sulphhydryl reagent, *p*-chloromercuribenzoate (PCMB). PCMB seemed to react with sulphhydryl groups in proteins of the sugar receptor, but not at the sugar-binding site; it apparently blocked a change in the permeability of the receptor membrane. Shimada *et al.* have now made a detailed study of the effects of PCMB on the electrophysiological response of single receptor hairs, to fructose, glucose and other sugars.

It turned out that there is a striking difference in the effect on the response to glucose and fructose: response to glucose was completely repressed; response to fructose was unaffected (see figure). This constitutes the first direct demonstration of the existence of a 'glucose site' and a 'fructose site' as suggested since Dethier (1955). When a range of other monosaccharides was tested it was found that these fell into two groups: D-fructose, D-fucose, and D-galactose react chiefly with the 'fructose site'; D-arabinose, L-fucose, L-arabinose, L-sorbose, D-xylose, L-glucose and D-glucose mostly with the 'glucose site'. In the disaccharides sucrose and maltose the glucose moiety predominates and both react with the 'glucose site'. High concentrations of glucose exert no specific protective effect against PCMB treatment: this supports the view that PCMB may not react at the glucose-binding site but at a different site necessary for stimulation by D-glucose.



Depression of responses to fructose and to glucose by treatment with *p*-chloromercuribenzoate at graded concentrations. Ordinate: response relative to that before treatment. Duration of treatment with PCBM was always 3 min. △, 0.1M fructose; ●, 0.2M glucose.

From a discussion of these results and the known equilibrium changes in the conformations of a single sugar in aqueous solution, Shimada *et al.* adopt the more precise terms 'pyranose site' and 'furanose site' (instead of 'glucose site' and 'fructose site') representing the sites which respond especially to sugars in the pyranose and furanose forms respectively. The pyranose group was further divided into two subclasses according to the presence or absence of three successive equatorial hydroxyl groups regardless of their positions.

Positive control in the test tube

from Benjamin Lewin
Molecular Genetics Correspondent

TURNING on gene expression *in vitro* has in general proved to be more difficult than switching it off, for although several systems have been developed in which specific repression takes place in the test tube, characterisation of positive control systems has been less successful. The positive control system investigated in most detail to date is the catabolite activator protein stimulated by cyclic AMP, whose mediation is necessary for expression of several inducible and repressible operons. Although this system seems to act on the initiation of transcription, little is known about the molecular details of its activity. Several papers in the March issue of the *Proceedings of the National Academy of Sciences* now take the analysis of positive control *in vitro* a stage further in three systems.

The first positive control system to be suggested was that of the arabinose operon, in which genetic analysis implied that a repressor protein might be converted by arabinose into an inducer protein (instead of simply suffering the inactivation characteristic of negative control systems). Lee *et al.* now report (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 634; 1974) the development of a system in which the regulator protein, activated by arabinose, and the catabolite activator protein, stimulated by cyclic AMP, are both needed for initiation of transcription. Using a template of phage λ carrying the arabinose genes, synthesis of *ara* messenger RNA can be followed by hybridisation first with λ DNA (to remove phage sequences) and then with λ *ara* DNA. This system seems completely dependent on the catabolite activator protein, is stimulated by the arabinose regulator protein in the presence of arabinose, and is inhibited by fucose (an anti-inducer of the operon). These results support previous suggestions that both the regulator protein and the catabolite activator protein must join with RNA polymerase to initiate transcription. Further analysis of this system may be important in helping to define how regulator proteins may interact with each other and with DNA.

Phage lambda itself provides a system under both negative control (lambda repressor prevents expression of integrated lysogenic lambda genomes) and positive control (needed both for the establishment of lysogeny and also for lytic development). Establishment of lysogeny depends on two phage functions (*cII* and *cIII*) in addition to the repressor coded by the *cI* gene, and also upon host genes. One of the host functions has now been identified by Belfort and Wulff (*ibid.*, 779), who have isolated *hfl*⁻ mutants of *Escherichia coli* that show very high frequencies of lysogenisation after lambda infection. Because the *hfl*⁻ mutation is recessive, it is likely that it is caused by the loss of an active *hfl*⁺ gene product, which usually prevents establishment of lysogeny. Because λ phage DNA lacking an active *cIII* gene continues to achieve the same high frequency of lysogenisation in *hfl*⁻ cells that is displayed by wild type λ, it is possible that the *hfl*⁺ function is usually antagonised by the *cIII*⁺ gene product.

That the host catabolite activator system also is implicated in establishment of lysogeny is suggested by the reduction in lysogenisation frequency in mutants defective in this system. Belfort and Wulff found that whereas these host mutants display a slight reduction in frequency of lysogenisation by λ⁺ phages, they show a massive reduction in lysogenisation by *cIII*⁻ mutants. That the catabolite activator and *hfl* systems both act in the same pathway as the *cIII* phage gene product is shown by the observation that the inability of catabolite activator host mutants to lysogenise λ *cIII*⁻ mutants is reversed by the introduction of an *hfl*⁻ mutation. Because catabolite re-

pression is normal in *hfl*⁻ strains, it is possible to exclude the idea that this gene specifies a component of the catabolite repression system. The roles of the catabolite activator protein and the *hfl* gene product in establishing lysogeny are not clear, but it is likely that these systems may be more intricate than previously suspected, and further components may remain to be identified.

A different form of positive control, so far demonstrated only with the mRNAs of phages T3 and T7, is exercised by processing systems. The RNA polymerase enzyme of the host, *E. coli*, transcribes one large messenger *in vitro* from the early region of these phages (corresponding to 20% of the DNA, located at its left end). But infected cells contain five, much smaller, early T7 messengers. Recently it has seemed likely that the discrepancy is due to the presence in the cell of an enzyme, RNase III, which cleaves the large precursor RNA (that is the product *in vitro*) into mature messengers. Hercules *et al.* (*ibid.*, 840) now report that this cleavage represents a step essential for phage gene expression, since only the mature mRNAs and not their large precursor, are active as templates for translation *in vitro*. This concept is supported by the observation that the early phage genes are only poorly translated in RNase III⁻ mutants of *E. coli*. Why the precursor RNA cannot be translated is obscure; but this system may well prove interesting from the viewpoint of translational control as well as for its processing mechanisms. And although no bacterial systems have yet been shown to suffer precursor maturation as a prerequisite for translation, it is an interesting possibility that RNase III may play this part in uninfected bacteria.

Turning to the transcription of T3 and T7 DNAs which takes place by the phage specified polymerase later during infection, Golomb and Chamberlin (*ibid.*, 769) have mapped the principal units of gene expression. With a template of T7 DNA, the T7 RNA polymerase forms six size classes of mRNA (molecular weights 5.5, 4.5, 2.0, 0.8, 0.4 and 0.2×10^6) equimolar in proportion except for the 2×10^6 species which seems to comprise two classes of mRNA. When the T7 DNA template is shortened by the removal of regions from its right end with nucleases, the smallest mRNA is no longer produced, and the mRNAs of 5.5, 4.5 and (one of) 2.0×10^6 daltons are shortened. Since all these messengers are complementary only to the r-strand of T7 DNA and can be synthesised in spite of deletions at the left end, it seems likely that they are derived from overlapping units of transcription; transcription may start at promotor sites located at 56, 64 and 83% along the phage DNA from its left end, and may end at a common termination site near the right end of the molecule.

When T3 DNA is used as a template for the T7 RNA polymerase, a single major RNA species is formed of 2.0×10^6 daltons. Hybrids between T3 and T7 DNA show various patterns of transcription, consistent with an overlapping arrangement of transcription units. Since hybrid templates can be examined by heteroduplex mapping to define precisely which regions are derived from T3 and which from T7, future use of this technique may allow the start and stop points of the polymerase to be mapped more precisely. There are two classes of late gene in T7, and so it is tempting to speculate that they may be represented by the two classes of mRNA, one comprising the overlapping transcription units and the other corresponding to the remaining messengers. Although previous suggestions have quite often been made that transcription might start at one point and end at any one of several sites to allow coordinate or noncoordinate expression of adjacent genes, the late phage overlapping messengers seem to achieve the same end by reversing the roles of the start and stop points. It remains to be seen whether this has wider significance for the uninfected bacterium.

How algae survive desiccation

from Peter D. Moore
Plant Ecology Correspondent

FEW habitats are subjected to the extremes of environmental conditions experienced by the intertidal zones of coasts. Plants and animals living in these regions face wide fluctuation in temperature, insolation, salinity and desiccation with each ebb and flow of the tide. Most of these organisms are essentially marine and therefore it is the period of emersion which represents the stress phase which must be tolerated to ensure survival. The effect of this stress is not laid evenly over the eulittoral zone, but is most acute at its higher levels, where the emersion period is most protracted. It is this variation of stress intensity with vertical height which, in conjunction with the factor of interspecific competition, accounts for the well known intertidal zonation of benthic marine algae (see Zaneveld, *Am. Zool.*, **9**, 367; 1969).

Many of the algae of the upper eulittoral suffer emersion periods of several days, sometimes even weeks, during which they become thoroughly desiccated. The physiological mechanisms which enable these morphologically simple plants to survive such stresses have been a source of interest to botanists for some while.

One aspect of algal physiology which has received attention is that of carbon balance, especially during emersion periods. Chapman (*Proc. 5th Int. Seaweed Symp. Halifax N.S.*, 217; 1966) found that the respiration rate of *Carpophyllum* decreases as desiccation proceeds, falling to 20% of the hydrated rate at 80% desiccation. Bidwell and Craigie (*Can. J. Bot.*, **41**, 179; 1963) also demonstrated that carbon fixation is reduced during emersion in *Fucus vesiculosus*. Ogata (*J. Shimonosaki Univ. Fish.*, **16**, 89; 1968) has since reported that the depression of respiration rate during desiccation is found in many algae, but that rehydration results in an almost instantaneous resumption of normal respiration.

The current *Carnegie Institution of Washington Year Book*, which covers the years 1972–73, contains information concerning the effect of desiccation on the photochemical reaction of photosynthesis in an intertidal alga. Fork and Hiyama (page 384) have made a study of *Porphyra perforata* and *P. tenera*, species of the upper eulittoral zone. Under normal, hydrated conditions cytochrome *f* is oxidised by light and becomes reduced in darkness. This ceased to happen if the plants had been dried in the sun, which could mean that no electrons were available from the oxidation of water, or possibly that there was a physical uncoupling of cytochrome *f*. Rewetting of the alga immediately resulted in light induced oxidation of cytochrome *f*, indicating that the primary photochemical apparatus had survived the drying process.

The stability of the photosynthetic equipment of *Porphyra* comes as no surprise to botanists who are familiar with this upper shore alga which daily suffers desiccation yet survives. This work does, however, demonstrate most precisely the nature of this stability. This plant's resilience is even more remarkable when one considers its apparently delicate structure; unlike the robust fucoids, *Porphyra* forms sheets of tissue just one cell in thickness. For this very reason *Porphyra* may prove to be an eminently suitable subject for future studies on subcellular responses to extreme environmental conditions.

Correction

IN the News and Views article "Palindromes in eukaryotic DNA" (*Nature*, **248**, 733; 1974), the sequence in the third paragraph should read:

5′ .... A B C *t* C′B′A′ .... 3′
3′ .... A′B′C′ *t* C B A .... 5′

and not as printed.

Two plates in Africa during the Cretaceous?

Kevine Burk & John F. Dewey
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Plate tectonic theory is based on the idea of the conservation of displacement at the ends of major faults[1]: ridges, trenches and triple junctions are linked by transform faults so that a plate boundary may not end except by joining two others at a triple junction. This is a consequence of the motion of torsionally rigid plates in which large permanent strains are restricted to narrow boundary zones. Although brittle intraplate deformation occurs[2], excellent fits between rifted continental margins[3] and between pairs of magnetic anomaly isochrons[4] show that plates do not generally suffer significant torsional strains.

Provided that the zones in which large, permanent deformations occur are narrow relative to the plates they surround, plate tectonics is a useful theory. The behaviour of wide zones of interplate deformation may be approximated either by 'soft' regions such as the Himalayas and Tibet[5] or by complex microplate systems such as the present Mediterranean[6].

It is important to understand to what extent relative plate displacements can be registered as strain in a zone too wide and diffuse to be realistically termed a plate boundary. We describe here a wide zone of strain that developed in Africa during the Cretaceous, recognition of which leads us to the view that Africa, from about 125 Myr ago to about 80 Myr ago, was not behaving as a single rigid plate.

Evolution of the Benue Trough

PLATE accretion began in the South Atlantic about 125 Myr ago, during the late Valanginian–early Hauterivian[7]. Aptian rock salt and carnallite in the Gabon Basin overlie a mainly non-marine sequence and are themselves overlain by marine shales and carbonates (Fig. 1). This provides evidence of a narrow proto-South Atlantic with restricted circulation during late Aptian times[8]. Plate accretion and separation of South America from Africa followed an early Cretaceous phase of intracontinental rifting and magmatism. During this phase rifts were formed, along which plate accretion subsequently occurred. Rifts, some of which contain up to 9 km of sediment, were generated striking at high angles to the continental margin[9]. In Africa and South America, nodal alkaline and peralkaline magmatism and widespread basaltic and rhyolitic volcanism occurred during this rifting phase. The rifting and magmatic activity have been related to the development of domes, crested by rift systems with three branches, which occur at hot spots[10]. Plate accretion began on two rift arms, leaving the third as a failed arm or aulacogen[11].

The Benue Trough (Fig. 2) is one such rift but it differs from others in that plate accretion occurred along it during early Cretaceous times. Three pairs of magnetic anomalies strike north-eastward beneath the Niger Delta[12,13]; we suggest that they are late Keathley sequence anomalies developed in Hauterivian–Barremian time during the early opening of the Benue Trough, when a ridge–ridge–ridge triple junction existed in the proto-Gulf of Guinea. The oldest sediments exposed in the Benue Trough are Albian shales (Fig. 1) which pass up into a sequence of Albian to early Santonian shales with minor Turonian alkali basalts and thick Cenomanian deltaic sequences (Fig. 1). We suggest that this mainly sedimentary sequence was deposited in an already open, or slowly opening, narrow ocean. Near the top of the sequence, late Coniacian andesites are intercalated in shales. The whole sequence was folded during the Santonian (Fig. 1) about axes which strike north–east[14]. We interpret the late Coniacian andesites as evidence of subduction of part of the oceanic floor of the Benue Trough, and the Santonian folds as indicating closure of the trough. We are unsure of relative motion, if any, across the Benue Trough during Albian to Turonian times, though the Turonian alkali basalts may indicate very slow extension[15]. Twice, therefore, during the Cretaceous evolution of Africa, a plate margin passed into the continent from a triple junction in the Gulf of Guinea, so that there must have been two African plates at these times, one south-east and one north-east of the Benue Trough. To where did these plate margins continue in Africa, and how and over what area was relative displacement between the two African plates expressed as strain?

Cretaceous Africa north-east of Benue Trough

There is no evidence of a discrete well-defined plate margin north-east of the Benue Trough during either the Hauterivian–Barremian extensional phase or the Coniacian–Santonian convergent phase. We will discuss the extensional phase first and then the convergent phase.

Extensional phase. The Benue Trough divides at the Chum and Poli triple junctions to form three rifts[16], one of which, the Poli, continues as a subsurface feature marked by a positive Bouguer anomaly (Fig. 2). Louis[17] modelled this gravity

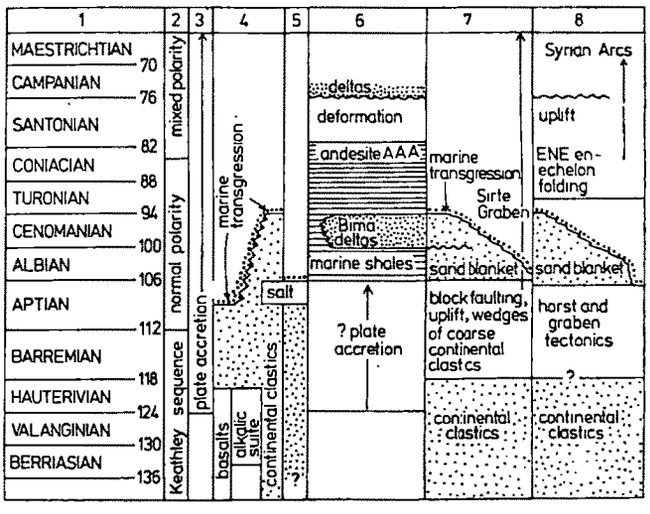


Fig. 1 Correlation chart showing relationships between magmatism, tectonics and sedimentation during the Cretaceous in northern and western Africa and eastern South America. 1, Cretaceous stages; 2, geomagnetic behaviour; 3, plate accretion in South Atlantic (ref. 7); 4, development of eastern continental margin of South America (based mainly on refs 37–39); 5, Gabon Basin (ref. 8); 6, Benue trough (refs 12–15); 7, Algerian and Libyan Sahara (refs 22–26); 8, Egypt and Levant (refs 24, 27). Note that Early Cretaceous magmatism largely predates the development of accreting plate margins and the associated distension of northern Africa.

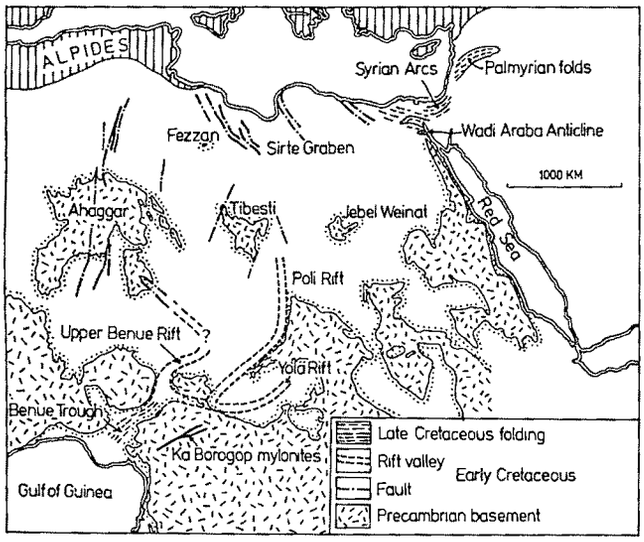


Fig. 2 Northern part of the African continent showing Early Cretaceous rifting and distensional structures and Late Cretaceous folding and compressional structures (based mainly on refs 36, 28 and 17).

anomaly as a basaltic dyke up to 30 km wide. Burke and Whiteman[16] interpreted it as one of the axial dykes related to extension, which are becoming widely known in rift valleys[18-21]. The Upper Benue and Yola intracontinental rifts contain Cenomanian continental sandstones equivalent to the Bima deltas of the Benue Trough that prograded south-westwards along the narrow Benue Ocean. From the Berriasian until the Hauterivian, most of North Africa was covered by a thin veneer of continental deposits. During the Barremian–Aptian, block faulting and differential regional uplift affected a wide region of North Africa from the Algerian Sahara to Egypt[22] (Figs 1, 2 and 3*B*). Most of the block faulting took place on reactivated basement faults trending roughly north-south. Tibesti was uplifted and shed sand wedges[23], and north–south block faulting in the Ahaggar was accompanied by local spreads of coarse clastics in graben, and erosion or nondeposition over horsts[24]. The Jebel Weinat Massif was also rising at this time[25]. In north-west Libya, gentle folding of early Cretaceous rocks occurred before the deposition of late Aptian sediments[26] and, in the Fezzan, Cenomanian sediments rest unconformably on older Cretaceous rocks. These strong differential movements over the Algerian Sahara and Libya, ceased by Albian times. An Albian sandstone sheet of fairly uniform thickness completely covers the Barremian–Aptian structures[24]. The Sirte graben complex of northern Libya (Fig. 2) controlled sedimentation during the middle and late Cretaceous. In Egypt, faults trending north–west were active during Barremain–Aptian times, producing a series of graben and horsts with up to 2 km of shale. The Barremian–Aptian activity suggests that a large region of northern Africa (Fig. 2) was slowly distending, between two African plates, in response to the same displacement that was being accommodated by plate accretion in the Benue Trough.

Compressional phase. The Cretaceous deformation of the Benue Valley is dated as Santonian (Fig. 1). Deformation was also occurring elsewhere in northern Africa at this time[24]. The Syrian Arcs, the Wadi Araba anticline and related structures (Fig. 2) began to form late in the Turonian and grew during the Coniacian and Santonian (Fig. 1). An important sub-Campanian unconformity records this deformation phase in Egypt (Fig. 1). In the Levant, the Santonian rests directly on gently folded Turonian and older rocks[27]. The Ka Borogop mylonite belt (Fig. 2) is not Precambrian, as was previously thought, but affects Cretaceous conglomerates[28]. Its upper age limit is unknown but we here suggest that it was developed, during the Coniacian–Santonian closing of the Benue Trough, perhaps with a significant strike-slip component (compare with ref. 29). Therefore, although convergence of the two African plates was accommodated by subduction in the Benue Trough it was apparently taken up by intracontinental compression over at least part of the region of northern African affected by Barremian–Aptian distension. Although the relative displacement of the two plates may not have been large we are unable to determine its magnitude or precise direction. The Benue Trough was a structure which narrowed towards the continent during the early Cretaceous and all early Cretaceous strain between the Benue Trough and Egypt seems to have been extensional. This suggests that the pole of relative motion between the two African plates at this time lay north-east of Egypt. In plate tectonic terms, the broad zone of extension may be regarded as either a plate boundary up to 2,000 km wide or as a series of smaller intracontinental plates. If nothing were known of the history of the Benue Trough this extensional zone might be regarded as an enigmatic zone of intraplate, intracontinental rifting.

Stresses unrelated to latitudinal motion

Intraplate earthquakes, well away from possible plate boundary effects, yield dominantly thrust fault and strike-slip first motion solutions on all present major plates, indicating that plates are undergoing local, brittle deformation in response to compressive stresses[2]. There is no clear pattern of compression directions, but compression has been deduced at localities from latitudes of about 60°N to 40°S. Intraplate extension has been deduced only for eastern Baffin Island and southern Africa.

We have argued[10] that intraplate extension which yields intracontinental rift-valley systems is related to the growth of hot spot domes and periclines. As the domes grow, they are characterised by alkaline and peralkaline magmatism and eventually by crestal rifting, commonly in three-armed systems. This hot spot phase of intracontinental rifting and magmatism may be protracted and abortive but it may lead to a phase in which rift valleys propagate and link up, followed by continental fragmentation and the opening of oceans by plate accretion[30]. Burke and Wilson[31] have argued that extensive hot spot activity before continental separation may indicate

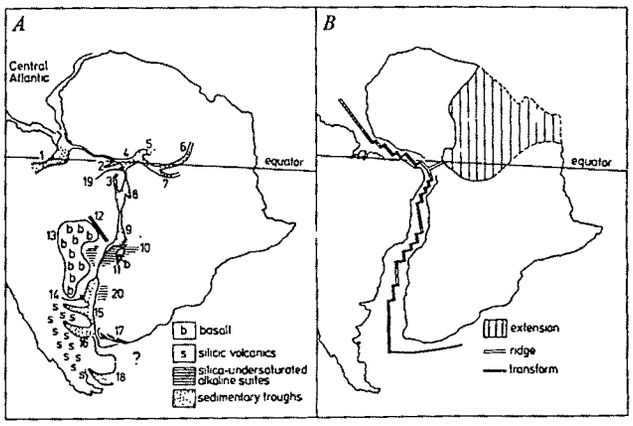


Fig. 3 *A*: Berriasian–Valanginian magmatism, tectonics and sedimentation on the site of the future South Atlantic. 1, Marajo basin (ref. 37); 2, Potiguar basin (ref. 37); 3, Jucano basin (ref. 37); 4, Benue trough (ref. 12); 5, Upper Benue rift (ref. 16); 6, Poli rift (ref. 17); 7, Yola rift (ref. 16); 8, Sergipe-Gabon basin (ref. 39); 9, Cuanza basin (ref. 40); 10, Angola alkaline province (ref. 41); 11, Kaokoveld basalts (ref. 42); 12, Goiana arch (refs 37 and 38); 13, Parana basalts (ref. 37); 14, Pelotas basin (ref. 37); 15, Rio Salado basin (ref. 9); 16, Bahia Blanca basin (ref. 9); 17, Algoa and associated Cape Province basins (ref. 43); 18, Malvinas basin (ref. 9); 19, Cabo granite and associated silicic alkaline rocks (ref. 37); 20, Messum alkaline province (ref. 44). *B*: Aptian plate boundaries in the South Atlantic and Africa. The circle with a dot lies in the general area of a pole describing the Aptian motion of north-western Africa with respect to south-eastern Africa.

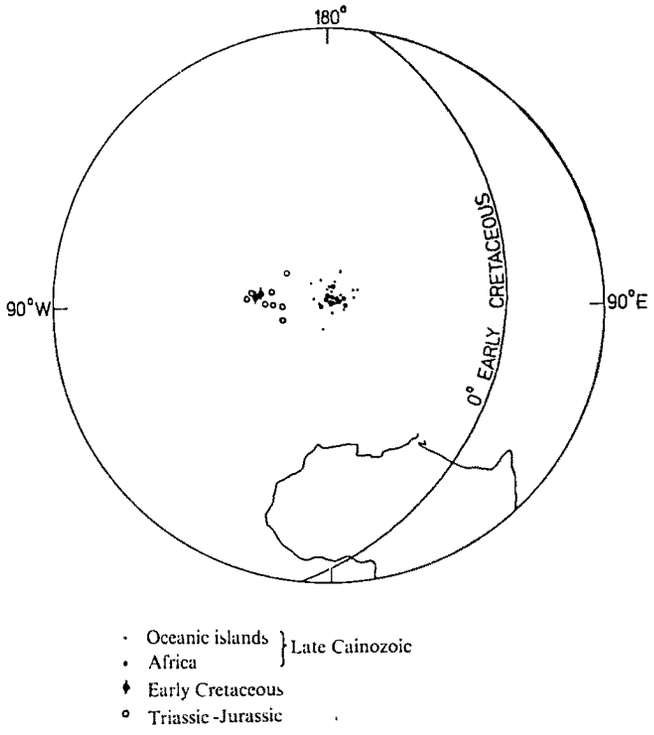


Fig. 4 Palaeomagnetic poles for the African plate (refs 33 and 34) form two groups marking times in the Mesozoic and Tertiary when the plate was at rest with respect to the spin axis. Between about 100 and 25 Myr ago African plate motion with respect to the main dipole field was approximately a 30° rotation about a pole in the Gulf of Guinea. Rifting and igneous activity dominated the continent while it was at rest and were minimal while it was moving.

that the continent, or plate, in question was stationary or moving very slowly with respect to underlying mantle plumes. Once plate accretion between two continental fragments begins, hot spot activity may diminish or cease on one or both of the fragments; if motion relative to the plume population is fast. Our model of rift valley generation involves a hot spot phase with dominantly vertical movements and only mild extension, followed by a phase of extension leading to continental break up.

Turcotte and Oxburgh[32], using membrane stress theory, have adopted a different view of intraplate deformation. They argue that as plates, considered as thin elastic membranes, change their radius of curvature as they change latitude, stresses build up. If, for instance, a portion of a plate were moving to a lower latitude at a rate of 1 cm yr^{-1}, then membrane stresses would equal the tensile strength of the lithosphere after 30 Myr, provided that the stresses were not dissipated. An increase in latitude of 90° would, however, be required before membrane stresses were about equal to the compressive strength of the lithosphere. Membrane stresses thus cannot account for the widespread intraplate compressive failure of the lithosphere[2] although they could, theoretically, generate tensional failure to form rift valleys. If intracontinental extension and rifting results from the propagation of cracks developed in response to membrane stresses, extension should predate magmatism. In rift valleys generated by hot spots, magmatism should predate significant extension. We here show that the early Cretaceous tectonic–magmatic history leading to the separation of Africa and South America and the opening of the Benue Trough favours the hot spot interpretation.

From about 160 to 120 Myr ago, magmatism was widespread in the general region along which Africa separated from South America (Fig. 3*A*). Alkaline and peralkaline igneous rocks were intruded and extruded in distinct nodes (Cabo Granite, Rio de Janeiro, Messum); basalts (Parana, Kaokoveld) and rhyolites (southern Argentina) formed extensive volcanic provinces. During and after this magmatic phase, great thicknesses of non-marine Valanginian to Barremian sediments accumulated in troughs with faulted boundaries (rifts and aulacogens) parallel and at high angles to the future continental margins of South America and Africa. The beginning of plate accretion in the South Atlantic overlapped the waning phases of magmatism and aulacogen formation (Fig. 1) yet nonmarine sedimentation persisted until middle to late Aptian times. The sea had invaded the Benue Trough by earliest Albian times and it is likely that the Benue Trough opened during the extension of North Africa (Figs. 1 and 3*B*). Significant extension leading to the separation of South America and Africa, the opening of the Benue Trough and the distension of North Africa, postdated most of the early Cretaceous magmatic–aulacogen phase.

Late Triassic to early Cretaceous palaeomagnetic poles for Africa[33] cluster closely around a mean at 265°E, 65°N (Fig. 4). While Gondwana was breaking up there were no systematic changes to lower latitudes of the kind needed for an explanation in terms of membrane stress of the associated early Mesozoic rift systems. Africa, during approximately the last 25 Myr of its history, has been characterised by a similar episode of hot spot uplift, volcanism and rifting. African palaeomagnetic poles for this time interval[33,34] similarly cluster around a mean near the present pole (Fig. 4). Rifting and magmatism in Africa have developed at times when Africa did not change its latitude. Between 110 and 25 Myr ago Africa changed its position, relative to the spin axis, in a way that may be approximated by an anticlockwise finite difference rotation of 30° around a pole at 0°N, 10°W (Fig. 4). During this time there was little or no rifting or magmatism on the African continent.

The closing of the Benue Trough and the compression of North Africa occurred at a time of major changes in relative plate motion. As a result of the opening of the Labrador Sea, the relative motion of Africa with respect to Europe changed to an anticlockwise rotation about a pole at 25.4°N, 163.8°E (ref. 35). Plates moving with respect to underlying plumes may generally be in compression whereas those that are at rest with respect to underlying plumes develop tensional features related to hot spot activity.

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1 Wilson, J. T., *Nature*, **207**, 343 (1965).
2 Sykes, L., and Sbar, M., *Nature*, **245**, 298 (1973).
3 Bullard, E., Everett, J. E., and Smith, A. G., *Phil. Trans. R. Soc.*, **258**, 41 (1965).
4 Pitman, W. C. III, and Talwani, M., *Bull. geol. Soc. Am.*, **83**, 619 (1972).
5 Dewey, J. F., and Burke, K., *J. Geol.*, **81**, 683 (1974).
6 McKenzie, D., *Nature*, **226**, 239 (1970).
7 Larson, R. L., and Ladd, J. W., *Nature phys. Sci.*, **246**, 209 (1973).
8 Belmonte, Y., Hirtz, P., and Wenger, R., in *Salt Basins around Africa*, (edit. by Ion, D. C.), 55 (Institute of Petroleum, London, 1965).
9 Ludwig, W. J., Ewing, J. I., and Ewing, M., *Bull. Am. Ass. Petrol. Geol.*, **52**, 2337 (1968).
10 Burke, K., and Dewey, J. F., *J. Geol.*, **81**, 406 (1973).
11 Hoffman, P., Dewey, J. F., and Burke, K., in *Modern and Ancient Geosynclinal Sedimentation*, (edit. by Dott, R. H., and Shaver, R. H.), (Society of Economic Palaeomtolagists and Mineralogists, Tulsa, in the press).
12 Burke, K., Dessauvagie, T. F. W., and Whiteman, A. J., *Nature phys. Sci.*, **233**, 51 (1971).
13 Burke, K., Dessauvagie, T. F. W., and Whiteman, A. J., in *African Geology, Ibadan*, 1970 (edit. by Dessauvagie, T. F. J., and Whiteman, A. J.), 187 (University of Ibadan, Ibadan, 1972).
14 Murat, R. C., in *African Geology, Ibadan*, 1970 (edit. by Dessauvagie, T. F. J., and Whiteman, A. J.), 251 (University of Ibadan, Ibadan, 1972).
15 Carter, J. D., Barber, W., Tait, E. A., and Jones, G. P., *Bull. geol. Surv. Nigeria*, **30**, (1963).
16 Burke, K., and Whiteman, A. J., in *Implications of Continental Drift to the Earth Sciences*, **2** (edit. by Tarling, D. H., and Runcorn, S. K.), 727 (Academic Press, London, 1973).
17 Louis, P., *Contribution geophysique à la connaissance geologique du bassin du lac Tchad* (Memoir de la Organisation de la Recherche Scientifique dans les Territoires d'outre Mer, n° 42, Paris, 1970).

[18] Griffiths, D. H., King, R. F., Khan, M. A., and Blundell, D. J., *Nature phys. Sci.*, **229**, 69 (1971).
[19] Searle, R., and Gouin, P., *Tectonophysics*, **15**, 279 (1972).
[20] Ocola, L., and Meyer, R. P., *J. geophys. Res.*, **78**, 5173 (1973).
[21] Ramberg, I., *Nature phys. Sci.*, **240**, 149 (1972).
[22] Karpoff, R., *Bull. Soc. geol. Fr.*, Ser. 7, **7**, 469 (1965).
[23] Lelubre, M., *Bull. Soc. geol. Fr.*, Ser. 5, **19**, 251 (1949).
[24] Sander, N. J., in *Geology and History of Sicily*, (edit. by Alvarez, W., and Gohrbandt, K. H. A.), 43 (Petroleum Exploration Society of Libya, 1970).
[25] Kiltzsch, E., in *African Geology, Ibadan*, 1970 (edit. by Dessauvagie, T. F. J., and Whiteman, A. J.), 423 (University of Ibadan, Ibadan, 1972).
[26] Busson, G., in *Guidebook to the Geology and History of Tunisia*, 131 (Petroleum Exploration Society of Libya, 1967).
[27] Bentor, Y. K., Grader, P., Parnes, A., Reiss, Z., Shiftan, Z., and Vroman, A., (*Lexique Stratigraphique International*, **3**, 8 (1960).
[28] Le Marechal, A., and Vincent, P. M., in *African Geology, Ibadan*, 1970, (edit. by Dessauvagie, T. F. J., and Whiteman, A. J.), 229 (University of Ibadan, Ibadan, 1972).
[29] Fitch, T. J., *J. geophys. Res.*, **77**, 4432 (1972).
[30] Dewey, J. F., and Burke, K., *Geology*, **2**, 57 (1974).
[31] Burke, K., and Wilson, J. Tuzo, *Nature*, **239**, 387 (1972).
[32] Turcotte, D. L., and Oxburgh, E. R., *Nature*, **244**, 337 (1973).
[33] McElhinny, M. W., *Palaeomagnetism and Plate Tectonics* (Cambridge University Press, Cambridge, 1973).
[34] Piper, J. D. A., and Richardson, A., *Geophys. J. R. astr. Soc.*, **29**, 147 (1972).
[35] Dewey, J. F., Pitman, W. C. III, Ryan, W. B. F., and Bonnin, J., *Bull. geol. Soc. Am.*, **84**, 3137 (1973).
[36] *Tectonic Map of Africa*, 1:5M (Association des Services Geologiques Africains—UNESCO, Paris, 1968).
[37] Almeida, F. F. M., *Twenty-fourth International Geological Congress, Montreal*, **3**, 339 (1972).
[38] Neill, W. M., *Nature phys. Sci.*, **245**, 104 (1973).
[39] Wardlaw, N. C., and Nicholls, G. D., *Twenty-fourth International Geological Congress, Montreal*, **6**, 43 (1972).
[40] Brognon, G. P., and Verrier, G. R., *Bull. Am. Ass. Petrol. Geol.*, **50**, 108 (1966).
[41] Rodrigues, B., in *African Geology, Ibadan*, 1970, (edit. by Dessauvagie, T. F. J., and Whiteman, A. J.), 149 (University of Ibadan, Ibadan, 1972).
[42] Siedner, G., and Miller, G. A., *Earth planet. Sci. Lett.*, **4**, 451 (1968).
[43] Rigassi, D. A., and Dixon, G. E., in *African Geology, Ibadan*, 1970, (edit. by Dessauvagie, T. F. J., and Whiteman, A. J.), 513 (University of Ibadan, Ibadan, 1972).
[44] Marsh, J. S., *Earth planet. Sci. Lett.*, **18**, 317 (1973).

Repeating sequences in aminoacyl-tRNA synthetases

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Some aminoacyl-tRNA synthetases have elongated by fusing two very similar sequences.

AMINOACYL-tRNA synthetases are responsible for the specificity of translation of the genetic code. They each catalyse a similar reaction in which a particular amino acid reacts with ATP probably forming a 5′-aminoacyl adenylate, which then transfers to the 3′-terminal adenosine of a particular tRNA, eliminating AMP. Their specificities are also similar in that different tRNAs have similar structures[1] and that each enzyme requires an unsubstituted α-amino acid and ATP for the complete reaction.

To avoid errors in protein synthesis, however, they must be completely specific for a particular amino acid and a particular tRNA. Since the genetic code seems to be universal, this specificity has been conserved over a very long period. We might therefore expect these enzymes to be a family with homologous primary and tertiary structures that are under enormous selective pressure to conserve their essential features.

The accumulation of data on the protomeric units of several synthetases has revealed an objection to this view, however. Broadly, four classes of synthetases can be defined on the basis of protomer size and binding capacity. (1) Those containing a single polypeptide chain with a molecular weight of about 120,000 and containing one binding site for amino acid and tRNA, for example, isoleucyl-tRNA synthetase from *Escherichia coli*[2,3]. (2) Those containing two identical protomers each with a molecular weight of about 90,000 and possessing one binding site for amino acid and tRNA per protomer, for example methionyl-tRNA synthetase from *E. coli* (ref. 4, and G.K., unpublished). (3) Those containing two identical protomers each with a molecular weight of about 40,000, for example, tryptophanyl-tRNA synthetase from *E. coli*[5]. (4) Those containing dissimilar protomers, for example phenylalanyl-tRNA from yeast[6], glycyl-tRNA synthetase from *E. coli*[7]. This wide diversity in the sizes and organisation of the protomers does not seem consistent with the concept of evolution from a common precursor since the known classes of homologous protein generally contain protomers of very similar size. These apparent inconsistencies have led us to isolate several aminoacyl-tRNA synthetases from a single source, the thermophile *Bacillus stearothermophilus*, and to search for sequence homologies between them.

Enzymes from *B. stearothermophilis*

Aminoacyl-tRNA synthetases are present in low amounts in most cells and many of them are denatured during the lengthy purification that is necessary. The thermophilic organism, *B. stearothermophilus* was chosen as a source because proteins from this species exhibit remarkable stability. A purification procedure that yields about 100 mg of pure leucyl-, methionyl-, tryptophanyl-, tyrosyl- and valyl-tRNA synthetases from 5 kg wet weight of these cells has been developed in collaboration with the Microbiological Research Establishment, Porton Down, Wilts. All enzyme samples used in these studies were more than 95% pure.

Before determining any of the chemical parameters of these enzymes their subunit structures had to be established. The molecular weights of the native proteins determined by gel filtration[8] were usually sufficiently accurate (Table 1). On the other hand, it was essential to estimate the sizes of the protomers on proteins which had been completely de-

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Table 1 Subunit structures of *B. stearothermophilus* synthetases

Aminoacyl-tRNA synthetase	Molecular weight (native)	Molecular weight (subunit)	Subunit structure
Tryptophanyl-	70,000 (E, F)	35,000 (S, G)	2 × 35,000
Tyrosyl-	95,000 (F)	44,000 (S)	2 × 44,000
Methionyl-	135,000 (E, F)	66,000 (S, G)	2 × 66,000
Valyl-	100,000 (F)	110,000 (S, A)	1 × 110,000
Leucyl-	100,000 (E)	110,000 (S, A)	1 × 110,000

E, Equilibrium sedimentation; F, Sephadex gel filtration; G, equilibrium sedimentation in 6 M Guanidine hydrochloride; S, SDS gel electrophoresis; A, agarose gel filtration in 0.1% SDS.

natured and dissociated. For this purpose the proteins were either boiled in 1% SDS or preoxidised with performic acid and boiled in 1% SDS. The molecular weights of the protomers were determined by electrophoresis in SDS-polyacrylamide gels[9] or by gel filtration on columns of SDS-agarose (Table 1). Investigations of the tryptophanyl-enzyme were hampered by autolysis in the stored preparations. The size of the protomers in methionyl-tRNA synthetase was confirmed by equilibrium ultracentrifugation in the presence of 6 M guanidine hydrochloride[10]. The results show that valyl- and leucyl-tRNA synthetase are monomeric proteins

Table 2 Amino acid compositions of *B. stearothermophilus* synthetases

Amino acid	Aminoacyl-tRNA synthetase Tyrosyl-	Methionyl-	Valyl-	Leucyl-
Cys†	2	5	8	6
Asp	29	56	91	93
Thr	23	27	52	50
Ser	18	24	48	33
Glu	44	69	117	120
Pro	6	17	26	30
Gly	33	50	76	72
Ala	33	44	91	67
Val	23	41	80	64
Met	11	18	32	33
Ile	26	39	66	51
Leu	41	54	97	77
Tyr	17	29	30	29
Phe	18	22	38	34
Trp‡	11	19	19	24
His	9	12	31	26
Lys	27	45	68	65
Arg	28	34	69	47
Molecular weight	45,000	66,000	110,000	110,000

* Calculated on the basis of the molecular weights of the protomers.
†From analyses of the oxidised protein, using oxidised chymotrypsinogen as standard. This procedure can give over estimates of up to 15% for cysteic acid.
‡ Estimated by the method of Edelhoch[17].

with molecular weights of about 110,000 whilst tyrosyl- and methionyl-tRNA synthetase are dimers whose protomeric units have molecular weights of 45,000 and 66,000 respectively.

To facilitate the discussion the amino acid compositions of the proteins shown in Table 2 have been calculated on the basis of the molecular weight of the protomers present in each protein. Since the cysteine (cysteic acid) contents of the enzymes proved small, special precautions were taken to ensure the accuracy of its analyses. Hydrolysates of chymotrypsinogen and *B. amyloliquefaciens* RNase[11] (which contains no cysteine) were used for calibration and the values shown are the averages of several analyses.

Repeating sequences

The first indication that some of the protomers might contain repeating sequences was found in maps of the tryptic peptides but the most convincing evidence for their existence came from estimates of the molar yields of several tryptic peptides from each of the proteins. It was assumed that yields greater than 1 mol of peptide per mol protomer would prove the existence of at least two copies of the corresponding sequence in the intact protomer. Since the approach relies on the isolation of pure peptides in high yield, certain precautions had to be taken. Preliminary experiments had shown that trypsin digestion was more complete when the proteins were denatured by oxidation rather than carboxymethylation. The trypsin was purified on trypsin inhibitor-Sepharose columns in order to minimise nontryptic cleavages in the digest, and wherever possible only those peptides which were pure after a single purification step were considered since we could not correct for losses during purification.

The tryptic digest of oxidised methionyl-tRNA synthetase was fractionated by ion-exchange chromatography on Locarte L1/5 resin using a gradient of pyridinium-acetate for elution[12]. This procedure (Fig. 1) resolved several peptides which proved pure by paper electrophoresis, paper chromatography and amino acid analysis. The amino acid analyses also gave their compositions and yields. The molar yield of each peptide calculated from them was then used to determine the mol peptide per mol of protomer (Table 3). A similar experiment was performed with tryptic digests of oxidised leucyl-tRNA synthetase using a combination of SP-Sephadex chromotography and Sephadex G-50 gel filtration to purify the peptides. This procedure was preferred to the one used with methionyl-tRNA synthetase because it selectively purified the larger acidic peptides in high yield (Table 3).

The yields of tryptic peptides from valyl-tRNA synthetase were estimated by a different procedure. The protein was carboxymethylated with ^{14}C-iodoacetate[13], digested with trypsin and the radioactive peptides purified by paper electrophoresis and chromatography. By using radioactive peptides losses during purification could be corrected. Note that the yields (Table 3) have been calculated from the amino acid analyses of the purified peptides and not from the yield of radioactivity which is an unreliable criterion. Nevertheless, the radioactive label was useful in enabling corrections for losses to be made at each step in purification.

The results show that the protomers of methionyl-, valyl- and leucyl-tRNA synthetase gave several peptides with yields between one and two per protomer (Table 3).

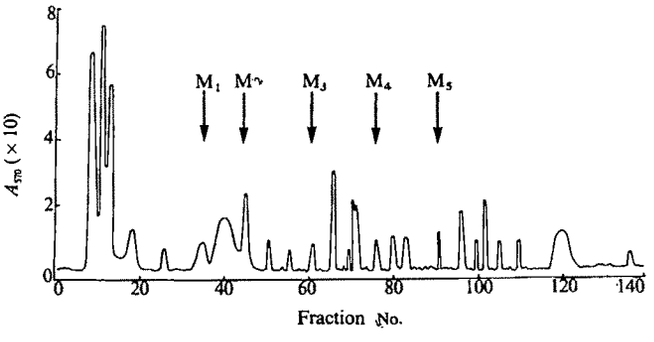


Fig. 1 Separation of the tryptic peptides from methionyl-tRNA synthetase. Enzyme (26 mg) was oxidised with performic acid, digested with trypsin (1:100, w/w) for 4 h at 37° C in 0.5% NH_4HCO_3 and lyophilised. The peptides were dissolved in 0.2 M pyridine–acetate *p*H 3.1 and applied to a column (1 cm × 60 cm) of Locarte L1/5 resin equilibrated with the same buffer. The peptides were eluted with a gradient of pyridine–acetate

$$\frac{175 \text{ ml}\quad 0.2 \text{ M pyridine–acetate } pH\ 3.1}{350 \text{ ml}\quad 2 \text{ M pyridine–acetate } pH\ 5.0}$$

One-sixth of the effluent was monitored automatically for peptides by the ninhydrin reaction after alkaline hydrolysis, and the rest collected in fractions which were pooled on the basis of the elution profile. Each subfraction was tested for homogeneity by paper electrophoresis at *p*H 6.5 and 2.1. The material from each of the arrowed peaks contained only one peptide.

The relatively low yields for the peptides from methionyl-tRNA synthetase are probably a result of losses during purification. All the same, the yields provide strong evidence for more than one copy of each of those peptides in the protomer.

Distribution of repeated sequences

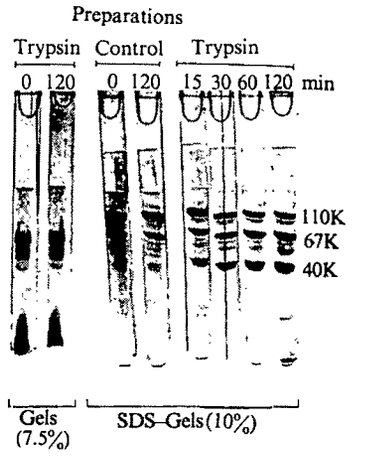
The distribution of the repeated sequences might indicate how the proteins have evolved, but would seem to need knowledge of the entire primary structures. We have attempted a short cut which relies on splitting each protomer into a few large fragments and then surveying the fragments for certain peptides. If a peptide is contained within more than one large fragment then the repeating units which make up the protomer are large. If it is contained within one fragment, then the repeated regions must be relatively short and close to one another.

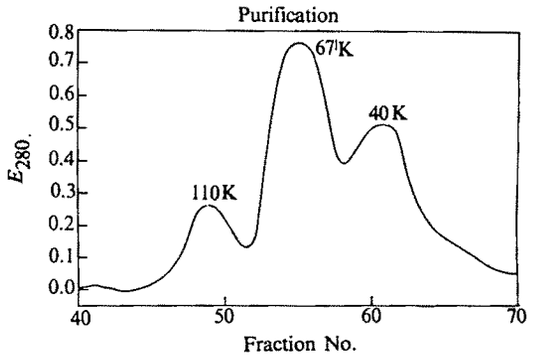
This approach has proved to be useful with valyl-tRNA synthetase. The native enzyme consists of a single polypeptide chain of about 1,000 residues and is extremely sensitive to trypsin which splits the chain into two fragments of unequal size (Fig. 2). The electrophoretic behaviour of the split protein is identical to that of the native enzyme showing that degradation is not extensive. The larger fragment,

Table 3 Estimation of the yields of some tryptic peptides from the synthetases

Protein sample	Peptide + composition	Yield (nmol)	Mol peptide per mol protomer
Methionyl-tRNA synthetase (210 nmol protomer)	M_1 D T S E G I F R	290	1.39
	M_2 E P G L K	260	1.25
	M_3 D E A K	240	1.14
	M_4 E A R	240	1.14
	Mf M S K	240	1.14
Leucyl-tRNA synthetase (90 nmol protomer)	L_1 D^5 T_2 S El P_2 G_3 A_2 V_3 I L_2 K R	140	1.55
	L_2 D_2 K	160	1.78
	L_3 D E G V K	150	1.67
	L_4 D_2 S E_3 A L K	150	1.67
Valyl-tRNA synthetase (67 nmol protomer)	V_1 C T E_2 G Y R	128	1.91
	V_2 C E A V K	120	1.79
	V_3 C D_2 E P G A L	120	1.79

The peptides from methionyl-tRNA synthetase were purified according to the procedure shown in Fig. 1. The peptides from leucyl-tRNA synthetase were isolated from a tryptic digest of the oxidised enzyme by passage of the digest through columns of SP-Sephadex C25 equilibrated with 0.17 M pryidine-acetate *p*H 4.7 and Sephadex G50 equilibrated with 1% pyridine. The peptides from valyl-tRNA synthetase were isolated from a tryptic digest of the ^{14}C-carboxymethylated protein. The digest was subjected to paper electrophoresis at *p*H 6.5 and *p*H 2.1 (see Fig. 2), which completely separated the radioactive carboxymethylcysteine peptides. The peptides were eluted with 1 M ammonia and purified to homogeneity by paper chromatography. The pure peptides were then analysed after hydrolysis and the yield of each calculated. The yield was corrected for losses during purification by comparing the amount of radioactivity in each pure peptide with that present in the same peptide in the unfractionated digest. The single letter notation used for amino acids is that proposed in ref. 18.





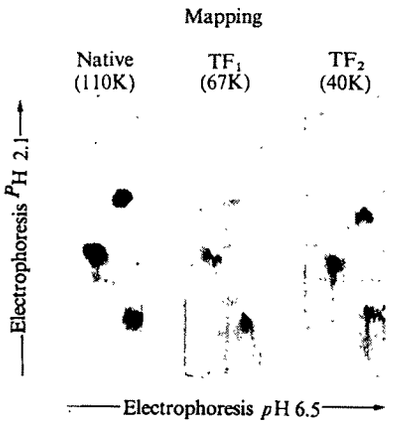


Fig. 2 The distribution of the carboxymethylcysteine sequences between the fragments of valyl-tRNA synthetase. Enzyme (40 mg in 20 ml of 0.1 M Tris-HCl *p*H 7.5) was incubated with trypsin (1:2500 w/w) at 37° C for 2 h. Aliquots were removed at various intervals and subjected to polyacrylamide gel electrophoresis in the presence and absence of SDS. During incubation the native enzyme was split into two fragments with molecular weights of about 67,000 and 40,000. The digested enzyme was made 2% in SDS and 2% in 2-mercaptoethanol, immersed in boiling water for 5 min and passed through a column of agarose 1.5 M equilibrated with 100 mM Tris-HCl, 0.1% SDS and 10 mM 2-mercaptoethanol. Material from the 67K and 40K peaks was pooled and recycled to yield homogeneous preparations. Samples of the native enzyme, valyl-TF_1 (67K) and valyl-TF_2 (40K) were reduced and carboxymethylated with ^{14}C-iodoacetate in 6 M guanidine hydrochloride, digested with trypsin (1:100 w/w) in 0.5% NH_4HCO_3 for 4 h at 37° C and the soluble peptides subjected to two-dimensional mapping by paper electrophoresis. The regions of the maps containing the major radioactive peptides from each sample are shown. The apparent inhomogeneity of the sample is due to considerable overloading of the gels.

valyl-TF_1 (molecular weight 67,000), and the smaller, valyl-TF_2 (molecular weight 40,000), were dissociated and separated from one another by gel filtration on columns of agarose 1.5 M equilibrated with 0.1% SDS. The amino acid analyses show eight half cystines in the native protomer, four half cystines in the large fragment and three half cystines in the small fragment[14]. The three proteins were reduced, ^{14}C-carboxymethylated, digested with trypsin and subjected to two dimensional mapping. Autoradiographs of the maps were prepared to identify the carboxymethylcysteine peptides (Fig. 2). The same radioactive peptides stand out in all three maps showing that both fragments contain the same carboxymethylcysteine sequences. This means that the repeated peptides are distributed between widely separated regions of the protomer and that it is made up of long repeating units and not short, clustered repeats.

When digested with low concentrations of subtilisin, methionyl-tRNA synthetase is converted into a fully active fragment which is composed of a single polypeptide chain with a molecular weight of about 55,000. Methionine is the amino terminus of both the native enzyme and the fragment, indicating that the trypsin digestion has removed a segment of about 100 residues from the C-terminus of the native protomer. The differences between the amino acid analyses of the native enzyme and the fragment show that the excised region contains five lysine and 11 arginine residues and should therefore yield 17 tryptic peptides. Except for the relative intensities of some of the spots, however, two-dimensional maps of the tryptic peptides from the two proteins display almost complete homology (Fig. 3). Such a result would be expected if the native protomer were composed of two long homologous sequences fused end to end, digestion of the excised region from the C-terminus having removed one copy of the duplicated peptides and left the qualitative features of the fingerprint unchanged.

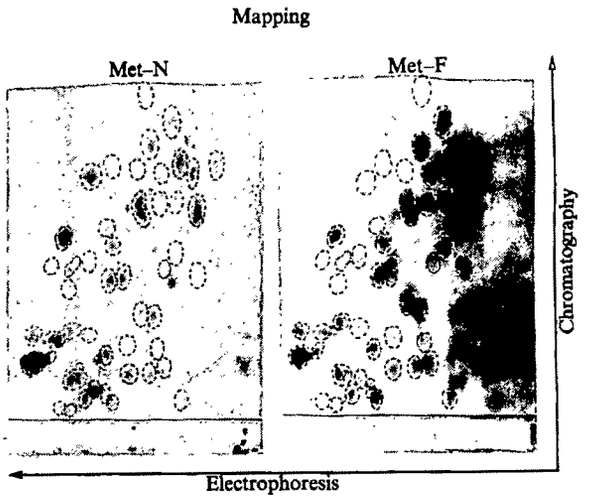


Fig. 3 Two-dimensional maps of the tryptic peptides from methionyl-tRNA synthetase and its subtilisin fragment. The fragment was prepared from the native enzyme by digestion with subtilisin (1:2500 w/w) in 100 mM Tris-HCl *p*H 7.5, and purified by gel filtration on Sephadex G150. Samples of the homogeneous fragment and the native enzyme were oxidised with performic acid, digested with trypsin (see legend to Fig. 1) and the soluble tryptic peptides (over 95% of the proteins used) subjected to paper electrophoresis (formic acid–acetic acid buffer *p*H 2) and paper chromatography (butanol–acetic acid–water–pyridine 30:6:24:20). Maps were stained with 1% ninhydrin in acetone.

Homology of the repeated sequences

To find the degree of homology of the repeating units and the proportion of each protomer made up by the repeating sequences, we tried to determine the number of peptides obtained upon trypsin digestion of each protein by high resolution mapping. Once again oxidised proteins were used since they did not give significant amounts of insoluble material after digestion with trypsin, making nearly all of the protein amenable to mapping.

The tryptic digests of the proteins were first fractionated by ion-exchange chromatography on a column of Locarte L1/5 resin. The peptides were eluted with a linear *p*H gradient, the effluent monitored automatically by the ninhydrin reaction and fractions collected. The fractions containing the material in each of the ninhydrin-positive peaks were pooled, concentrated and each peak subjected to two-dimensional mapping by paper electophoresis at *p*H 6.5 and 2.0. The results obtained with methionyl-, valyl- and leucyl-tRNA synthetase showed that this procedure fractionates the peptides in the digests very well and that the chance of low estimates of the number of tryptic peptides as a result of coincidence is negligible. Table 4 summarises the estimates of the number of tryptic peptides from each protein. Table 4 also shows estimates of the number of arginine and histidine peptides which were determined from two-dimensional maps of the tryptic peptides using the specific stains for these residues. Estimates of the number of cysteine peptides were obtained from studies on tryptic digests of the ^{14}C-carboxymethylated proteins. The number of carboxymethylcysteine peptides among the soluble proteins was determined after fractionation by ion-exchange chromatography followed by paper electrophoresis of the radioactive fractions (Fig. 4). Two radioactive peaks were obtained from methionyl- and leucyl-tRNA synthetase and electrophoresis showed that each contained a single radioactive peptide. Paper electrophoresis of the single radioactive peak obtained from valyl-tRNA synthetase showed two radioactive peptides of equal intensity. The recovery of radioactivity after ion-exchange chromatography exceeded 70% of that applied to the column which excludes the possibility of significant losses of radioactive peptides during

Table 4 Estimates of the number of tryptic peptides

Aminoacyl-tRNA synthetase	Total peptides		Arginine peptides		Histidine peptides		Cysteine peptides	
	Expected*	Observed	Expected*	Observed	Expected*	Observed	Expected*	Observed
Tyrosyl-	56	45-50	28	22	9	10	2	2
Methionyl-	80	40-45	34	19	12	6	5	3
Valyl-	138	50-55	69	32	31	12	8	3
Leucyl-	113	40-45	47	24	26	10	6	2-3

* Calculated from the amino acid analyses shown in Table 2.

The total number of tryptic peptides obtained from each protein was determined from digests of the oxidised proteins. The soluble peptides, comprising over 90% of the protein used for each digest, were first fractionated according to the procedure described in Fig. 1. The material in each peak was then subjected to two-dimensional mapping on paper, the peptides stained with ninhydrin and the number obtained from all the maps used to estimate the total number of tryptic peptides present in the digest. The number of arginine and histidine peptides was estimated by staining two dimensional maps on paper with *o*-phenanthroline and diazotised sulphanilic acid respectively. Estimates of the number of cysteine-containing peptides were determined from tryptic digests of the ^{14}C-carboxymethyl proteins (see Fig. 4).

fractionation. The insoluble peptides from methionyl-, valyl-, and leucyl-tRNA synthetase contained 35%, 20% and 5% of the total radioactivity in the carboxymethylated proteins, respectively. They were solubilised by digestion with thermolysin and subjected to paper electrophoresis. A single radioactive peptide was obtained from each of the insoluble fractions and comparisons with thermolysin digests of the soluble peptides indicated that they were unique. It was concluded that there are no more than three cysteine sequences in each protein of which only two of the three from leucyl-tRNA synthetase can be treated as major peptides. The minor peptide from leucyl-tRNA synthetase is

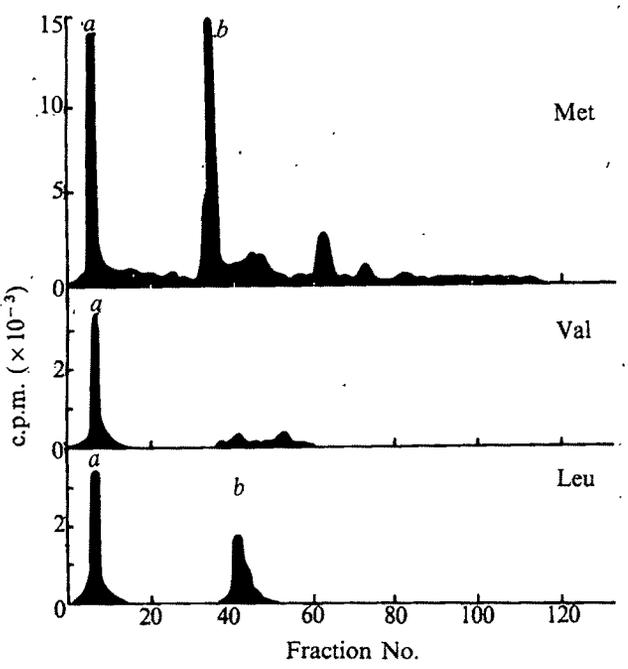


Fig. 4 Estimation of the number of cysteine peptides in tryptic digests of the enzymes. The proteins were reduced and ^{14}C-carboxymethylated in 6 M guanidine hydrochloride, digested with trypsin and the soluble peptides subjected to ion-exchange chromatography under the conditions described in Fig. 1. Aliquots of each fraction obtained from the column were counted for radioactivity and two-dimensional mapping showed that the major radioactive peaks from methionyl- and leucyl-tRNA synthetase contained one radioactive tryptic peptide each, whereas the major peak from valyl-tRNA synthetase contained two radioactive tryptic peptides.

probably a noncysteine peptide which was labelled by the rather drastic conditions used to ensure complete labelling.

It is clear from Table 4 that methionyl,- valyl- and leucyl-tRNA synthetase yield substantially fewer tryptic peptides than the expected number. In all cases the observed number is half, or less than half, the expected number. It is particularly significant that this applies to the histidine and (CM) cysteine peptides since the numbers concerned are quite small. These data, although of a negative nature, strongly suggest that the duplicated regions of these protomers are very similar.

Three lines of evidence

Our conclusion that the protomers of methionyl-, valyl- and leucyl-tRNA synthetase from *B. stearothermophilus* contain repeated sequences is based on three lines of evidence. First, each protomer contained more than one copy of several tryptic peptides. Second, widely separated regions of the protomers contained the same tryptic peptides. Third, all three protomers yield far fewer tryptic peptides than expected if they contained unique sequences over their entire lengths. Taken together, the data also suggest that the repeating units are about half the length of the protomers and that the units within each protomer are very similar in sequence.

These models are unusual since they suggest that in each case two very similar sequences have become fused to yield the functional protomer. Proteins can acquire such repeats by incorporating partial or complete duplications of their cistrons and some proteins have evidently elongated by this process, for example, immunoglobulins[15].

In most of the known cases, however, the sequences of the repeating units have subsequently diverged to an extent substantially beyond that envisaged for the aminoacyl-tRNA synthases which seem to resemble the haptoglobin α_2 chain which is also composed of two virtually identical sequences fused through gene duplication and fusion[16]. It is therefore plausible that the same mechanism has generated the elongated protomers present in some aminoacyl-tRNA synthetases although the possibility of post-transcriptional or post-translational fusion cannot be excluded.

The existence of long duplicated sequences in several of the aminoacyl-tRNA synthetases raises the possibility that other proteins, especially those with relatively long protomers, have elongated in this manner. In fact, there is evidence which suggests that rabbit muscle phosphofructokinase may be one such protein[17].

In none of these cases, however, is the functional advantage of such structures obvious and other information such as the folding of the 'intra-units' and the distribution of active sites between them will probably be necessary to clarify this question. One consequence of the existence of these structures, however, is that it disposes of the objection that the protomers of the aminoacyl-tRNA synthetases differ too much in size to have evolved from a common precursor, since the basic units of sequence are of comparable length although the physical lengths of the protomers are different.

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1 Holley, R., Apgar, J., Everett, G., Madison, J., Marquisee, M., Merrill, S., Penswick, J., and Zamir, A., *Science*, **147**, 1462 (1965).
2 Arndt, D. J., and Berg, P., *J. biol. Chem.*, **245**, 665 (1970).
3 Yarus, M., and Berg, P., *J. molec. Biol.*, **28**, 479 (1967).
4 Blanquet, S., Fayat, G., Waller, J. P., and Iwatsubo, M., *Eur. J. Biochem.*, **24**, 461 (1972).
5 Muench, K. R., and Joseph, D. R., *Miami Winter Symposium*, **2**, 172 (1971).
6 Fasolo, F., Befort, N., Boulanger, Y., and Ebel, J. P., *Biochim. biophys. Acta*, **217**, 305 (1970).
7 Ostrem, D. L., and Berg, P., *Proc. natn. Acad. Sci U.S.A.*, **67**, 1967 (1970).
8 Andrews, P., *Biochem. J.*, **91**, 222 (1964).
9 Laemmli, U. K., *Nature*, **227**, 680 (1970).
10 Ullmann, A., Goldberg, M. E., Perrin, D., and Monod, J., *Biochemistry*, **7**, 261 (1968).
11 Hartley, R. W., and Barber, E. A., *Nature new Biol.*, **235**, 15 (1972).
12 Schroeder, W. A., *Methods in Enzymology*, **25**, 203 (1972).
13 Crestfield, A. M., Stein, W. H., and Moore, S., *J. biol. Chem.*, **238**, 2413 (1963).
14 Edelman, G. M., Cunningham, B. A., Reeke, G. N., Becker, J. W., Waxdal, M. J., and Wang, J. L., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2580 (1972).
15 Black, J. A., and Dixon, G. H., *Nature*, **218**, 736 (1968).
16 Coffee, C. J., Aaronson, R. P., and Frieden, C., *J. biol. Chem.*, **248**, 1381 (1973).
17 Edelhoch, H., *Biochemistry*, **6**, 1948 (1967).
18 Edelhoch, H., *J. biol. Chem.*, **243**, 3557 (1968).

Direct measurement of electric current generation by cytochrome oxidase, H^+-ATPase and bacteriorhodopsin.

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SUMMARY. A method for association of proteoliposomes with planar phospholipid membrane has been elaborated, by which operation of molecular electric generators, such as cytochrome oxidase, H^+-ATPase and bacteriorhodopsin, can be followed using ordinary electrometer techniques.

THE existence of molecular electric generators in mitochondrial, chloroplast and bacterial membranes was postulated by Mitchell in 1966 who proposed that enzymes of respiratory and photosynthetic redox chains and ATPases localised in these membranes catalyse transmembraneous movement of electrons or protons against the electric gradient. As a result, the membranes should charge like a condenser and electrical energy accumulated in this way might be utilised for ATP synthesis or osmotic work.

This hypothesis is difficult to verify by direct methods because of the small size of intracellular organelles and bacterial cells and the complexity of the enzymic and ion transport processes occurring in these systems. To detect the electric membrane potential in organelles and bacteria, therefore, some indirect methods were developed, such as measurement of electrophoretic transport of penetrating ions, and the electrochromic shifts of carotenoid and chlorophyll spectra[2-8]. In this laboratory, the direct measurement of electromotive force (e.m.f.), produced by one of the molecular electric generators, (bacteriorhodopsin) has been accomplished[9]. *Halobacterium halobium* membrane sheets containing bacteriorhodopsin as the only protein were mixed with a decane solution of phospholipids. The mixture was used to form a planar artificial membrane onto the aperture in the Teflon septum separating two electrolyte solutions. Illumination of the bacteriorhodopsin-containing membrane was found to induce generation of a transmembrane electric potential difference which was measured by a sensitive voltmeter. All attempts to use the same procedure for making planar phospholipid membrane containing cytochrome oxidase and mitocondrial proton-translocating ATPase (H^+-ATPase) failed, however.

Direct measurement

Further experiments yielded a more universal method of direct measurement of the generation of an electric charge by membraneous proteins. The principle of this method is that, firstly, the protein is incorporated into the liposome membrane according to procedure of Racker and associates[10-12] which includes mixing cholate solution of a phospholipid and protein and removal of cholate by means of dialysis. Then, the resulting proteoliposomes are brought into contact with the planar phospholipid membrane. To induce fusion of the planar and proteoliposomal membranes, the negative surface charges of phospholipids are neutralised by Ca^{2+} ions. As voltmeter measurements showed, cytochrome oxidase, H^+-ATPase and bacteriorhodopsin, integrated with planar membrane in such a fashion, can generate a transmembrane electric potential difference at the expense of ascorbate – O_2 electron transfer, ATP hydrolysis and light energy, respectively.

Cytochrome oxidase was purified after the method of Yonetani[13] coupling factor F^1 after Horstman and Racker[14], ATPase hydrophobic proteins after Kagawa and Racker[10], and bacteriorhopsin membrane sheets after Oesterhelt and Stoeckenius[15]. For the procedure of the cytochrome oxidase and ATPase proteoliposome preparations, see ref. 16; for bacteriorhodopsin proteoliposomes, see ref. 17.

Electric potential difference across the planar membrane was measured with Ag/AgCl electrodes connected with the RFT VA J-51 vibrating capacitor electrometer and KSP-4 recorder. Planar phospholipid membrane was made of soya bean phospholipids (azolectin) solution in decane (70 mg azolectin per ml decane). The azolectin solution was applied to a 1 mm aperture in the Teflon septum which separated the experimental vessel into two compartments containing incubation mixture. After the planar membrane was formed, proteoliposomes were added into one of the compartments. In the 15-30 min required for the proteolipsomes to become associated with the planar membrane, an energy source was introduced and the membrane potential was measured.

Proteoliposome systems

The data of a typical experiment with cytochrome oxidase proteoliposomes are given in Fig. 1. As shown in Fig. 1*a*, ascorbate addition to the compartment, containing cytochrome oxidase proteoliposomes and cytochrome *c*, results in the formation of an electric potential difference across the planar membrane (plus in the compartment with proteoliposomes). Cyanide reverses the effect when added after ascorbate. In the experiment shown in Fig. 1*b*, ascorbate (– cytochrome *o*) and proteoliposomes were added to the two different compartments. In this case, no membrane potential was formed until the mixture was supplemented with a penetrating hydrogen atom carrier, phenazine methosulphate (PMS). Direction of the field was the same as in the preceding experiment. An electric potential of opposite polarity (minus on the proteoliposome side) was generated when cytochrome oxidase proteoliposomes containing cytochrome *c* inside were used. Again, PMS was found to be necessary (not shown in Fig. 1).

In Fig. 1*c* the result of the experiment with ATPase proteoliposomes is demonstrated. The addition of ATP to the com-

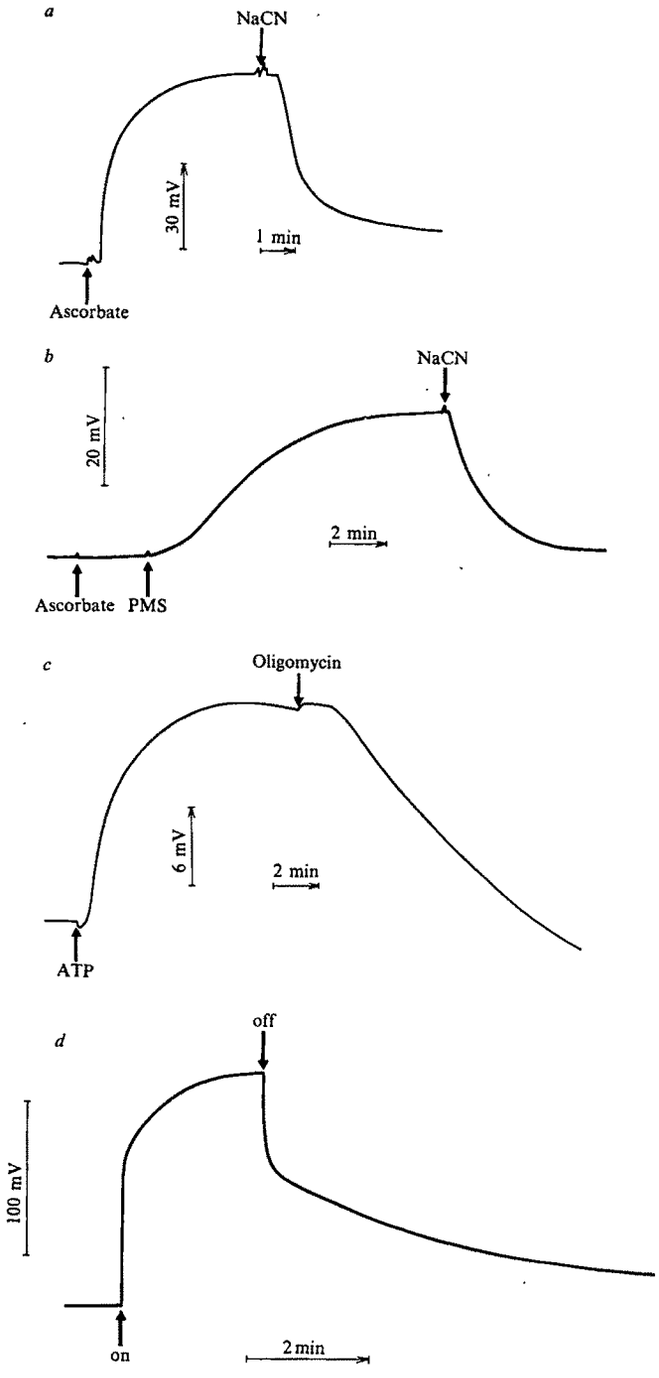


Fig. 1 Generation of transmembrane electric potential differences by cytochrome oxidase (*a*, *b*), H+κ-ATPase (*c*) and bacteriorhodopsin (*d*), *a*, Incubation mixture: 0.3 M sucrose, 5 mM Tris-citrate (*p*H = 7.2), 30 mM $CaCl_2$, 5 mM $MgSo_2$, 1×10^{-4} M cytochrome *c*. One of compartments was supplemented with cytochrome oxidase proteoliposomes (0.3 mg protein ml^{-1}. Additions: 10 mM ascorbate (added in the same compartment as proteolipsomes), 1 mM NaCN (added in both compartments) Planar membrane resistance (R) 1.5×10^{10} ohm, current at maximal potential difference (I) 5×10^{-12} A. *b*, Incubation mixture: 0.2 M sucrose, 0.05 M Tris-HCl (*p*H = 7.2), 30 mM $CaCl^2$, 7×10^{-5} M cytochrome c. One of compartments contained cytochrome oxidase proteoliposomes (0.25 mg protein ml^{-1}). Additions in the proteoliposome-free compartment: ^{5}mM ascorbate, 1.5×10^{-6} M PSM, in both compartments: 1.5×10^{-3} M NaCN. R = 1×10^{10} ohm, I = 2×10^{-12} A. *c*, Incubation mixture: 0.3 M sucrose, 5 mM Tris-citrate (*p*H = 7.2), 30 mM $CaCl_2$, 5 mM $MgSO_2$. One of compartments contained ATPase proteoliposomes (0.3 mg protein ml^{-1}). Additions: 2.5 mM ATP, oligomycin (30 μg ml^{-1}). R = 1×10^{10} ohm, I = 1.8×10^{-12} A. *d*, Incubation mixture: 0.2 M sucrose, 5 mM Tris-HCl (*p*H = 7.2), 30 mM $CaCl_2$, and in one of compartments, bacteriorhodopsin proteoliposomes (0.2 mg protein ml^{-1}). R = 2×10^{10} ohm, I = 7.5×10^{-12} A.

partment with proteoliposomes gives rise to the formation of electric potential difference (minus on the proteoliposome side). Oligomycin reverses the ATP action.

Figure 1*d* illustrates the light-induced generation of an electric field by bacteriorhodopsin proteoliposomes. In this case, minus was on the proteoliposome side. In the dark, the membrane potential dissipated.

In all cases, addition of a protonophorous uncoupler, trichlorocarbonylcyanidephenylhydrazone (2×10^{-7} M) or the shunting of the planar membrane by an external resistance greatly decreased the above responses, and accelerated their dissipation when no more energy was supplied.

The magnitudes of the membrane potential vary depending on the conditions and quality of proteoliposomes. The maximal potential values for cytochrome oxidase were ~ 110 mV, for bacteriorhodopsin ~ 150 mV. In the ATPase experiments, the membrane potentials were always lower, and more time was required for the proteoliposomes and planar membrane to fuse, the fact which may be due to the complex profile of the ATPase proteoliposome surface covered with the F_1 knobs.

Electrical response

The bacteriorhodopsin system has proved to be the most convenient experimental model, because the responses were large, steady and easily reproducible. This system was studied in some detail. In particular, the change of the photoeffect as the function of the potential difference generated across the planar membrane by an external source (ΔV_{ext}) has been measured. It was found (Fig. 2) that ΔV_{ext} of the same sign as that of the photo e.m.f. decreases, and of opposite sign increases, the light-induced response. At a certain ΔV_{ext}, the photoeffect becomes equal to zero. In these conditions, ΔV_{ext} should be equal to the bacteriorhodopsin photo e.m.f. if all bacteriorhodopsin generators have the same orientation. Photo e.m.f. should be higher than this value if some of generators are oriented in the opposite direction to the majority. According to Fig. 2, bacteriorhodopsin photo e.m.f. is $\geqslant$ 300 mV.

It seems most probable that these electrical responses are due to proteoliposomes attached to the surface of the planar phospholipidm embrane. As shown previously[18,19], the addition of Ca^{2+} induces the fusion of two planar phospholipid membranes resulting in the formation of an electrical contact between the membranes and a local decrease in the electrical resistance.

The fusion of proteoliposomes with planar membrane seems to be irreversible. In fact, experiments with the bacteriorhodopsin system showed that the photoeffect, if it appears, is retained after a solution without proteoliposome is substituted for the proteoliposome-containing mixture.

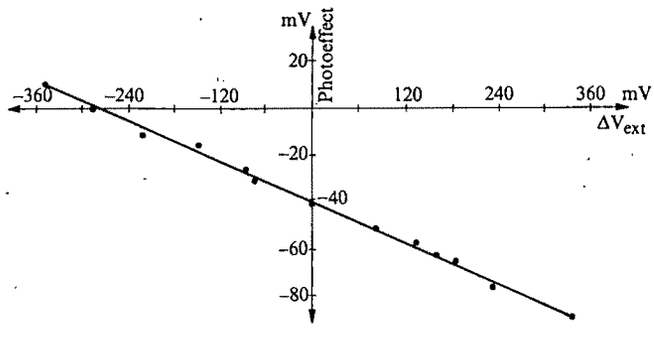


Fig. 2 Relationship between the bacteriorohodopsin-mediated photo effect and external ΔV. Incubation mixture: 0.3 M sucrose, 0.09 M $(NH_4)_2SO^4$, 5 mM Tris-HCl (*p*H = 7, 2), bacteriorhodopsin proteolipsomes in one of compartments (0.1 mg protein ml^{-1}).

Proteoliposomal and planar membrane fusion

The schemes for fusion of proteoliposomal and planar membranes are given in Fig. 3. Figure 3*A* indicates that cytochrome oxidase proteoliposome, associated with a planar membrane, carries out electron transfer from the extraproteoliposomal ascorbate to the intraproteoliposomal oxygen.

As a result, the proteoliposome interior charges negatively. H^+ ions or other cations of the proteoliposome interior move (down the electric gradient) across the proteoliposome membrane from the same compartment (where the proteoliposomes are), or, alternatively, from the opposite compartment situated on the other side of the planar membrane (Fig. 3, dotted lines). The latter situation should give rise to the formation of an electric potential difference across the planar membrane, namely, positive charging of the proteoliposome-containing compartment and negative charging of the opposite compartment. In agreement with this scheme, experiments on the proteoliposomes of different types showed that the proteoliposome-free compartment always charges like the proteoliposome interior. In fact, cytochrome oxidase proteoliposomes with cytochrome *c* inside, ATPase proteoliposomes and bacteriorhodopsin proteoliposomes generate positive charges, whereas the cytochrome oxidase proteoliposomes with cytochrome *c* outside generate negative charges in their interior[16,17]. The first three types of proteoliposomes charge the proteoliposome-free compartment positively, and the last charge it negatively.

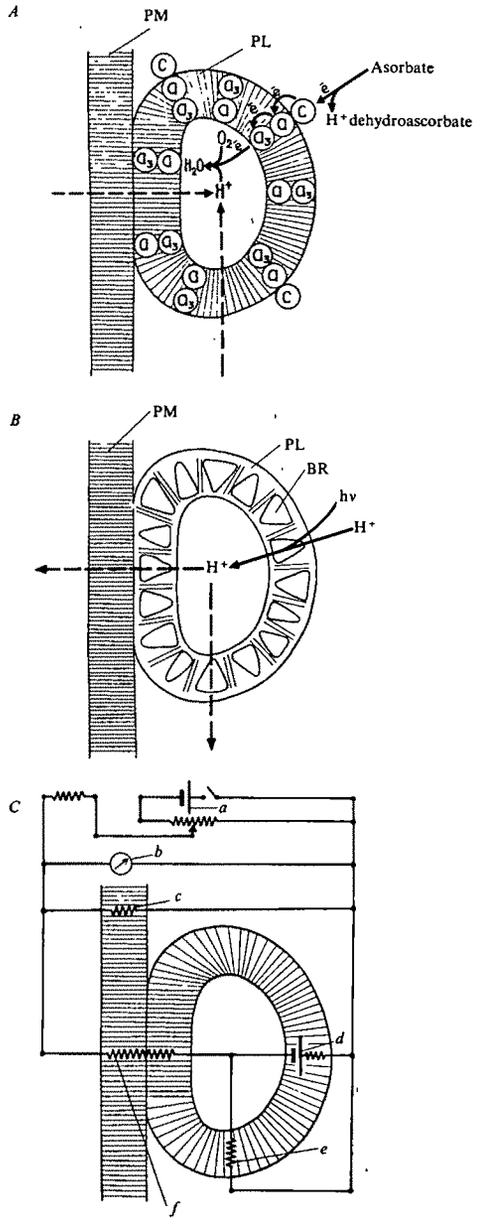


Fig. 3 Schemes illustrating interaction of the proteoliposomes (PL) with the planar membrane (PM). *A*, Cytochrome oxidase proteolipsomes with cytochrome *c* outside. *a*, a_3 and *c*—corresponding cytochromes; *B*, bacteriorhodopsin (BR) proteolipsomes; *C*, equivalent scheme for estimation of e.m.f. of the molecular electric generators. *a*, External battery; *b*, voltmeter; *c*, planar membrane resistance; *d*, molecular e.m.f. generator; *e*, proteoliposome resistance; *f*, resistance of fusion region.

The scheme given in Fig. 3*A* also explains why phenazine methosulphate is required for membrane potential to be supported by oxidation of ascorbate added into the proteoliposome-free compartment. Planar phospholipid membrane is impermeable to ascorbate (shown by special experiments) so reducing equivalents cannot reach cytochrome oxidase, if proteoliposomes and ascorbate are in different compartments, until a lipid-soluble hydrogen atom carrier is added (see Fig. 1*b*).

An important prediction of the scheme illustrated in Fig. 3*A* is the inefficiency of cytochrome oxidases localised in the region of fusion of the proteoliposome with the planar membrane. According to the procedure used in the cytochrome oxidase experiments, cytochrome *c* was added several minutes later than proteoliposomes, so that the fusion preceded the reconstitution of the cytochrome *a*–cytochrome *c* complex. Moreover, ascorbate concentration should be very low in the fusion region because of hydrophilic properties of this compound. Low membrane permeability for ATP and H^+ can be responsible for ineffectiveness of ATPase and bacteriorhodopsin molecules in the fusion region, respectively.

In the reconstitution of proteoliposomes, transmembrane orientation of proteins may be of two opposite directions. To actuate the systems oriented in one of these directions, non-penetrating substrates or catalytic factors may be used: ascorbate and cytochrome *c* in the case of cytochrome oxidase proteoliposomes; ATP and factor F_1 in the case of ATPase proteoliposomes. Asymmetric reconstitution of the bacteriorhodopsin proteoliposomes (Fig. 3*B*) may be a result of the difference in areas of the inner and outer surfaces of the vesicles (for discussion, see ref. 17).

In Fig. 3*C*, the equivalent electric scheme of the experiment on estimation of the e.m.f. of the molecular electric generators is given.

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1 Mitchell, P., *Chemiosmotic Coupling in Oxidative and Photosynthetic Phosphorylation*, Glynn Research, (Bodmin, 1966).
2 Mitchell, P., *Chemiosmotic Coupling and Energy Transduction*, Glynn Research (Bodmin, 1968).
3 Greville, G. D., *Curr. Top. Bioenergetics*, **3**, 1 (1969).
4 Skulachev, V. P., *FEBS Lett.*, **11**, 301 (1970).
5 Liberman, E. A., and Skulachev, V. P., *Biochim. biophys. Acta*, **216**, 30, (1970).
6 Skulachev, V. P., *Curr. Top. Bioenergetics*, **4**, 127 (1971).
7 Witt, H. T., *J. Bioenergetics*, **3**, 47 (1972).
8 Skulachev, V. P., *Energy Transformation in Biomembranes* (Nauka, Moscow, 1972).
9 Drachev, L. A., Kaulen, A. D., Ostroumov, S. A., and Skulachev, V. P., *FEBS Lett.*, (in the press).
10 Kagawa, Y., and Racker, E., *J. biol. Chem.*, **246**, 5477 (1971).
11 Racker, E., *J. Membrane Biol.*, **10**, 221 (1972).
12 Kagawa, Y., *Biochim. biophys. Acta*, **265**, 297 (1972).
13 Yonetani, T., in *Methods in Enzymology* (edit. by Estabrook, R. W., and Pullman, M. E.) **10**, 332 (Adademic Press, New York, 1967).

[14] Horstman, L. L., and Racker, E., *J. biol. Chem.*, **245**, 1336 (1970).
[15] Oesterhelt, D., and Stoeckenius, W., *Nature new Biol.*, **233**, 149 (1971).
[16] Jasaitis, A. A., Nemecek, I. B., Severina, I. I. Skulachev, V. P., and Smirnova, S. M., *Biochim. biophys. Acta*, **275**, 485 (1972).
[17] Kayushin, L. P., and Skulachev, V. P., *FEBS Lett.*, (in the press).
[18] Liberman, E. A., and Nenashev, V. A., *Biophysika* (USSR) **13**, 193 (1968).
[19] Blioch, Z. L., Glagoleva, I. M., Liberman, E. A., and Nenashev, V. A., *J. Physiol. Lond.*, **199**, 11 (1968).

Contractile units in vertebrate smooth muscle cells

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Evidence from isolated smooth muscle cells in rigor indicates that the contractile elements are grouped into separate fibrils or 'contractile units' arranged obliquely with respect to the cell axis.

FROM X-ray diffraction studies[1,2] of living vertebrate smooth muscle, the presence of myosin filaments during both relaxation and contraction is generally accepted but the form and arrangement of these filaments within the cell is far from settled. This latter dilemma arises because the appearance of the contractile apparatus as observed in electron microscope studies of whole muscle is extremely variable[3–15], a situation which derives mainly from the marked lability of the myosin-containing filaments under conditions of chemical fixation.

Another approach to the problem of smooth muscle structure was suggested by the demonstration[16] that viable smooth muscle cells which retain their spindle-like shape may be separated from toad stomach by conventional cell separation methods, utilising collagenase and trypsin. These cells were shown to contract on electrical stimulation[16,17]. If model cells could be produced from such preparations, corresponding to isolated myofibrils from striated muscle, whose ability to contract could be controlled through the external environment, then the contractile apparatus should be more accessible to both fine structural and biochemical studies. The same sort of approach has been particularly fruitful in investigations of nonmuscular motile systems[18–20].

With this object in mind, experiments were carried out to develop methods for the preparation of cells from smooth muscles such as chick gizzard, guinea pig taenia coli and guinea pig vas deferens which have already been the subject of several ultrastructural and biochemical investigations. The results from the first stage of this study are summarised in the present report. They show that under conditions whereby the cell membrane is made permeable, either through the cell dispersion process itself, by treatment with the nonionic detergent Triton X-100 or by glycerination, a very regular organisation of obliquely arranged birefringent fibrils is seen in the light microscope. From the ability of such cells to contract in the presence of ATP it is proposed that these fibrils represent the elemental 'contractile units' of the vertebrate smooth muscle cell.

The fibrils

Gizzards from chicks 2–3 weeks old, were thinly sliced and then diced into small pieces in Solution 1, a modified Hanks solution (NaCl, 137; KCl, 5; Na_2HPO_4, 1.1; KH_2PO_4, 0.4; $NaHCO_3$, 4 and glucose, 5.5 mM, respectively; Mg^{2+} and Ca^{2+} ions absent) buffered additionally with PIPES 5 mM (*p*H 7.1 – 7.4) and with added EGTA (2 mM). (The effect of EGTA was two fold: it acted first as a relaxant so that cells could be obtained at an extended length and second as an effective pretreatment for subsequent cell dispersion by collagenase[21].) In some experiments EGTA was replaced by hyoscine (10^{-6} g ml^{-1}). After 2–4 h at room temperature the muscle pieces were then transferred to 10–20 ml of a solution containing 1 mg ml^{-1} collagenase (Worthington CLS or Sigma type I) of the same composition as above except that EGTA and phosphate were omitted and $CaCl_2$ (5 mM) added to activate the enzyme. With slow stirring at 37° C up to three cell collections were made at 1 h intervals, fresh reaction solution being added to the muscle pieces after each harvest. The cells were gently pelleted and washed and stored in Solution 1.

In the light microscope these preparations showed a mixture of whole cells and cell fragments (Fig. 1*a*). The striking feature of both of these components was that they nearly all exhibited, in phase contrast, a regular criss-cross pattern of fine fibrils within the cytoplasm (Fig. 2*a*). The fibrils normally averaged about 0.4 μm in width (range 0.3 – 0.5 μm) with an average separation between parallel neighbours of about 0.9 μm (range 0.8 – 1.1 μm).

Evidently, for the cell fragments, the intracellular contents were in direct contact with the bathing medium and from the following observations it was concluded that the whole cells had also been rendered permeable by the separation procedure. The intense reddish colour of gizzard which is due predominantly to an intracellular pigment and not to the vasculature supplying this tissue, is gradually lost from the muscle pieces and is absent from pellets of the isolated cells. In addition, placing the cells in solutions of widely varying osmolarities caused no change in their dimensions. These general conclusions about the permeability of gizzard cells isolated with collagenase are consistent with the recent results of Kominz and Gröschel-Stewart[22].

In conditions for which the sarcolemma is disrupted and ATP and other soluble components lost from the cell, striated muscle adopts the well known 'rigor' state in which myosin crossbridges are firmly bound to actin[23]. That the cells from gizzard had adopted a similar rigor-like state was confirmed by unsuccessful attempts to stimulate them with a microelectrode (experiments carried out in collaboration with Dr R. Purves). Pushing the cells along with the microelectrode tip revealed that they were extremely stiff, rigid structures.

Among the various other methods tried, glycerinated gizzard pieces (immersed in 50% glycerol, 80 mM KCl, 5mM PIPES, *p*H 6.5 with or without 0.5 mM dithiothreitol and stored at −20° C for 4–6 weeks) were dissociated in the same manner as described above for fresh muscle. Such experiments yielded, by comparison with fresh muscle, a rather low proportion of 'intact' cells. These cells, however, showed the same regular fibrillar pattern as illustrated in Fig. 2.

In general, the mitochondria and nuclei in the gizzard myofibrils were rather swollen and sometimes partly obscured the fibrillar organisation. These organelles could

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Fig. 1 Isolated cell preparations (phase contrast) *a*, Chick gizzard cells and cell fragments (× 165); *b*, cells separated from extended taenia coli muscle (0.02% Triton X–100); average cell length 450 μm (× 218); *c*, cells separated from short taenia coli muscle (0.02% Triton X–100), average cell length 115 μm × 297).

however, be removed by brief treatment of the cells with the nonionic detergent Triton X-100 (0.1% (v/v) in Solution 1) to yield 'clean' cells showing only the presence of the fine fibrils (Fig. 2*b*).

For adult guinea pig taenia coli and vas deferens, a different result was obtained when using essentially the same procedure for these muscles as employed for fresh gizzard. Collagenase treatment (carried out by necessity in the presence of Ca) caused the cells in these muscles to shorten, and, if the treatment was long enough, to round up. Such shortened cells could then be grown successfully in tissue culture indicating that, in contrast to the gizzard cells, their membranes had not been ruptured by the separation procedure. But because of their short and irregular form, these cells were unsuitable for structural studies.

Rupture of cell membranes

As one means of obtaining cells at their *in situ* length, methods were employed to deliberately rupture the cell membranes before or during collagenase treatment on the assumption that this would induce them directly into a rigor-like state, comparable to that found for gizzard. This was most readily achieved by the addition of Triton X-100 during incubation of the muscle in collagenase. Of the various concentrations tried, the most suitable were found to be 0.01 – 0.03% (v/v) for taenia coli and 0.1% (v/v) for vas deferens. It was also found more satisfactory to leave these muscles intact and tie them at the desired length to plastic plates during processing in collagenase. After collagenase treatment and after transfer to Solution 1, the cells could be teased from the muscle with fine needles. A more detailed account of the various methods used will be described elsewhere.

The type of preparation obtained in this way from taenia coli muscles extended to their maximal length in the presence of EGTA is shown in Fig. 1*b*. The cells are very long, straight and spindle-shaped and, although not visible in this micrograph, their nuclei are intact. The extended length of cells from taenia coli averaged 450 μm (77 cells—range 320–570 μm) with a corresponding nuclear length of between 30 μm and 50 μm.

At relatively low magnifications with phase contrast microscopy (×300–×500), the cell cytoplasm appears essentially homogeneous but using a ×100 oil immersion objective (total magnification about ×1250) a regular fibrillar pattern is clearly resolved. Observation by polarisation optics showed the fibrils to be strongly birefringent and in these conditions they are most clearly demonstrated (Figs 3*c* and 4).

The average width of the fibrils was about 0.3 μm (range 0.26–0.39 μm) and the centre-to-centre separation between parallel neighbours about 0.5 μm (range 0.45–0.58 μm). No abrupt discontinuities such as banding could be detected along a single fibril.

Taenia coli strips shorten spontaneously at 37° C in a normal balanced salt solution or in a similar medium such as used here for collagenase digestion. Treatment of such strips with collagenase together with Triton X-100 as above yields shortened cells (Fig. 1*c*). The average length of these shortened cells was about 115 μm (100 cells—range 65–160 μm). In these cells the fine fibrils were seen to be considerably more oblique (Fig. 3, *a* and *b* forming angles up to about 24° (at about 115 μm) with respect to the

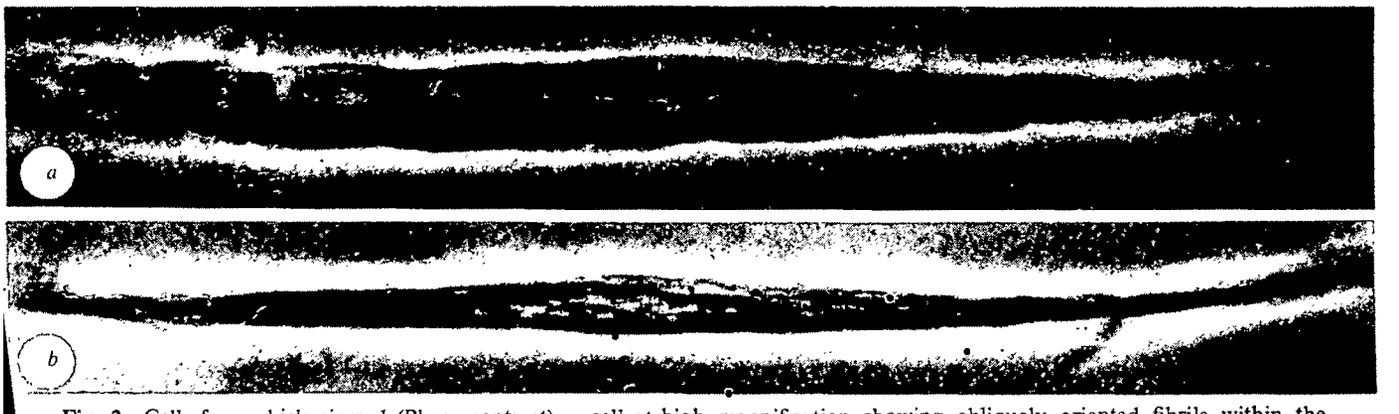


Fig. 2 Cells from chick gizzard (Phase contrast) *a*, cell at high magnification showing obliquely oriented fibrils within the cytoplasm (× 1246); *b*, gizzard cell from a preparation treated additionally with Triton X-100 (see text), again showing the fibrils (× 774).

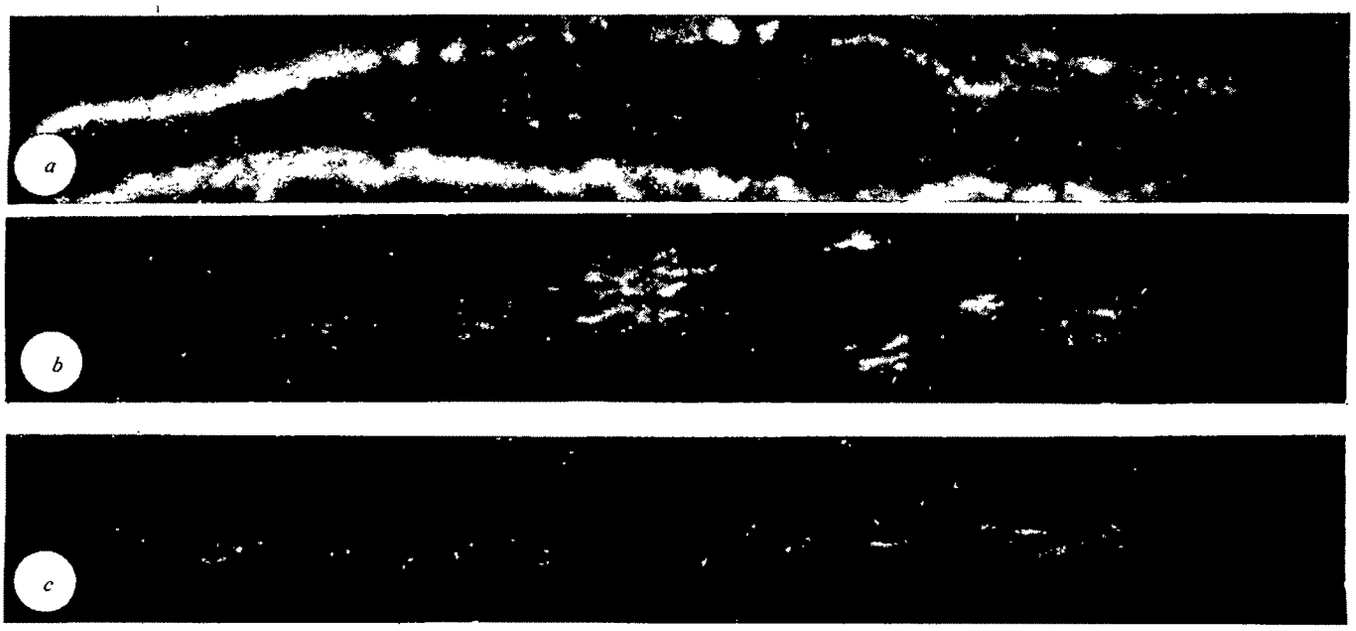


Fig. 3*b* and *c*, short cell from taenia coli (from a preparation such as shown in Fig. 1*c*). The cell length is about 90 μm. The fibrils are considerably more oblique with respect to the cell axis as compared to the extended cells (Figs 3*a* and Fig. 4). Polarisation optics and phase contrast respectively (× 1970). *c*, Part of an extended taenia coil cell (from a preparation such as shown in Fig. 1*b*) observed with polarisation optics. The fibrils are birefringent and subtend angles up to about 5° with respect to cell axis (× 2340).

cell axis, as compared to about 5° in the extended cells (Fig. 3*c*)). Cells could also be found which had contracted to as short as about 35 μm. These supercontracted cells had an irregular outline and their fibrils subtended angles up to 40° with respect to the cell axis. Cells from the vas deferens contained fibrils of similar dimensions to those observed in the taenia coli cells.

By comparison with the taenia coli and vas deferens, the fibrillar pattern in the gizzard cells was as already indicated, normally rather coarse and could be recognised at quite low optical magnifications. It was, however, not uncommon to find cells with a much finer fibrillar lattice with fibrils having a similar width to those observed in the taenia coli muscle. Some variation in fibril width was also recognised, but to a lesser extent, in cells from the taenia coli and vas deferens. The reason for these differences is at present unknown, but indicates that some subdivision or association of fibrils can occur.

Response to ATP

Efforts to cause gizzard and taenia coli cells to contract showed that they were capable of shortening in the presence of ATP. Cells suspended under a coverslip in 80 mM KCl, 4 mM $MgCl_2$, 5 mM PIPES, *p*H 6.5–6.8, could be induced to contract by the addition of 0.1–4 mM ATP in the same buffer to the coverslip edge.

The cells reacted to ATP by slowly shortening over a variable time period, but which was at the shortest in the order of about 1 min. Cells from taenia coli shortened to an average length of about 65 μm (40 cells, range 40–100 μm).

The changes in appearance of the cells that accompanied contraction as seen under phase contrast conditions were slightly variable and for taenia coli, two different responses could be recognised. In one case the initiation of contraction was accompanied by a roughening of the cell, apparently on its surface. Although this roughening effect tended to mask the internal details of the cell it seemed that there was also an associated change in the fibrils such that they could no longer be recognised, a situation which persisted throughout contraction.

In the second case, ATP caused a slight swelling of the cells, followed by slow shortening. In this situation the fibrils were seen to disperse and the cells became rather transparent. Roughening of the cell outline occurred as the cells approached their shortest length. For the gizzard preparations, the response was of the latter type described for taenia coli. After shortening had ceased, cells could then be found in which firils were again clearly visible and which then showed a considerable obliqueness with respect to the cell axis (that is, as in Fig. 3*a* and *b*).

In some cases, particularly with gizzard, the addition of ATP did not always produce contraction. With several preparations ATP addition caused some, or all, of the cells to swell up or to completely dissolve. Possible reasons for this variable response are the subject of further investigations. Of additional interest was that the inclusion of EGTA (2 mM) in the ATP-testing medium to chelate any contaminating calcium did not affect contraction in any way. This suggested that the Ca^{2+} regulatory mechanism of vertebrate smooth muscle[24,25] (R. D. Bremel and A. Sobieszek, M. K. Reedy, unpublished), had been lost or rendered ineffective during the cell preparation procedure.

Relaxed cells

By the use of ATP (0.5 mM) instead of Triton in the collagenase medium, extended cells were obtained from taenia coli which were similar in general appearance to the cells obtained from toad stomach by Bagby *et al.*[16] and Fay and Delise[17], being smoothly curved along most of their length. From their observed movement on a slide in a stream of bathing medium, these cells were seen to be very flexible, in contrast to their rigid counterparts obtained with Triton. Furthermore, they were found to shorten immediately on transfer from the EGTA-containing medium (Solution 1) to one in which EGTA was replaced by Ca^{2+}. This shortening could, alternatively, be observed under the microscope by the addition of the medium containing Ca^{2+} to the coverslip edge.

From these findings it was apparent that the cells obtained with ATP were essentially intact and could be considered, in the absence of Ca^{2+}, to be in a 'relaxed' state (The effects of ATP and calcium in this instance will be considered in detail elsewhere).

Under phase contrast or with polarisation optics, fibrils could not be recognised in these relaxed cells, neither could they be observed under phase contrast during contraction with Ca^{2+}.

The contractile apparatus

The present results represent the first demonstration in the light microscope of a regular fibrillar organisation within the vertebrate smooth muscle cell. Fibrils of similar dimension were often described in the early literature on smooth muscle[26–28] but were not seen to form any regular pattern within the cell. It is not unlikely that what the early histologists observed was a distorted arrangement of the fibrils described here.

From the ability of the cells to contract with ATP it is clear that the presence of the fibrils is not artifactual but represents a significant state of the contractile apparatus. As already indicated this state may be considered as essentially equivalent to rigor in striated muscle. In a very recent study, Kominz and Groschel-Stewart[22], using a similar technique, obtained isolated cells from gizzard which, by the use of an 'electric-sizing technique' were also shown to contract with ATP. But these authors did not report the presence of fibrils within the cells.

The most reasonable interpretation of the fibrillar pattern is that it reflects directly the gross organisation of the contractile apparatus. Thus, the contractile elements (the thick and thin filaments etc) are considered as being grouped into fibrillar units, that is the fibrils, within the cell. Furthermore, as there was no evidence for interconnections between the fibrils, it is most likely that each fibril represents a separate contractile unit, presumably attached at either end to the cell surface. These units are arranged obliquely across the cell and contraction is achieved by a shortening of the fibrils, which themselves, as a result of shortening, become progressively more angled with respect to the cell axis. Such changes in fibril orientation are fully consistent with the known decrease in birefringence of smooth muscle on shortening[29] and with the noted disorientation of myofilaments in the shortened cell[17].

The changes that occur when cells pass from rigor to the contracting state and which lead to a loss in visibility of the fibrils, clearly involve changes within the fibrils themselves. Myofibrils obtained from glycerinated striated muscle tend to swell when passing from rigor to the relaxed state[30]. A similar swelling response to ATP would cause the fibrils here to become both less refractile as well as closer neighbours, changes which could render them undetectable by light microscopy. From the results on 'relaxed cells' it is presumed that rather smaller changes occur in fibril dimensions between relaxation and contraction.

I suggest that the results presented here represent a significant step towards elucidating the organisation of the contractile apparatus of vertebrate smooth muscle. There is now strong evidence for an ordered grouping of the contractile elements into separate contractile units. On this basis, the problem of the mode of contraction in this muscle type reduces, in the first instance, to that for an individual fibril. Continuing studies are being aimed at further defining the functional significance of the fibrils and their detailed structural organisation.



Fig. 4 A group of extended taenia coli cells (as in Fig. 3*a*) showing the fibrils. Polarisation optics (× 1162).

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Note added in proof: By Triton treatment, in the presence of dithiothreitol, of 'relaxed' cells from taenia coli (produced with ATP) cells may be obtained which exhibit the same fibrillar organisation as cells rupture during collagenase treatment and which, further, show sensitivity to Ca^{2+} ion for contraction.

1 Lowy, Poulsen, F. R., and Vibert, P. P. *Nature*, **225**, 1053–1054 (1970).
2 Lowy, J., Vibert, P. J., Haselgrove, J. C., and Poulsen, F. R., *Phil. Trans. R. Soc.* **B265**, 191–196 (1973).
3 Cooke, P. H., and Fay, F. S., *J. Cell Biol.*, **52**, 105–116 (1972).
4 Devine, C. E., and Somlyo, A. P., *J. Cell Biol.* **49**, 636–649 (1971).
5 Garamvölgyi, N., Vizi, E. S., and Knoll, J., *J. ultrastruct. Res.*, **34**, 135–143 (1971).
6 Heumann, H.-G., *Cytobiologie*, **3**, 259–270 (1971).
7 Kelly, R. E., and Rice, R. V., *J. Cell Biol.*, **42**, 683–691 (1969).
8 Lowy, J., and Small, J. V., *Nature*, **227**, 46–51 (1970).
9 Pease, D. C., *J. ultrastruct. Res.*, **23**, 280–303 (1968).
10 Rice, R. V., Moses, J. A., McManus, G. M., Brady, A. C., and Blasik, L. M., *J. Cell Biol.*, **47**, 183–196 (1970).
11 Panner, B. J., and Honig, C. R., *J. Cell Biol.*, **35**, 303–321 (1967).
12 Shoenberg, C. F., *Phil. Trans. R. Soc.*, **B265**, 197–201 (1973).
13 Small, J. V., and Squire, J. M., *J. molec. Biol.*, **67**, 117–149 (1972).
14 Somlyo, A. P., Somlyo, A. V., Devine, C. E., and Rice, R. V., *Nature new Biol.*, **231**, 243–246 (1971).
15 Uehara, Y., Campbell, G. R., and Burnstock, G., *J. Cell Biol.*, **50**, 484–497 (1971).
16 Bagby, R. M., Young, A. M., Dotson, R. S., Fisher, B. A., and McKinnon, K., *Nature*, **234**, 351–352 (1971).
17 Fay, F. S., and Delise, C. M., *Proc. natn. Acad. Sci, U.S.A.*, **70**, 641–645 (1973).
18 Gibbons, B. H., and Gibbons, J. R., *J. Cell Biol.*, **54**, 75–97.
19 Mooseker, M. S., and Tilney, L. G., *J. Cell Biol.*, **56**, 13–26 (1973).
20 Naitoh, Y., and Kaneko, H., *Science*, **176**, 523–524 (1972).
21 Seglen, P. O., *Expl Cell Res.*, **74**, 450–454 (1972).
22 Kominz, D. R., and Groschel-Stewart, U., *J. Mechanochemistry Cell Motility*, **2** (in the press).
23 Huxley, H. E., in *The Cell* (edit. by Brachet, J., and Mirsky, A. E., **IV**, p. 365, (1960) Academic Press, New York and London.
24 Ebashi, S., Iwakura, H., Nakajima, H., Nakamura, R., and Ooi, Y., *Biochem. Z.*, **345**, 201-211 (1966).
25 Sparrow, M. P., Maxwell, L. C., Ruegg, J.C., and Bohr, D. F., *Am. J. Physiol.*, **219**, 1366–1372 (1970).
26 Heidenhain, M., *Ergebn. Anat. EntwGesch.*, **10**, 115 (1901).
27 McGill, C., *Am. J. Anat.*, **9**, 493–545 (1909).
28 Bloom, W. E., and Fawcett, D. W., *A Textbook of Histology*, 9th edition, 264 (Saunders, Philadelphia, 19xx).
29 Fischer, E., *J. cell. comp. Physiol.*, **23**, 113–130 (1944).
30 Bendall, J. R., *Muscles, Molecules and Movement*, 43 (Heinemann, London, 1969).

LETTERS TO NATURE

PHYSICAL SCIENCES

Relative-distance Machian theories

MACH's principle, in essence, requires that the dynamical law of the Universe be expressed ultimately in terms only of the relative distances between the observable entities in the universe. Here I propose a general framework for constructing theories that satisfy this postulate automatically. A simple model shows how the Newtonian world picture can be satisfactorily 'Machianised.' In principle, one could attempt to Machianise the post-1905 world picture along similar lines.

The key Kinematic concept is the relative configuration space (RCS) of the Universe. If, as in Newtonian theory, the Universe is assumed to consist of N particles in a fixed three-dimensional Euclidean space, the points of the RCS (the Newtonian RCS) are all the distinct relative configurations of these N particles. Many other RCSs are conceivable. For example, the points of an RCS could be all distinct three-geometries, and the RCS would then be the 'superspace' familiar in general relativity[1]. The general framework is not, therefore, tied to pre-1905 physics.

A kinematical history of the Universe (or RCS curve) is any continuous curve in the RCS. On any such curve, each point defines an 'instant of time' in the given history; the unfolding of time is but the fact of the universe's moving along some RCS curve (Leibniz's concept of time). Note that time — and therefore the possibility of defining relative velocities — is derived from within the RCS; note also the emphasis that is placed on the motion of the Universe as a whole.

If the dynamical law of the Universe is expressed solely in terms of elements taken from within the RCS, the theory must be Machian by construction. This is the general criterion that a Machian dynamics must satisfy. One possibility of realising such a dynamics is to seek a variational principle that contains only allowed kinematical elements and makes actually realised RCS curves extremal compared with neighbouring kinematically allowed curves. The initial conditions are specified by the direction of the RCS curve at a given point of the RCS. Boundary conditions do not arise.

In the case of the Newtonian RCS, space will play a geometrical but not dynamical role (in contrast to Newtonian mechanics). In a theory in which three-geometries constitute the points of the RCS, a Machian dynamics would automatically entail a variable three-geometry that unfolds in accordance with a Machian law. In this case, it would be more appropriate to speak of a relative-configuration (rather than relative-distance) Machian theory. Such an approach is not identical to the superspace approach to general relativity, in which it is assumed *a priori* that a sequence of three-geometries 'stacks' into a four-dimensional Riemannian space[1]. I see here the extraneous element (not taken from within the RCS) responsible for general relativity's failure to give an entirely satisfactory account of inertia.

It seems difficult to reconcile the universal time defined here with the local time of special relativity. But the flat-space approach to general relativity[2,3] has shown how the *a priori* assumed kinematical elements of a theory can, in fact, be rendered inobservable when a dynamical law of gravitation is imposed on a prescribed kinematical structure. In principle, something similar could happen here — a dynamical law, perhaps in conjunction with a particular class of plausible initial conditions, could lead to a dynamics in which only a local time can in fact be observed. The present approach reverses the priorities that Einstein had in mind when constructing general relativity. Instead of requiring special relativity to hold in the small under all conceivable circumstances in all possible universes (and hoping that this can somehow be reconciled with Mach's principle *via* the equivalence principle), the aim here is to construct theories that are Machian of necessity and then to see if any of these give rise to special relativity in the small in universes like ours.

In the case of a Newtonian RCS with N point particles $i = 1, \ldots, N$ of mass m_i (an intrinsic number associated with each particle; $\Sigma m_i = M$), the obvious allowed kinematical elements are the relative distances $r_{ij}(\lambda)$ between all pairs i and j of particles, and their derivatives $\dot{r}_{ij} = \mathrm{d}r_{ij}/\mathrm{d}\lambda$ with respect to an arbitrary time parameter λ along the RCS curve. The simplest nontrivial gravito-inertial dynamics is then defined by the Lagrange function

$$L = \psi\Gamma \tag{1}$$

where

$$\Gamma = \Big(\sum_{i<j} m_i m_j \dot{r}_{ij}^2\Big)^{1/2}, \quad i = 1, \ldots, N$$

$$\psi = \sum_{i<j} m_i m_j / r_{ij}$$

Equation (1) has a product form to ensure that $L\mathrm{d}\lambda$ is independent of the time parameter (a sum like $(\psi + \Gamma)\mathrm{d}\lambda$ would not be λ-independent).

The equations of motion deduced from (1) in three dimensions are too complicated to be readily comprehensible. Simple equations are obtained in one dimension by introducing Cartesian coordinates, whose origin may execute any suitably continuous motion whatsoever relative to the particles in their one-dimensional universe. The resulting Euler-Lagrange equations will describe the uniquely determined relative motion (which is all that is observable) in terms of the arbitrarily chosen coordinate system. Let $x_i(\lambda)$ be the coordinate of particle i; then L becomes

$$L = \psi\Big[\sum_{i<j} m_i m_j (\dot{x}_i^2 - 2\dot{x}_i\dot{x}_j + \dot{x}_j^2)\Big]^{1/2} \tag{2}$$

and the Euler-Lagrange equations

$$\frac{\mathrm{d}}{\mathrm{d}\lambda}(\partial L/\partial \dot{x}_i) = \delta L/\partial x_i$$

are

$$\frac{\mathrm{d}}{\mathrm{d}\lambda}\left\{\frac{\psi m_i}{\Gamma}\left[\sum_{j\neq i} m_j(x_i - x_j)\right]\right\} \equiv$$

$$\frac{\mathrm{d}}{\mathrm{d}\lambda}\left\{\frac{\psi m_i}{\Gamma}\left[\dot{x}_i(M - m_i) - \sum_{j\neq i} m_j\dot{x}_j\right]\right\} = \frac{\Gamma\partial\psi}{\partial x_i}$$

We can now specialise both λ, taking

$$\mathrm{d}\lambda = \mathrm{d}s = \Big(\sum_{i<j} m_i m_j \mathrm{d}r_{ij}^2\Big)^{1/2}, \quad \text{i.e. } \Gamma \equiv 1,$$

and the coordinate system, taking it such that

$$\Sigma m_i x_i = 0 \text{ (here } \mathrm{d}\lambda = \mathrm{d}s)$$

In these special frames, which are distinguished by the uniquely determined relative motion, the equations of motion simplify to

$$M\frac{\mathrm{d}}{\mathrm{d}s}(\psi m_i \dot{x}_i) = \frac{\partial\psi}{\partial x_i} \tag{3}$$

If only a few particles are present in the Universe, equations (3) clearly lead to motion that is very different from the Newtonian. On the other hand, in an environment broadly similar to ours (very many stars distributed uniformly over a large region), ψ

will be effectively constant and s will be indistinguishable from Newtonian time. Then equation (3) becomes

$$m_i \, d\dot{x}_i/dt = (1/M\psi)\partial\psi/\partial x_i \quad (4)$$

where $\gamma = 1/M\psi$ is a 'gravitational constant' that is determined by the actual distribution of matter in the Universe. A different model (in preparation) turns out to have more interesting properties, so I shall not attempt to estimate γ here. In the neighbourhood of the Sun, say, the motion predicted by equation (4) is essentially indistinguishable from the Newtonian if γ has the correct value.

This model is, I believe, interesting for several reasons. First, it explains inertia (resistance of a body to rectilinear acceleration relative to the remaining bodies in the Universe) solely in terms of relative distances and relative velocities and demonstrates that a complete dynamics can be expressed in such terms. For the discussion of how Mach's principle should be implemented, this may be important, for Einstein, in particular, doubted whether the Newtonian world picture could be Machianised in this manner[4]. It is particularly satisfying that preferred frames (the inertial frames of Newtonian theory) are distinguished by a motion whose dynamics is expressible solely in terms of relative distances.

Second, although two features of Newtonian gravitation are introduced *a priori* in equation (1) — all gravitational 'charges' are of the same sign and are proportional to the inertial 'masses' — the theory itself determines the actual strength of gravity (because a coupling constant cannot enter the product L) which must be attractive. (In the model in preparation, gravity and inertia are truly subsumed into a single mechanism and the correct order of magnitude of the gravitational constant is predicted.)

Third, whereas Newtonian theory and general relativity provide equations of motion for essentially inobservable quantities in the limit of universes containing only a few particles, no such problem arises in the present approach. If there is just one particle in the Universe, the RCS does not even exist, so there is no theory at all. If there are two particles, the RCS is the positive half axis, but no equation of motion can be formulated because equation (1) becomes trivial; the particles may approach or recede but no meaning can be attached to the rate at which this happens. Nontrivial dynamics first becomes possible when there are three particles, and even then the motion is quite unlike the Newtonian. Many particles in an isotropic background are required to build up the inertial forces as we know them in this universe.

Other forces could be included readily by adding a term linear in the r_{ij}s to L (gyroscopic-type forces) or multiplying L by another factor $\Phi = \Phi(r_{ij})$. Indeed, in an attempt to include electrostatics, take $L' = \Phi\Psi\Gamma$, where $\Phi = \sum_{i<j} e_i e_j / r_{ij}$, with $e_i = 1$ for $i = 1, \ldots N/2$ and $e_i = -1$ for $i = N/2 + 1, \ldots N$. Then the equations of motion corresponding to the conditions under which equation (4) hold are

$$m_i \, d\dot{x}_i/dt = (1/M\Psi)\,\partial\Psi/\partial x_i + (1/M\Phi)\,\partial\Phi/\partial x_i \quad (5)$$

As L' is a product, the relative strength of the new forces is again determined by the theory for each particular universe. In fact, if the particles in this case have equal masses and are distributed more or less uniformly, then $|\Psi/\Phi| \sim N$, because all the $\sim N^2$ terms in Ψ are positive, whereas there are just N more negative than positive terms in Φ. Thus, the electrostatic forces in equation (5) are $\sim N$ times stronger than the gravitational forces and like charges repel and opposites attract (provided $\Phi < 0$; I do not know whether distributions could exist for which $\Phi > 0$). As it happens there are $N \sim 10^{80}$ baryons in the observable Universe[5], whereas we require a strength ratio $\sim 10^{40}$, but the fact that we get the square of the right answer is perhaps an indication that product Lagrangians, which arise naturally (if not uniquely) in such a global approach to motion, could perhaps provide an explanation for the famous cosmic coincidences[5].

If the general framework is in fact the right approach to the problem of inertia, radical changes in theoretical physics are almost certainly necessary, and it is unlikely that the simple model presented here could bear any more resemblance to an ultimately successful theory than Bohr's original model of the hydrogen atom does to modern quantum field theory.

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[1] Wheeler, J. A., in: *Gravitation and Relativity* (edit. by Chiu, H. - Y. and Hoffmann, W. F.), (Benjamin, New York, 1966).
[2] Thirring, W. *Ann. Phys.*, **16**, 96 (1961).
[3] Mittelstaedt, P., and Barbour, J. B., *Z. Phys.*, **203**, 82 (1967).
[4] Einstein, A., *The Meaning of Relativity*, chapter 3 (Methuen, London, 1922).
[5] Bondi, H., *Cosmology* (Cambridge University Press, 1960).

Upper mass limit for quasar

The radio source 4C11.50=1548 + 115 is reported to be a pair of quasars only 5 arcs apart[1,2]. One quasar, 4C11.50a, has a redshift $z = 0.4359$ and is approximately 17 mag, the second quaser, 4C11.50b has a redshift $z = 1.901$ and is an object of 19 mag. I wish to point out that if the redshifts are cosmological the mass of 4C11.50a must be less than about $4 \times 10^{12} M_\odot$.

The light ray from the more distant b passes close to a. The impact parameter for the light ray depends somewhat on the cosmological model but may be taken as about 40 kpc ($H_o = 50$ km s^{-1} Mpc^{-1}). If a quasar has a mass as large as a giant galaxy then the gravitational deflection of the light ray may be noticeable. Gravitational imaging has been discussed at length by many authors but the discussion presented by Press and Gunn[3] is entirely adequate for this consideration. For simplicity, it can be assumed that both quasars are point masses and point light sources. When a light ray from a distant quasar b passes close to the quasar a then two images of b may be seen located on opposite sides of a. When a, b and an observer are exactly aligned, then b is seen as a ring with quasar a located at its centre. The angular radius of the ring, φ_o, is of the same order of magnitude as the deflection angle of the light ray. Therefore, there is an approximate relationship: $\varphi_o \approx 2'' \, (M_a/M_\odot)/(l/R_\odot) \approx 5 \times 10^{-8''} \, (M_a/M_\odot)/(l/pc)$, where M_a is the mass of the quasar a and l is the impact parameter. If a, b and the observer are not exactly aligned, then the observer sees two images of b. If the angular separations from a of the brighter and the fainter image are φ_1 and φ_2 respectively, then

$$\varphi_1 > \varphi_0 > \varphi_2$$

Combining the two relationships provide an estimate of the mass of a:

$$M_a/M_\odot < (\varphi_1/5 \times 10^{-8''})/(l/pc)$$

The observed image of 4C11.50b is almost certainly the brighter, as there is no other object visible within about 1 arc min on the opposite side of 4C11.50a (ref. 2). Inserting $\varphi_1 = 5''$, $l = 4 \times 10^4$ pc into the latter formula gives an upper mass limit for the quasar 4C11.50a:

$$M_a < 4 \times 10^{12} M_\odot$$

This mass limit is not excessively large. Combining the observed luminosities for cluster galaxies of the first rank[4] with the luminosity-to-mass ratio for ellipticals[5] gives masses of $10^{13} M_\odot$.

If it were possible to detect the second image of 4C11.50b the mass of 4C11.50a could be calculated. For the point-mass scatterer there is a simple relationship between the luminosity ratio and the separation ratio of the two images[3]. If the observed ratios did not agree with those predicted for the point

masses then information could be obtained about the mass distribution around the quasar 4C11.50a. If quasars are active nuclei of giant galaxies[6] the existing observations indicate that the total mass within 40 kpc of the nucleus of 4C11.50a is less than about $4 \times 10^{12}\ M_{\odot}$. There is a weak emission in 4C11.50a at the same wavelength as a strong CIV emission in 4C11.50b (ref. 1). It may be because of the unresolved second image of 4C11.50b.

I thank Professor W. Zonn for informing me about the discovery of a close pair of quasars.

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[1] Wampler, E. J., Baldwin, J. A., Burke, W. L., Robinson, L. B., and Hazard, C., *Nature*, **246**, 203 (1973).
[2] Hazard, C., Jauncey, D. L., Sargent, W. L. W., Baldwin, J. A., and Wampler, E. J., *Nature*, **246**, 207 (1973).
[3] Press, W. H., and Gunn, J. E., *Astrophys. J.*, **185**, 397 (1973).
[4] Sandage, A., *Astrophys. J.*, **178**, 1 (1972).
[5] Page, T., *Astrophys. J.*, **136**, 685 (1962).
[6] Kristian, J., *Astrophys. J. Lett.*, **179**, L61 (1973).

Transit of Mercury across the solar disk

THE transit of Mercury across the solar disk on May 9, 1970 (4 h 20 min 30 s – 11 h 12 min 0 s UT), was observed by recording the radio emission at a wavelength of 8 mm using a 22-m paraboloid radio telescope[1]. The pencil beam of the antenna is of width $D=1'.6$ and the sensitivity of the radiometer $\simeq 1.5$ K. The antenna was steered so that Mercury was in the centre of the beam. Mercury obscures the inhomogeneities of radio emission when passing across the solar disk and some fluctuations of the flux of radio emission can occur. The characteristic time of these fluctuations and their amplitude characterise the sizes and temperatures of condensations respectively. In Fig. 1 we show a sample of original (averaged over 15 s) records of fluctuations during the passage of Mercury across the relatively active regions on the disk. Similar records, but with slightly slower fluctuations of smaller amplitude have been obtained for quiet regions of the disk. The auto-correlation curves for active (A) and quiet (Q) regions (Fig. 2) reveal details of the nature of the emission.

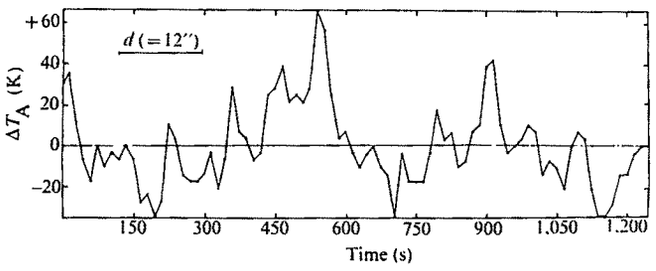


Fig. 1 Fluctuations of radio noise observed as Mercury transits the Sun.

For Q regions the characteristic sizes corresponding to maxima are 3″.4 for the lag $m=0$ and 32″.2 and 41″.3 for the lags $m=44$ and $m=53$. We note that the characteristic size l of condensations is approximately determined from the relations: $l=V\tau_m/2$, if $d>1$, and $l=V\tau_m-d$ when $d<1$, where $\tau_m=m\Delta t$, ($\Delta t=15$ s), v is the velocity of the transit of Mercury across the disk ($=0''.067\ s^{-1}$) and d is the size of Mercury (12″). (We assume, of course, that the size of condensations of the order of the spacing between them).

For A regions the corresponding maxima appear at lags $m=0$, 5, 29, and 36 which correspond to the sizes 1″.8, 2″.51, 17″.4, and 24″.2, respectively. Condensations in A regions are a little more compact than in Q regions. The smallest condensations in both regions have a size of 2″ to 3″ which coincides with the size of smallest 'elements' of the general magnetic field[2]. The group of larger condensations (from 17″ to 41″), although in the size range characteristic of larger 'elements' of general magnetic fields, reflects, we believe, the characteristic sizes of chromospheric supergranules (10^4 to 3×10^4 km[3]).

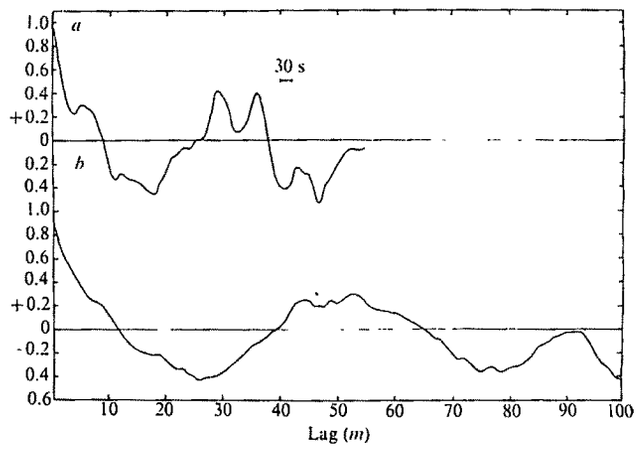


Fig. 2 Autocorrelation curves for: *a*, A region; *b*, Q region.

The characteristic amplitude of fluctuations or excess of brightness temperature ΔT_B over the background (temperature $T_{B0}=8{,}000$ K for $\lambda=8$ mm) determined as the r.m.s. of all fluctuations (deviations from the mean) depends on the ratio d/l and the value of D, and also on the character of eclipse ('central' or 'partial') of condensations by Mercury. The corresponding approximate formulae for excess of antenna temperature ΔT_A are $\Delta T_A \simeq (d^2/D^2)\ \Delta T_B$ when $d<l$ and

$$\Delta T_A \simeq 2\,(d/l)/(D/l)^2 \Delta T_B$$

when $d>l$. The brightness temperature excess ΔT_B for small condensations ($l<3''$) is $\simeq 5{,}000$ K in Q regions and 8,950 and 6,420 K in two observed A regions. For large condensations connected with supergranules the corresponding temperatures ΔT_B are 2,830 K for Q regions and 2,690 for A regions. As the background temperature is 8,000 K the true brightness temperature 8,000 $+\Delta T_B$ is 13,000 K for small condensations (Q regions) and is 14,000–17,000 K in A regions. The values are characteristic of upper chromospheric temperatures and transition layer temperatures. For supergranular structures the true T_B is about 10,700 – 10,800 K in A and Q regions, typical of the lower chromosphere. In other words small condensations, which are more compact in A regions, are appreciably hotter than supergranular structures; the latter are practically of the same temperature in both regions. Condensations of radio emission do not form a continuous sequence in the regions examined and all are concentrated around characteristic size of granules and supergranules.

Could the effect observed be due to the time variations of radio emission from the whole area D. To exclude this possibility scans along the same path were repeated without Mercury. These records did not show appreciable fluctuations similar to that of Fig. 1. Moreover we have examined the cross correlation of these fluctuations with those without Mercury; the cross-correlation coefficient never reached 0·05. The fluctuations we consider here cannot therefore, be

connected with possible time variations of the radio emission of the Sun as a whole[4].

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[1] Efanov, V., Moiseev, I., and Severny, A., *Int. astr. Union Symp. No.* 35 (edit. by Kiepenheuer, K. O.), 588 (1968).
[2] Severny, A., *Q. Jl R. astr. Soc.*, **12**, 363 (1971).
[3] Leighton, R., Noyes, R., and Simon, G., *Astrophys. J.*, **135**, 471 (1962).
[4] Durasova, M., Kobrin, M., Losovsky, J., Chandajev, A., and Judin, O., *Astr. Tsirk. Byuro astr. Soobshch.*, **531**, (1969)

Ionisation ledges in the equatorial ionosphere

A CUSP formation on topside ionograms recorded at equatorial latitudes by means of the satellite Alouette I, has been discussed by earlier workers[1, 2]. The cusps are associated with an ionisation ledge lying along the magnetic field line passing through the peaks of the equatorial anomaly.

We here describe observations of cusps associated with echoes reflected from a feature aligned with the field below the anomaly field line and we suggest how the ledge of ionisation in the equatorial topside ionosphere may be formed.

On ISIS-2 ionograms from the dip latitude region 9°N to 7.5°S, recorded at Ahmedabad on October 6, 1972. The ionograms were reduced to electron density profiles by the 'parabolic in log N method'[3].

Figure 1 shows one of the sequence of ionograms exhibiting a cusp, and Fig. 2 shows the corresponding electron density profile. The ledge, which is an enhancement of the electron density over the 'normal' level shown by the dashed line is clearly evident. The horizontal arrow (Fig. 2) indicates the height of maximum percentage deviation of the electron density in the ledge region and the tip of the vertical arrow indicates the height from which the cusp echoes were reflected. The cusp echoes were returned from below the ledge.

Figure 3 shows the heights of constant electron density contours in an altitude against dip latitude diagram. The equatorial anomaly is well developed and the dashed line in the diagram shows the magnetic field line passing through the peaks of the equatorial anomaly. The height of the anomaly field line over the equator is 860 km. The heights at which the maximum percentage deviation of the ledge electron density

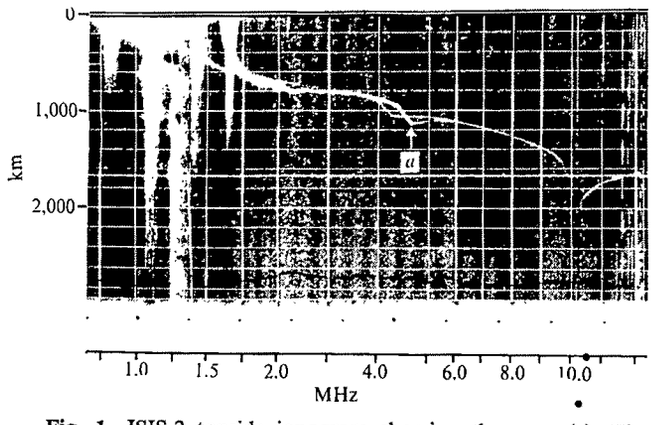


Fig. 1 ISIS-2 topside ionogram showing the cusp (*a*). The x axis is the frequency (MHz), the y axis is the virtual depth (km) below the satellite.

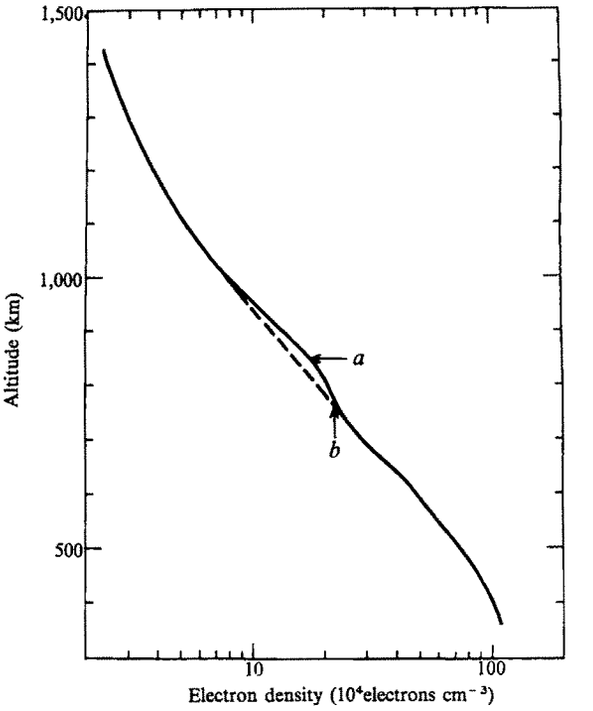


Fig. 2 The electron density profile obtained from the ionogram shown in Fig. 1. recorded October 6, 1972 (UT, 08 h 00 min 32s; LT 1240). Longitude, 70° E; latitude, 7.6° N; dip latitude, —1.42°. *a*, Height of maximum deviation of electron density; *b*, altitude of echoes associated with cusps.

occurred at different dip latitudes are shown by squares in the diagram and they fall on or closely to the anomaly field line. The maximum percentage deviation was observed to increase towards the dip equator, reaching a maximum value of 16%.

The circles in Fig. 3 indicate the heights at which the cusp echoes were reflected. The locus of the 'cusp points' are aligned along a magnetic field line crossing the magnetic equator 775 km above the ground, that is, below the anomaly field line. The ionograms were accurately scaled and the error in evaluating the true height of the cusp points is not more than 15 km. A possible mechanism leading to the formation of a ledge of ionisation such as that observed, can be described.

Recent observations of the neutral densities of molecular

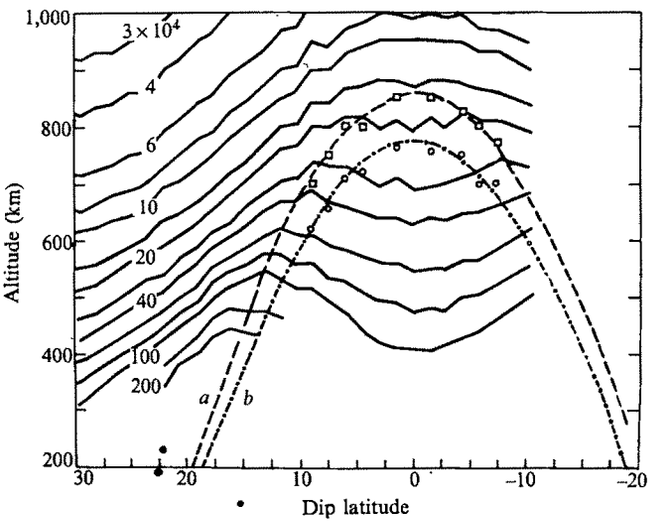


Fig. 3 Latitudinal variation of the height of contours of constant electron density. □, Ledge maxima; ○, heights at which echoes associated with the cusps were reflected. *a*, Locus of anomoly peak; *b*, locus of cusps.

nitrogen and atomic oxygen using a mass spectrometer on the satellite, OGO 6, reveal an equatorial anomaly at an altitude of about 450 km, similar to that in the equatorial ionosphere; density maxima (crests) occur on either side of a minimum (trough) at the magnetic equator. Typical values of the differences between the densities of N_2 and O at the trough from those at the crests, observed at $\pm 20°$ magnetic latitude, are 20% and 10% respectively. It seems likely that the neutral density enhancements will extend above 450 km, along a magnetic field line in a manner similar to the ionisation enhancements, and will merge above the magnetic equator. The neutral temperatures derived from the observed densities show enhancements of 3 to 4% at the neutral density crests.

The existence of enhancements aligned with the field in the neutral density will decrease the diffusion of plasma along the field lines involved. Ionisation in the equatorial ionosphere is lifted vertically during the day by the $\mathbf{E} \times \mathbf{B}$ effect, where $\mathbf{E}$ and $\mathbf{B}$ are the electric and magnetic fields respectively, and diffuses downwards along the magnetic field lines with an ambipolar diffusion coefficient[4]:

$$D_a = [(T_e + T_i)/T_i]\,[7 \times 10^{18}/n(\mathrm{O})]\,[T_n/1{,}000]^{\frac{1}{2}}\ \mathrm{cm^2\,s^{-1}} \quad (1)$$

where T_n, T_i and T_e are the neutral, ion and electron temperatures respectively, and n(O) is the number density of atomic oxygen. The value of D_a increases exponentially with altitude as the neutral density, which is predominantly atomic oxygen in the topside ionosphere, decreases, and can be represented by $D_a = D_0 e^z$ where z is measured in units of the atmospheric scale height H and D_0 is the value of the diffusion coefficient at any known lower level.

The ionisation velocity, V, along the field line, resulting from diffusion in the absence of temperature gradients and winds, is given by[5]

$$-V = (D_a/H)\,[(1/N)\,(\mathrm{d}N/\mathrm{d}s) - (1/2)\sin I] \quad (2)$$

where I is the dip angle and s the distance along the field line measured in units of H and taken positive in the northward direction, so that $-\mathrm{d}z/\mathrm{d}s = \sin I$.

Equation 2 shows that at a given dip angle V is directly proportional to D_a which varies inversely with the density of neutral atomic oxygen (equation 1). Away from the equator neutral density enhancements occur along the ionisation anomaly field line. Along this field line the diffusion coefficient, and therefore the diffusion velocity parallel to the field line, will be decreased by about 10% because of the enhanced O concentration. Any increase in neutral temperature along the same field line will increase the scale height. This further decreases the diffusion velocity. We suggest that these processes will result in the enhancement of the electron density around the field line on which the neutral density is greatest. The field line with which the ionogram cusps are associated may thus occur at the beginning of the sharp rise in the neutral density.

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[1] Lockwood, G. E. K., and Nelms, G. L., *J. atmos. terr. Phys.*, **26**, 569 (1964).
[2] King, J. W., Smith, P. A., Eccles, D., Fooks. G. F., and Helm, H., *Proc. R. Soc.*, **A281**, 464 (1964).
[3] Jackson. J. E., *Proc. IEEE*, **57**, 960 (1969).
[4] Hedin, A. E., and Mayr, H. G., *J. geophys. Res.*, **78**, 1688 (1973).
[5] Rishbeth, H., and Garriott, O. K., *Introduction to Ionospheric Physics*, 144, (Academic Press, New York and London 1969).

Lightning incidence in Britain and the solar cycle

The existence of a correlation between solar activity and meteorological or climatological parameters seems no longer in doubt and a credible explanation has recently been suggested[1]. It has also been shown that the mean monthly or yearly number of sunspots gives a crude but effective index of the solar activity and that the most marked variation is cyclic with a period of approximately 11 yr.

It has been deduced from lightning-counter records (F. Popolansky's, unpublished) and from analysis of faults caused by lightning in overhead electricity supply systems[2,3] that the mean incidence of lightning (usually expressed as the number of flashes per unit area per year) is proportional to the mean number of annual thunderstorm days recorded by observers, raised to the power 1.9. The data from 40 Meteorological Office observers chosen to be representative of Britain were used to obtain means of the annual number of thunderstorm days for the years 1930 to 1973, and the square of the mean for each year was taken as a rough index of the annual incidence of lightning. The 5-yr running means of both the annual values of this index and the mean annual sunspot numbers are plotted in Fig. 1. In spite of the year-to-year variations in

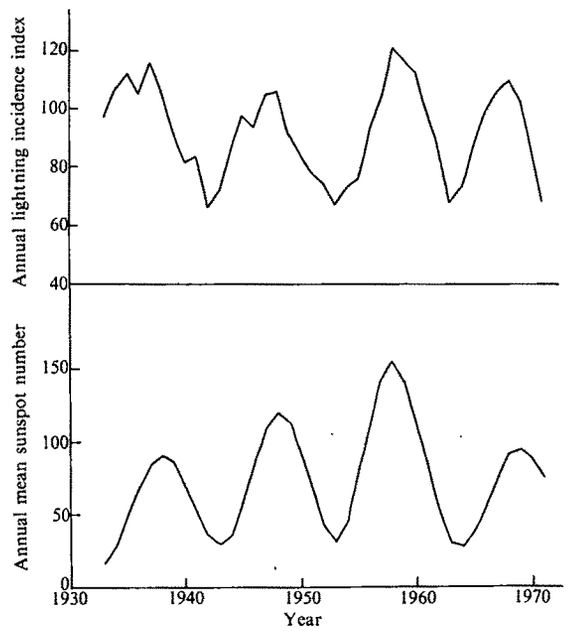


Fig. 1 Annual variation of: *a*, 5-yr running means of lightning incidence; *b*, sunspot number.

the incidence of lightning, there is an underlying cyclic variation with a period of about 11 yr which is in phase with the solar cycle and has an amplitude of $\pm$ 30% of the mean. The correlation obtained between these smoothed data is 0.8, a result of statistical significance despite the smoothing. During the period 1964 to 1972 the trend in faults attributed to lightning on the British electricity distribution network also followed the lightning incidence/sunspot cycles but these fault data are too few to be statistically significant.

The conclusion that the underlying trend in lightning activity is related to the solar cycle is not only of interest to the atmospheric physicist but also to those electricity supply and civil engineers who are concerned with long-term planning of lightning protection. Of more immediate

interest is the fact that mean lightning incidence in Britain reached a minimum in 1973 and, neglecting year-to-year variations, can be expected to reach a peak at the peak of the next solar cycle with a magnitude of about twice the 1973 level.

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[1] King, J. W., *Nature*, **245**, 443–446 (1973).
[2] Forrest, J. S., *Q. J. L. R. meteor. Soc.*, **329**, 277–286 (1950).
[3] Stringfellow, M. F., *Electricity Council Report*, No. ECRC/R602 (1973).

Major and trace elements in the Allende meteorite

WE have determined major and trace element abundances for seven Ca,Al-rich aggregates, ten melilite chondrules, and an olivine chondrule and two olivine-rich aggregates from the Allende meteorite, a type III carbonaceous chondrite. Major element abundances were determined with the electron microprobe technique described by Reed and Ware[1]. An MS7 spark source mass spectrometer was used for the determination of trace element abundances. The technique used was that described by Taylor[2] but with improved and extended data reduction techniques. The precision is about ±5% for the rare earth elements (REEs) and the accuracy about ±10%, but varies somewhat, being poorer for elements below mass number 105 than for those with higher mass numbers.

The Allende samples can be grouped in various ways using chemical, mineralogical and textural parameters. Our results distinguish four distinct groups, as follows: group I, melilite-rich chondrules; group II, CaAl-rich aggregates with fractionated chondrite-normalised REE abundance patterns; groups III, Ca,Al-rich aggregates with unfractionated chondrite-normalised REE abundance patterns, except for negative Eu and Yb anomalies; group IV, olivine-rich chondrules and aggregates.

The range and average of the elements studied for each group and for the bulk meteorite are given in Table 1. Chondrite-normalised REE abundances of the average for each group and for the bulk meteorite are shown in Fig. 1.

The melilite-rich chondrules in group I are spherical aggregates of melilite, Ti-rich fassaite, spinel and anorthite with a coarsely crystalline igneous texture, and have high Al_2O_3, high CaO and an unfractionated REE pattern averaging 10–15 times chondritic abundances but with a small positive Eu anomaly. In contrast the olivine-rich chondrules and aggregates of group IV have an REE abundance pattern averaging three times that of the chondrites, with possibly a slight positive Ce anomaly and a slight negative Eu anomaly. The major and trace element abundances of these two chemically distinct types of chondrules indicate that the melilite chondrules may represent the initial condensates from a cooling gas of solar composition and the olivine chondrules later low-temperature condensates. (Condensation sequences for a cooling gas of solar composition have been calculated by Lord[3], Larimer[4] and Grossman[5].)

The fine-grained Ca,Al-rich aggregates, composed mainly of spinel, fassaite, melilite and/or grossular, and minor amounts of nepheline and sodalite, can be divided into two groups on the basis of their trace element abundances. Two of the seven examined, classified as group III, have relatively unfractionated REE abundances at about 20 times those of chondrites, but with large negative Eu and Yb anomalies. Even though the chemistry of these aggregates, like that of the melilite chondrules, suggests they would be the first condensate from a cooling gas of solar composition,

Table 1 Chemical composition (oxides in weight per cent, elements in p.p.m.) for selected materials from the Allende metoerite.

	Group I (10)		Group II (5)		Group III (2)		Group IV (3)		
	Range	Average	Range	Average	Range	Average	Range	Average	Bulk
SiO_2	24.9–35.1	30.0	17.7–34.5	28.9	20.5,24.5	22.5	38.8–45.2	42.0	34.28
TiO_2	1.0–1.5	1.2	0.30–1.1	0.64	0.74,0.96	0.85	0.17–0.30	0.22	0.15
Al_2O_3	21.5–32.6	28.6	19.3–44.0	30.3	43.3,40.3	41.8	3.4–9.4	5.7	3.29
FeO	0.4–3.1	1.7	1.3–12.2	7.1	3.7,3.9	3.8	9.6–11.0	10.5	27.15
MgO	6.9–12.6	9.9	10.1–16.6	12.8	15.4,13.6	14.5	23.5–36.6	31.8	24.63
CaO	23.3–33.3	27.7	8.6–24.0	13.2	11.4,11.4	11.4	2.7–8.3	4.7	2.59
Na_2O	0.11–0.92	0.38	0.39–4.7	3.1	1.3,1.8	1.6	0.45–2.7	1.5	0.46
K_2O	0.00–0.08	0.02	0.02–0.26	0.12	0.04,0.05	0.05	0.03–0.20	0.09	0.03
Rb	0.05–0.97	0.33	0.42–6.1	3.2	1.2,1.1	1.2	1.1–20	6.9	1.3
Sr	110–200	136	18–73	37	28,43	35	13–20	18	12
Y	15–29	23	0.86–3.0	1.7	36,41	39	1.6–4.3	3.5	3.1
Zr	35–72	56	1.9–7.2	4.1	99,113	106	3.8–11	8.4	10
Nb	1.9–5.1	3.7	0.94–2.6	1.7	1.9,9.1	5.5	0.54–0.89	0.77	0.65
Mo	7.4–14	11	<0.7	<0.7	4.2,9.8	7.0	0.9–1.1	1.0	1.5
Ba	23–104	57	6.2–22	15	22,10	16	5.3–9.8	9.0	4.1
La	3.2–4.7	4.1	3.7–13	7.8	6.5,5.8	6.2	0.79–1.9	1.2	0.51
Ce	8.2–12	11	10–31	19	11,15	13	2.5–4.8	4.0	1 3
Pr	1.2–1.9	1.6	1.6–4.7	3.0	2.2,2.1	2.2	0.32–0.72	0.45	0.21
Nd	5.4–9.0	6.8	6.2–20	13	11,9.9	10	1.6–3.1	2.1	0.97
Sm	1.7–3.2	2.2	2.2–6.2	4.0	3.1,3.1	3.1	0.49–1.1	0.73	0.34
Eu	0.76–1.3	1.0	0.17–0.62	0.28	0.25,0.36	0.31	0.13–0.19	0.16	0.10
Gd	2.2–4.0	3.1	0.84–3.8	2.3	4.5,4.6	4.6	0.46–1.1	0.75	0.42
Tb	0.41–0.71	0.53	0.12–0.52	0.31	0.81,0.82	0.82	0.08–0.17	0.12	0.08
Dy	2.5–4.8	3.7	0.66–2.6	1.6	6.5,6.5	6.5	0.53–0.92	0.75	0.42
Ho	0.59–1.2	0.94	0.07–0.21	0.14	2.3,1.7	2.0	0.13–0.19	0.16	0.10
Er	1.9–3.8	2.8	0.15–0.43	0.26	6.0,4.9	5.5	0.38–0.60	0.47	0.29
Tm	0.30–0.46	0.38	<0.2	<0.2	0.74,0.71	0.73	<0.2	<0.2	0.05
Yb	2.1–3.4	2.6	0.37–1.6	0.81	0.74,1.9	1.3	0.47–0.70	0.60	0.31
Hf	0.83–1.5	1.3	<0.3	<0.3	2.2,2.5	2.4	<0.3	<0.3	0.2
Th	0.34–0.61	0.45	0.14–0.72	0.46	0.70,0.72	0.71	0.06–0.17	0.12	0.07

The numbers in brackets after the groups are the number of specimens analysed: the figures for the bulk composition are from a compilation by E. Jarosewich (unpublished).

it is apparent from their fine-grained texture and the presence of grossular that these aggregates cooled rapidly, certainly more rapidly than the primeval solar nebula. It would seem therefore that the melilite chondrules and the aggregates of Group III have been subjected to similar high energy events, which vaporised already condensed material, the chondrules cooling from a gas through the liquid state to the solid whereas the aggregates, because of conditions of lower pressure, cooled directly from a gas to a fine-grained solid.

The remaining five Ca,Al-rich aggregates, however, classified as Group II, exhibit remarkable REE abundance patterns. Although the light REE (La-Sm) show similar abundances in groups II and III, group II aggregates have rapidly diminishing abundances of the heavier REEs. Superimposed upon this are a marked negative Eu anomaly and a large positive Yb anomaly. Group II aggregates are also depleted in Y, Zr, Mo and possibly Nb with respect to those of group III. Tanaka and Masuda[6] have described an Allende aggregate with a group II REE pattern. Like the group III Ca,Al-rich aggregates, the aggregates of group II have a fine-grained texture and contain metastable minerals, indicating they cooled rapidly, probably directly from the gas phase to a fine-grained solid.

It is very difficult to produce the fractionated REE abundance patterns of group II either by fractional condensation or evaporation at a suitable temperature range based solely on the boiling points of the REE metals or any of their compounds for which there are sufficient data. For example, even assuming that Eu and Yb exist in the divalent state and are located in different lattice sites to the remaining trivalent REEs, Sm and Dy are not depleted relative to the other REEs yet have much lower boiling points. It is possible that a property of the REEs for which we, as yet, do not have sufficient information might, under certain conditions, give rise to the fractionated REE patterns observed.

An intriguing alternative is that the group II aggregates may contain a component which has had a different history of nucleosynthesis to other matter in the Solar System. The light REEs are produced by an *s* process inside stars whereas the heavy REEs are produced predominantly by the *r* process during supernova explosions. Clayton *et al.*[7] on the basis of oxygen isotope anomalies, have postulated a separate history of nucleosynthesis for some of the Ca, Al-rich material of the Allende meteorite. They found enrichment of ^{16}O in all anhydrous minerals in C2 and C3 meteorites, however, whereas we find depletion of heavy REEs only in group II Allende aggregates, and thus there seems to be no correlation between these two anomalies. Critical examination of our data on isotopic abundances of the REE shows no indication of preferential depletion in *r*-process nuclides, and Gray *et al.*[8] found no evidence of lack of isotopic homogeneity in Rb and Sr execpt for variation in ^{87}Sr abundance produced by radioactive decay of ^{87}Rb.

The differences in minor and trace element distributions evidently reflect differing conditions of genesis of these components. The melilite chondrules seem to represent material segregated at an early high temperature stage; the olivine chondrules and aggregates at a slightly later stage, and the aggregates of groups II and III may represent residual material which condensed after the previous components had segregated from the initial material. The complex pattern of trace element distributions in these Allende components indicates a complex history of formation of this meteorite.

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1 Reed, S. J. B., and Ware, N. G., *X-Ray Spectrom.*, **2**, 69 (1972).
2 Taylor, S. R., *Geochim. cosmochim. Acta*, **35**, 1187 (1971).
3 Lord, H. C., *Icarus*, **4**, 279 (1965).
4 Larimer, J. W., *Geochim. cosmochim. Acta*, **31**, 1215 (1967).
5 Grossman, L., *Geochim. cosmochim. Acta*, **36**, 579 (1972).
6 Tanaka, T., and Masuda, A., *Icarus*, **19**, 523 (1973).
7 Clayton, R. N., Grossman L., and Mayeda, T. K., *Science*, **182**, 485 (1973).
8 Gray, C. M., Papanastassiou, D. A., and Wasserburg, G. J., *Icarus*, **20**, 213 (1973).

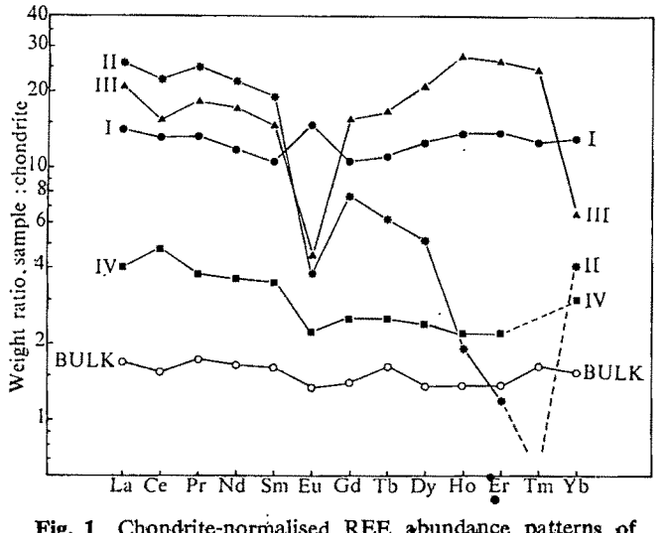


Fig. 1 Chondrite-normalised REE abundance patterns of the average for each group and of a bulk sample of the Allende meteorite. Group I: melilite-rich chondrules; groups II and III: Ca,Al-rich aggregates; group IV: olivine-rich chondrules and aggregates. Note the Eu and Yb abnormalities in groups II and III.

Chandler wobble and viscosity in the Earth's core

JEFFREYS[1], and Munk and MacDonald[2], have stated that viscosity of the core cannot account for the observed damping of the Chandler wobble. This statement may be in error.

Jeffreys's statement is based on a simple algebraic error on page 257 of the fourth edition of *The Earth*[1]. He starts from the assumption that there exists, at the core–mantle boundary, an Ekman boundary layer of thickness $(\nu/\omega)^{1/2}$, where ν is the kinematic viscosity and ω the angular velocity of rotation of the earth. If V is the differential velocity between the core and the mantle, the rate of dissipation for unit area is of the order of $\rho V^2 (\nu\omega)^{1/2}$, where ρ is the density. If α is the small angle between the axes of rotation of core and mantle, $V = a\alpha\omega$, where a is the radius of the core. The total rate of dissipation, F, is then

$$F = 4\pi\rho a^4\omega^2\alpha^2(\nu\omega)^{1/2} \quad (1)$$

This is Jeffreys's equation (13) except for the factor 4π and his inadvertent writing of ω^3 instead of ω^2. This error is unfortunately carried into the numerical result on the following line (Jeffreys's equation (14)); the damping time, which is proportional to $1/F$, is consequently too long.

Munk and MacDonald[2] (page 170) proceed in essentially the same way as Jeffreys, except that they write $V = \alpha a \sigma$, substituting the frequency, σ, of the Chandler wobble for ω. The substitution is hard to justify. Munk and MacDonald justify it with Jeffreys and Vicente's[3] demonstration that, to a first approximation, the core remains motionless, which is, however, precisely the reason that Jeffreys[1] adopted $V = \alpha a \omega$.

Returning to Jeffreys' corrected expression (1), and comparing F with the wobble energy, E_w of the mantle:

$$E_w = \frac{1}{2} A_m \frac{(C_m - A_m)}{C_m} \omega^2 \alpha^2 \quad (2)$$

where A_m and C_m are the principal moments of inertia of the mantle. The time, τ, needed to reduce the energy by $1/e$ is

$$\tau = \frac{E_w}{F} = \frac{A_m}{8\pi\rho a^4} \frac{(C_m - A_m)}{C_m} (\nu\omega)^{-\frac{1}{2}} \quad (3)$$

and the time needed to reduce the amplitude in the same ratio is 2τ. Observations indicate that $2\tau \sim 30$ yr. Equation 3 then gives for the kinematic viscosity of the core

$$\nu = 0.026 \text{ cm}^2 \text{ s}^{-1} \quad (4)$$

Leppaluoto[4], using the significant-structure theory of liquids, finds that the dynamic viscosity, $\eta = \nu\rho$, of liquid iron at its melting point and at the pressure of the core–mantle interface (1.4 Mbar) probably lies between 0·1, and 0·5 poise. The density at the top of the core is approximately 10 g cm^{-3}, and the corresponding limits on ν are therefore 0.01 and 0.05 cm^2 s^{-1}, which nicely bracket the value of equation 4, even though Leppaluoto's values should be corrected for the presence in the core of elements other than iron (for example, sulphur), and for temperature, which, at the core–mantle boundary, is unlikely to be precisely the melting temperature of pure iron. Gans[5], using somewhat questionable approximations, suggests $\nu = 0.066$ cm^2 s^{-1} as a typical value of the viscosity of the liquid core at the inner-core boundary.

The third (1952) edition of *The Earth* does not have the ω^3 error[6]. Assuming $\nu = 5 \times 10^7$ cm^2 s^{-1}, Jeffreys consequently found a damping time that was too short and concluded that the viscosity of the core must be less than 10^5 cm^2 s^{-1} to account for the observed damping. He did not conclude at that time that viscous damping of the Chandler wobble is impossible, as indeed it does not seem to be.

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[1] Jeffreys, H., *The Earth*, fourth ed. (Cambridge University Press, Cambridge, 1959).
[2] Munk, W. H., and MacDonald, G. J. F., *The Rotation of the Earth* (Cambridge University Press, Cambridge, 1960).
[3] Jeffreys, H., and Vincente, R., *Mon. Not. R. astr. Soc.*, 117, 142 (1957).
[4] Leppaluoto, D., thesis, Univ. California, Berkeley (1972).
[5] Gans, R. F., *J. geophys. Res.*, 77, 360 (1972).
[6] Jeffreys, H., *The Earth*, third ed. (Cambridge University Press, Cambridge, 1952).

Montmorillonite in surface detritus

QUANTITATIVELY, clay minerals are usually the most important components of those fractions of deep-sea sediments which are smaller than 2 μm. There are specific patterns in the distributions of the principal groups of clay minerals in both deep-sea sediments[1] and in aeolian dusts from the overlying marine atmosphere[2], which have been used to evaluate the origin of the clays. It is considered[1–3] that the clay minerals kaolinite, illite and chlorite are largely detrital in origin and are transported to the oceans from the continents.

Less is known, however, about the origin of montmorillonite. Deep-sea sediments of some regions, for example, the South Pacific, show extensive evidence that montmorillonite has formed from the *in situ* alteration of volcanic debris on the ocean floor[1], that is the montmorillonite is authigenic. A similar explanation has been proposed for the origin of montmorillonite in Indian Ocean deep-sea sediments[1], where this mineral makes up approximately 41% of the total clays smaller than 2μm. There is, however, speculation about the origin of montmorillonite in deep-sea sediments from the South Atlantic, where it comprises approximately 26% of the total clays smaller than 2 μm (ref. 1). It is believed[1–3] that either the montmorillonite or the parent volcanic material is transported to the region by aeolian and river mechanisms or that the mineral is derived from volcanic material from the Mid-Atlantic Ridge region. These theories are based on observations of the distribution of montmorillonite in deep-sea sediments; there is little information available on its concentration in marine detritus from the World Ocean.

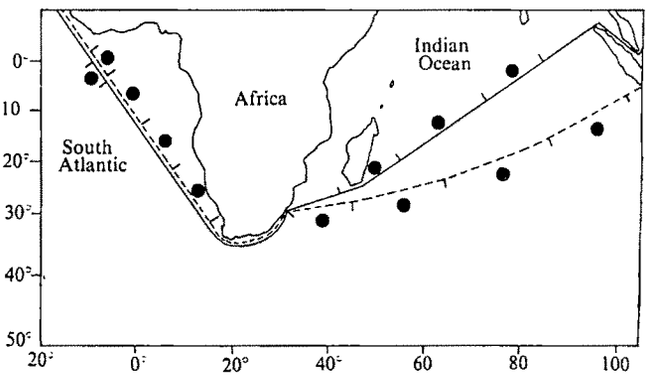


Fig. 1 Partial track of the MV Cyclops. Solid line, UK–Japan run; broken line, Japan–UK run. Detritus samples were collected over the intervals indicated by the lines perpendicular to the track; ●, central positions of sampling sites.

Here, we present preliminary data on the relative concentrations of montmorillonite in 'open-ocean' surface water samples (0–5 m) from the South Atlantic and Indian Oceans. Collections were made during March and July, 1972 on board MV Cyclops by pumping water from a position in the ship's bows (which minimised contamination from the ship's sides) through plastic tubing. The particulate matter was separated by continuous centrifuging. The centrifuge bowl was lined with plastic and after each collection the liners were removed, placed in plastic bags and stored in a deep freeze. The detritus was later removed from the liners and dried at about 40° C, and representative samples taken. A fraction of the samples smaller than 2 μm was separated, treated with hydrogen peroxide to remove organic matter, and the clay minerals were determined by X-ray diffraction. The ship's track and the sampling positions are shown in Fig. 1 and the average montmorillonite concentrations in the detritus are listed in Table 1 together with those of the underlying deep-sea sediments.

On average, the concentration of montmorillonite is similar in detritus samples from both the South Atlantic and Indian Oceans, and constitutes approximately 14% of the total clays smaller than 2 μm. This is considerably less montmorillonite than is present in the underlying deep-sea sediments. Furthermore, the relative distributions of montmorillonite vary between the detritus and deep-sea sediments of the two regions; in the South Atlantic there is about one and a half times as much montmorillonite in the sediments as in the detritus, and in the Indian Ocean about three times as much in the sediments.

There are a number of explanations for the deficiency of montmorillonite in the detritus relative to the sediments. For example, other clay mineral species, and/or volcanic

Table 1 Average concentration of montmorillonite*

South Atlantic Ocean	
Detritus	~14%
Deep sea sediments[2]	~26%
Indian Ocean	
Detritus	~14%
Deep sea sediments[1]	~41%

* The concentrations of montmorillonite are given as a percentage of the four clay minerals montmorillonite, illite, chlorite and kaolinite in the fraction of the detritus and sediments smaller than 2μm.

ash, could be transported to the surface of the ocean and could subsequently undergo modification to form montmorillonite both in the water column and at the sediment surface. Alternatively, montmorillonite could be formed from the *in situ* modification of locally derived volcanic debris at the sediment surface. Whatever processes are operative, however, it may be concluded from the present results that only about half of the montmorillonite in South Atlantic deep-sea sediments, and only about a third of that in Indian Ocean sediments, have a continental detrital origin.

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[1] Griffin, J. J., Windom, H., and Goldberg, E. D., *Deep-Sea Res.*, **15**, 433 (1968).
[2] Chester, R., Elderfield, H., Griffin, J. J., Johnson, L. R., and Padgham, R. C., *Mar. Geol.*, **13**, 91 (1972).
[3] Biscaye, P. E., *Yale Univ., Geochem. tech. Rep.*, 8 (1964).

Revised continental fit of Australia and Antarctica

I HAVE already presented a morphological reconstruction of the continental fragments of Gondwanaland in the south-western Pacific[1]. The reassembly was based on the computer-generated fits of Australia and Antarctica[2,3] (which match the 1,000-fathom and 500-fathom isobaths). The method used in each case[2,3] is a 'least squares' geometric fit of the selected isobath. Another fit has recently been proposed[4], for this fit an instantaneous rotation pole is determined from fracture zones, at 10.8° N, 32.2° E, but the Australia–Antarctica fit is based on a 'least squares' geometric pole at 6° S, 40.5° E. I suggest that all of these fits may be in error by about 250 km, as present-day isobaths only define poorly the predrift continental edges.

A better method of making continental reassemblies is to 'reverse' seafloor spreading, using a mapped pattern of magnetic lineations and fracture zones. Such a pattern is fairly well defined between Australia and Antarctica[5,6]. Weissel and Hayes[6] show several stages of drift (see their Fig. 13), but in the initial step they have accepted the computer fits[2,3] as the constraint. They therefore delineate a westward bend in fracture zones south of Australia, amounting to some 250 km (see their Fig. 2). Hayes and Ringis[7] attempted a regional continental reconstruction but erroneously implied that the Australia–Antarctica fit has been "rather precisely determined" using only the reversal of the magnetic patterns, whereas Weissel and Hayes[5,6], in fact, used the computer fit[2,3]. They note that the South Tasman Rise overlaps the Iselin Plateau, presuming that both are continual crust, and then suggest that relative movement of West and East Antarctica occurred during drift.

In the light of these problems and inconsistencies, I have revised my original reconstruction[1] (Fig. 1). The fit was made using a 1:15 million scale Lambert's Equal Area Azimuthal projection, centred at 160° E, 60° S (near Macquarie Island). Continental boundaries were initially defined at the 2,000-m isobath, and fracture zones plotted from ref. 6, except that each fracture zone was projected straight towards the Australian coast. Australia was then

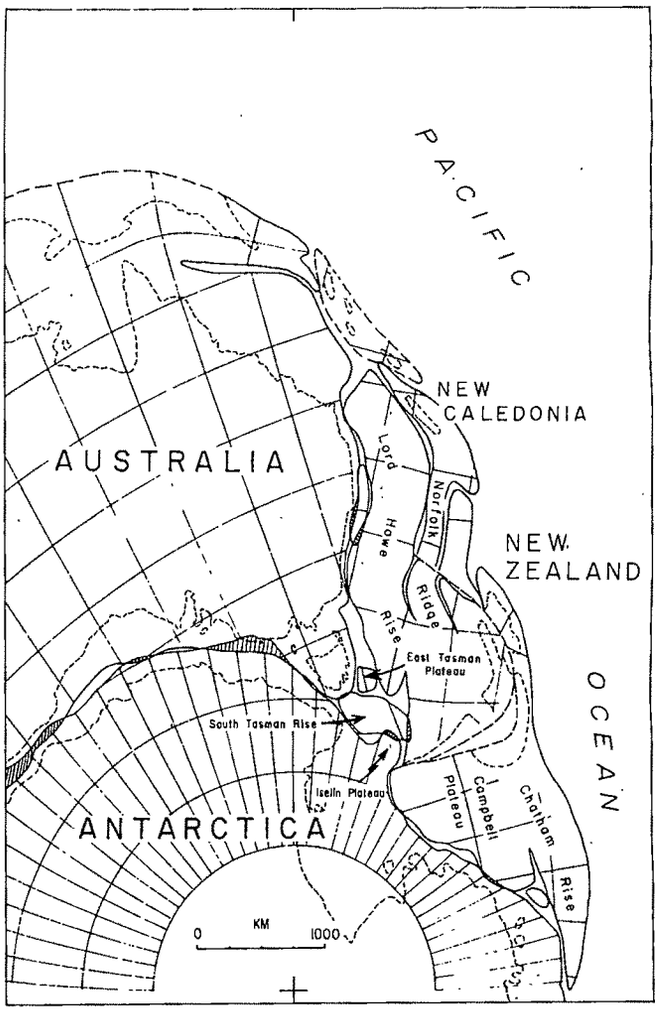


Fig. 1 Reconstuction of continental fragments in the south-western Pacific. Continental outlines and 2,000-m isobaths (smoothed) shown, taken from Lambert's Equal Area Azimuthal projection, centred at 160° E, 60° S. Present latitude and longitude lines (5 °spacing) shown for reference. Hatching denotes overlap.

fitted to Antarctica by reversal of motion along the intervening fracture zones. This operation places Australia some 250 km west (relatively) of its position in earlier computer fits[2-4] and allows the continental South Tasman Rise[8] to fit neatly between Tasmania and the Iselin Plateau (Fig. 1).

The fit was checked by rotating contiguous points to their present positions using a 50-cm stereographic net, and it was found that rotation about a pole at 10.8° N, 32.2° E

(determined from fracture zones in ref. 4) gave results consistent with the fit proposed here, and significantly different from the least squares geometric fits. The shape of mutually opposed coasts on the Lambert's Equal Area projection were compared with their computer-drawn equivalents by enlarging the latter optically to a scale of 1:15 million. No significant distortion was found.

The Campbell Plateau was fitted against Antarctica as before[1] and the Tasman Sea closed by a 23° rotation of the Lord Howe Rise about a rotation centre at 145° E, 15° S. This is broadly consistent with the Tasman Sea magnetic pattern but implies that the orientation of fracture zones, particularly in the north, is different from that of Hayes and Ringis[7]. This suggests that their interpretation may be in error in this region. A 5° grid (present coordinates) is superimposed on the fragments in the reconstruction (Fig. 1). This shows that neither the Campbell Plateau nor the Lord Howe Rise have been deformed to achieve the fit. The only significant deformation required is along the Alpine Fault Zone through New Zealand.

The modified fit (Figs 1 and 2) still has one major overlap, where the bulge of the Antarctic 2,000-m isobath overlaps Australia. A similar mismatch (though placed further west) was noted by Sproll and Dietz[2], who suggested that either the Antarctic bathymetry was substantially in error in this area, or that postrift volcanics or sediments form the bulge. A detailed compilation of available oil-company exploration data from the south-eastern Australian continental margin shows, however, that with the fit suggested here, this bulge largely overlaps an Upper Mesozoic and Cainozoic sedimentary sequence in the Otway Basin of south-eastern Australia[9]. These are typical rift-fill sediments (refs 9, 10, and K. A. Richards, and B. M. Hopkins, ECAFE Symposium, Canberra, 1969) which represent an unusually wide development of the continental margin in this area. On Fig. 2, I have delineated the 3,000-m isopach of Upper Mesozoic and Cainozoic sediments along the Australian margin, selecting this line as more representative of the predrift margin. The correspondence of this isopach with the Antarctic 'bulge' is quite clear.

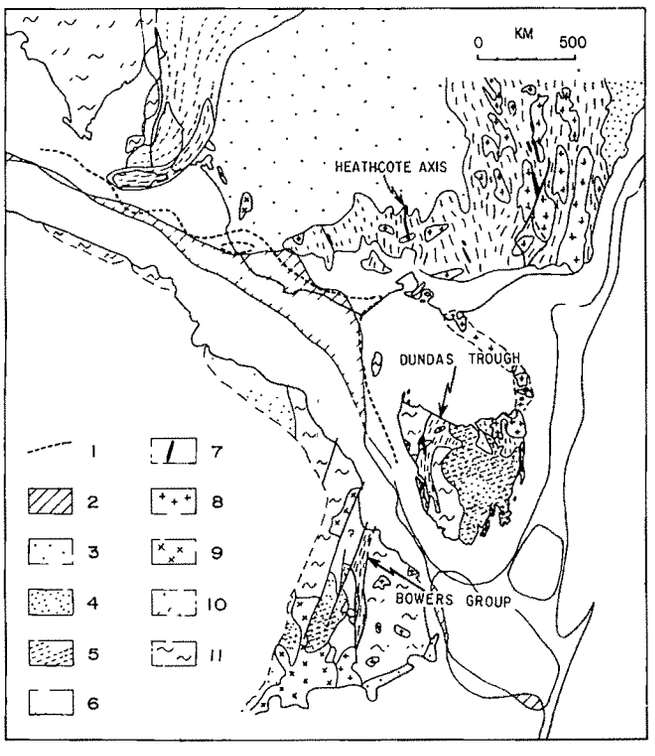


Fig. 2 Detailed fit of south-eastern Australia and Antarctica, showing major geological elements. 1, 3,000-m isopach of Upper Mesozoic Cainozoic sediments of south-eastern Australia; 2, overlap of 2,000-m isobaths; 3, Cainozoic sedimentary cover; 4, Upper Palaeozoic and Mesozoic basins (essentially undeformed sediments); 5, areas of Jurassic dolerite intrusions; 6, Palaeozoic of Tasman Orogenic Zone; 7, ultramafic masses; 8, Silurian-Devonian granites; 9, Cambrian–Ordovician granites; 10, late Proterozoic sediments of the Adelaide Geosyncline (moderately folded); 11, exposed Precambrian "basement". Some major faults are also shown by heavier solid lines. Data taken from refs 11 (Australia) and 12 (Antarctica).

The outlined fit (Figs 1 and 2) is therefore preferred to computer fits based on present isobaths[2,3] and points to the obvious need to establish the geological status of lines of fit before using computer techniques. Isopachs of marginal sediments are probably a more realistic definition of prerift continental shapes. The fit also eliminates the source of several 'problems' in the region. For example, the South Tasman Rise is incorporated without any movement of West Antarctica.

Figure 2 also shows the major geological elements of mainland Australia, Tasmania and Antarctica[11,12]. No certain correlations can be made yet. One speculative correlation, however, is that of the Dundas Trough (Cambrian sediments, ophiolites and Andean-type calc-alkaline volcanics[13]) with the Heathcote Axis (Cambrian greenstones and calc-alkaline volcanics[14]) and Bowers Group (Cambro–Ordovician sediments[15,16]). This suggestion offers a chance to correlate narrow units, without depending on vague lithological similarities, but needs further evaluation.

There has been some discussion of alternative reassemblies and argument for and against major transcurrent movements active during the Palaeozoic and Mesozoic[17–20]. These articles do not allow for any possible error in the computer fits[2,3] and generally make very vague and generalised lithological correlations to support their conclusions. Many of the supposed difficulties in regional correlations using earlier fits[2,3] diminish considerably if the revised fit presented here is valid. The occurrence of Precambrian basement rocks in Tasmania also suggests that the island is uplifted relative to Victoria and Antarctica, probably as a result of Cainozoic seafloor spreading and therefore a relatively deeper erosion level is observed at present in Tasmania[9].

The present knowledge of the regional geology, seems to provide little to support unequivocally hypotheses involving major transcurrent movements[17,19,20].

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1 Griffiths, J. R., *Nature*, **234**, 203 (1971).
2 Sproll, W. P., and Dietz, R. S., *Nature*, **222**, 345 (1969).
3 Smith, A. G., and Hallam, A., *Nature*, **225**, 139 (1970).
4 McKenzie, D., and Sclater, J. G., *Geophys. J. R. astr. Soc.*, **24**, 437 (1971).
5 Weissel, J. K., and Hayes, D. E., *Nature*, **231**, 518 (1971).
6 Weissel, J. K., and Hayes, D. E., *Antarctic Oceanology II: the Australian–New Zealand Sector, Antarctic Research Series*, **19**, 165 (American Geophysical Union, Washington, 1972).
7 Hayes, D. E., and Ringis, J., *Nature*, **243**, 454 (1973).
8 Kennett, J. P., *et al.*, *Geotimes*, **18**, 14 (1973).
9 Griffiths, J. R., thesis. Univ. Tasmania (1973).
10 Griffiths, J. R., *J. Austr. Petrol. Explor. Ass.*, **11**, 75 (1971).
11 *Tectonic Map of Australia and New Guinea 1:5 000 000* (Geological Society of Australia, 1971).
12 *Geologic Map of Antarctica 1:5 000 000* (American Geographical Society, 1972).
13 Solomon, M., and Griffiths, J. R., *Proceedings of the Tasman Geosyncline Symposium, Brisbane* (in the press).
14 Talent, J. E., and Thomas, D. E., *Regional Guide to Victorian Geology, Second Edition* (edit. by McAndrew, J., and Marsden, M. A. H.), 65 (University of Melbourne, Melbourne, 1973).

[15] Sturm, A., and Carryer, S. J., *N.Z. Jl Geol. Geophys.*, **13**, 408 (1970).
[16] Laird, M. G., Andrews, P. B., Kyle, P., and Jennings, P., *Nature*, **238** 34 (1972).
[17] Crawford A. R., and Campbell, K. S. W., *Nature*, **241**, 11 (1973).
[18] Daily, B., Jago, J. B., and Milnes, A. R., *Nature*, **244**, 61 (1973).
[19] Crawford, A. R., and Campbell, K. S. W., *Nature*, **244**, 64 (1973).
[20] Harrington, H. J., Burns, K. L., and Thompson, B. R., *Nature*, **245**, 109 (1973).

Archaean tectonics

THERE is compelling evidence that Archaean convective motions were very different in scale from the motions which we associate with modern plate tectonic phenomena. The ancient motions resulted in a distinctive surface expression. Many lines of evidence suggest that the Earth is cooling by heat and mass transfer which is reflected in magmatic phenomena at or near the surface. Evidence of convective mixing in all regions of the deep Earth is accumulating. Today, the major sites of mass and energy transfer are at the ocean ridges where new crust is formed. This motion towards the surface is balanced by the return of crustal material at the subduction zones. There are few sites of crust formation. They are all elongated, with linear dimensions comparable with the circumference of the Earth, and they are widely spread.

Geological maps of the crust of more than 3×10^9 yr ago indicate a totally different tectonic-magmatic situation. Such regions are well documented from southern Africa and Greenland[1]. The sedimentary and igneous rock types in these regions have, in general, modern counterparts, and they provide evidence of the existence of an extensive hydrosphere. The rocks of the granite family, however, dominate 80–90% of the geologic maps and there are no significant areas of ophiolitic rocks; no analogues of the modern ocean crust[2]. Metamorphic rocks of the granulite facies are very common and are often associated with anorthosite complexes[3]. Metamorphic events reflect steep thermal gradients. Extensive linear features are rare and volcanic–sedimentary basins involve complex mixtures of volcanic types from ryholites and andesites to basalts and komatiites. This is not a modern situation.

A model for the early crust must take account of three concepts. Runcorn[4] has summarised evidence which indicates that convective motions have been quantised through time. The evidence from age distribution patterns is compelling. Elder[5] has indicated how the thermal structure of the Earth is appropriate to a body cooling from the surface and in a state of vigorous convection. The models involve a rather thin surface region with a steep thermal gradient followed by a deep, essentially isothermal region. Where the gradient changes rapidly, there is a layer of high thermal turbulence with large fluctuations of temperature (a possible low velocity or melting region). With time, this region will sink into the cooling body and the amplitude of fluctuations will decay. Finally, Fyfe and Leonardos[6] point out that basalt will not continuously penetrate a fusible granitic layer; fusion of the granite, will form a viscous low density layer, and this will turn off injection or encapsulate the heavier magma. I use the term encapsulate in the sense in which it is used in the ceramic or metallurgical industries to describe the situation in which a heavy material is protected from the environment by a lighter, viscous, glassy layer.

The Archaean model proposed here is in part a result of an attempt to explain the map and synthesis proposed for the Rhodesian craton[7] which is similar to many cratonic regions of a comparable age. Before any quantum jump

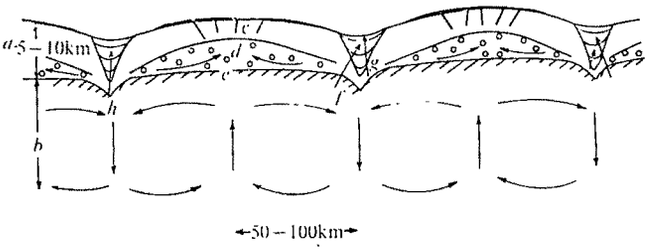


Fig. 1 Model of convective motion in the early crust and upper mantle, *a*, granitic crust; *b*, low velocity zone of upper matle; *c*, region of basaltic penetration of the crust; *d*, granitic domes; *e*, granulites and anorthosites; *f*, basalts; *g*, rhyolites; *h*, andesites.

in the convective pattern[4], the dynamic structure could be as shown in Fig. 1. A continuous, thin, highly radioactive, easily fusible granitic layer, 5–10 km thick (Fig. 1*a*), overlies a high-level melting zone in the upper mantle (Fig. 1*b*). In the melting zone partial melting occurs on a large scale, and various products of melting result from a high level of thermal turbulence. Convection cells in the mantle have a short wavelength pattern (about 100 km) as is implied by the separation of basins in Rhodesia[7]. Basalt penetration of the crust (Fig. 1*c*) is sporadic and is terminated by crustal fusion and the formation of granitic domes (Fig. 1*d*). Basalt flow is concentrated beneath the encapsulating acid layer. The basalt tends to underplate the acid crust and fractionates to produce anorthosite complexes which themselves will be associated with granulites, the crustal fusion residue (Fig. 1*e*). Above regions of return flow where the crust is thinned, volcanic rocks may be injected; basalts, rhyolites, or, where mixing occurs, andesites (Fig. 1*f*, *g*, *h*). During periods of maximum thermal turbulence and high partial fusion, the ultramafic komatiites may erupt.

The situation after the convective pattern changes might represent that pertaining only for a short time before the change. The quantum jumps are considered to be towards larger cells (compare with ref. 4). In the model of early patterns, heat–mass transfer in the upper layers involves a two-storey motion, with basic magmas adding heat to the acid layer which itself overturns by magmatic processes. Erratic convective motion could lead to thrusting of the mini-continents, which would produce the type of tectonics frequently observed when basement rocks are exposed.

It is proposed here that modern ophiolitic plate tectonics began only after the thermal regime of the acid crust allowed stable thickening to the point where granitic materials did not cover most of the earth. This idea does not conflict with palaeomagnetic results discussed by Piper *et al.*[8] and perhaps provides the "supplement to plate theory of orogenesis" discussed in their work. It could be predicted that future convective patterns will lead to a further thickening of acid materials and a more extensive covering the basaltic crust.

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[1] Sutton, J., and Windley, B. F., *Phil. Trans. R. Soc.*, **A273**, 315 (1973).
[2] Church, W. R., *Publication of the Earth Sciences Branch, Department of Energy, Mines and Resources, Canada*, **42**, 71 (1972).
[3] Fyfe, W. S., *Phil. Trans. R. Soc.*, **A273**, 457 (1973).
[4] Runcorn, S. K., *Phil. Trans. R. Soc.*, **A258**, 228 (1965).
[5] Elder, J. W., *J. Earth Sci., Leeds*, **8**, 287 (1972).
[6] Fyfe, W. S., and Leonardos, O. H. jun., *Nature*, **244**, 501 (1973).
[7] MacGregor, A. M., *Proc. geol. Soc. S. Afr.*, **54**, 27 (1951).
[8] Piper, J. D. A., Briden, J. C., and Lomax, K., *Nature*, **245**, 244 (1973).

Electrons and the emission of soot from flames

AN increasing body of evidence suggests that soot formation is associated with ionisation in flames and may be controlled electrically. Work in which sooting flames are subjected to large electric fields[1–3] shows that all soot particles are charged (at least in the presence of an applied field) and can therefore be manipulated by fields. This applies not only to fully formed particles but also to their growth and burning up in flames. Thus by applying fields so as to remove particles rapidly from the zone in which they are formed, their size as well as the total yield is greatly reduced. The former is ascribed to the decreasing residence times and the latter to the removal of surface on which growth can occur. Conversely, very large particles can be produced by holding them stationary against the gas flow by means of a field and thereby increasing their residence times. Also, any appreciable field intensity removes the charged nuclei on which carbon particles tend to grow[3].

Many practical systems are not amenable to the application of large electric fields and a great many research projects have concerned themselves with the effect of additives (refs 4–8 and E. M. Bulewicz, D. G. Evans, and P. J. Padley, personal communication). The most effective additives have generally proved to be substances which have low ionisation potentials or form particles of low work functions. Thus the alkali metals, when introduced to the extent of parts per million, alter the 'structure' of carbon black and the effectiveness of the additive in breaking long chains is in the inverse order of its ionisation potential. Although the order of effectiveness within each group of elements is determined by the ionisation potential, this does not apply across groups, the alkaline earths, for example, differing from the alkali metals, barium additives which produce $BaOH^+$ as the positive ion being the most effective. Also, the same additive can produce suppression of soot formation, or its promotion, depending on the amount and the position in which it is introduced. Where the position of addition is not localised, the pattern is that small amounts of additive produce promotion whilst large amounts produce suppression with a crossover point of zero effect in between. When the additive is introduced locally—on a wire, say—insertion at the base of the flame produces suppression; introduction higher up results in an increase of soot produced[6].

Chemical—as an alternative to electrical— effects have been considered responsible for some of these observations[5]. Since electronic structure is inevitably related to chemistry, the issue is most likely to be resolved by the application of electric fields. Different groups, however, have used either additives, or applied fields—not both simultaneously. We now propose a hypothesis based on free electrons and then test it by the combination of the two techniques. Although the results bear out the hypothesis and, furthermore, offer a novel practical method for controlling soot emission, this does not, of course, constitute a proof that chemical effects could not also be important.

Most of this work has been carried out in diffusion flames —in which the reactants are initially separate the maximum temperature and the main reaction zone occurring close to the stoichiometric contour. This is also close to the surface along which ionisation will peak, being a strong function of temperature. It is not, however, the region of carbon formation, since conditions for pyrolysis are most favourable on the fuel side, where there is a lack of oxygen. Thus if ionisation is to play a major part in soot formation, the diffusion coefficient of the charge carrier must be a most important parameter. Of the particles involved, it is the electron that has by far the largest diffusion coefficient and it is known that the negative charge carrier in the high temperature zones of flames is an electron[2]. We therefore suppose, as an initial hypothesis, that the effect of additives is entirely due to the free electrons which they produce. We shall then attempt to account for all previous experimental observations in terms of such a hypothesis and, if that is successful, design some further crucial experiments.

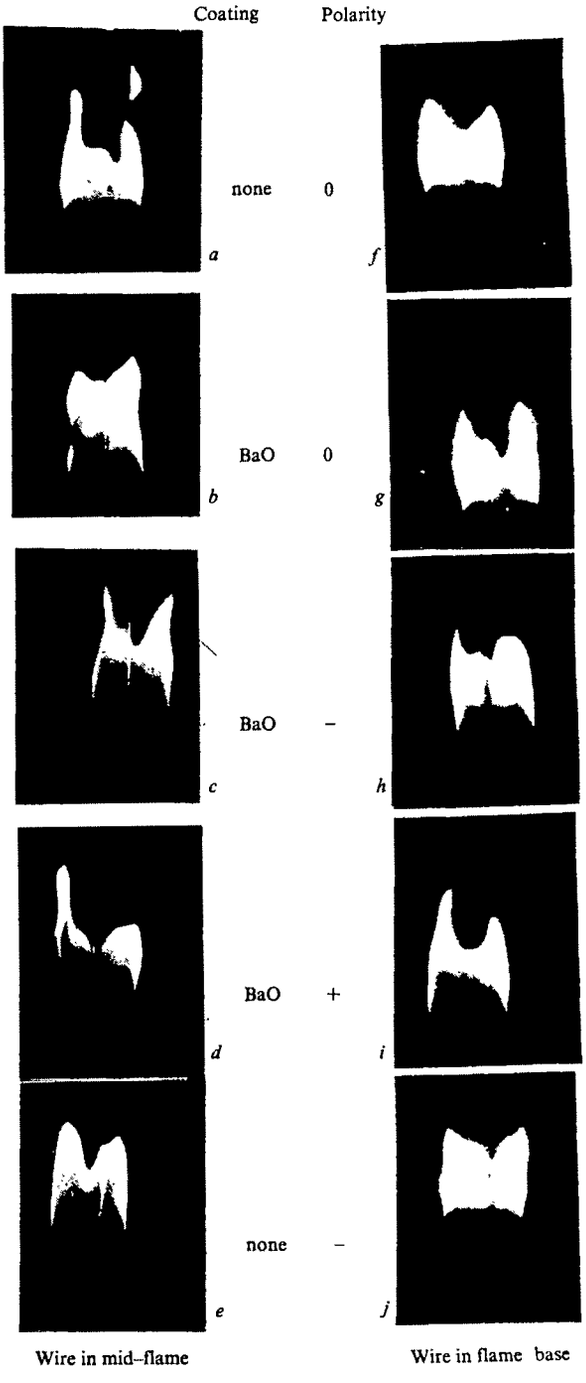


Fig. 1 Effect of coating and polarity on soot formation above wires.

Although the chief role of an influx of additional electrons into the pyrolysis zone is likely to be that of neutralising positive charges, this can have two diametrically opposed consequences. Where all or most of the particles[1–3], or

chains of particles, are positively charged, neutralisation of positive charges at their extremities would stop them repelling one another and thereby remove the obstacle to agglomeration. Agglomerates of appreciable size are much less likely to burn away during their subsequent passage through the high temperature zones in the presence of oxygen. When only a fraction of the chains is charged, on the other hand, agglomeration occurs by dipole action, the rate reaching a maximum when the fraction charged is a half. For smaller proportions of charged particles, electrons will therefore decrease agglomeration rates.

This proposition accords extremely well with the observed facts. Large electron concentrations will eventually always inhibit agglomeration and thereby suppress soot formation. Electron concentrations insufficient to neutralise a very large proportion of particles under conditions where the majority of particles is charged will act mostly as a 'glue' in encouraging small particles to aggregate and thereby increase the mass of soot escaping.

The dependence on the mobility of the positive ion also falls into place, although the role attributed to it is diametrically opposed to what has been supposed hitherto. In terms of the above hypothesis, the positive ion acts chiefly through recombining with the electrons it meets away from the high temperature zone (it is of course much more likely to encounter an electron than a carbon particle). The larger the ion the smaller its excursion.

Although this tentative hypothesis thus accounts for all the experimental observations, a much more direct test would be one in which the additive would be discouraged from migrating by being held on a wire at a relatively low temperature, whilst the charges emanating from it could be controlled by an electric potential applied to the wire.

Nichrome wires coated with barium oxide and uncoated wires of diameter 0.2 mm were inserted at various heights into diffusion flames of propane diluted with argon, burning with air. The flames consisted of single, approximately flat, faces, which were contrived very simply by attaching a 'fish-tail' nozzle to the fuel burner tube and blowing off the second flame face by an adjacent argon stream emerging from a similar nozzle. The results are shown in the photographs of Fig. 1 and the current–voltage characteristics of Fig. 2.

The results are in exact accord with the predictions of the hypothesis, in that it is the electron which produces effects characteristic of the additive. At heights where the additive promotes soot formation (Fig. 1*b* as compared with Fig. 1*a*) application of a negative potential, encouraging the emission of electrons, greatly increases the effect (Fig. 1*c*) and the application of a sufficient positive potential prevents it altogether (Fig. 1*d*). It transpired from the current–voltage characteristics that positive potentials not only prevented electron emission but led to the emission of positive ions (Fig. 2) and some ionic wind effects came into evidence at higher currents. The absence of soot formation in the case of positive ion emission casts doubt on previous suppositions that positive inorganic ions themselves act as nuclei; they do of course mop up electrons.

We tried, unsuccessfully, to explain these effects in terms of what is already known about the effects of electric fields on carbon formation. According to this, the negatively charged wire would be expected to collect the positively charged carbon particles—and this is indeed precisely what happens in the case of the uncoated wire (Fig. 1*e*)—rather than to increase soot formation. Once a layer of carbon is built up on the wire, of course, electron emission sets in.

Figure 1(*f–j*) illustrates the effects observed when wires are inserted into zones in which suppression is produced by the additive in the absence of an applied potential (Fig. 1*g*). Although these are the reverse of the pattern of Fig. 1*a–e* they also can be summarised by the observation that the behaviour associated with additives is manifested by free electrons. It is accentuated when the applied field promotes electron emission and tends to reverse when electron emission is prevented by positive polarity of the support.

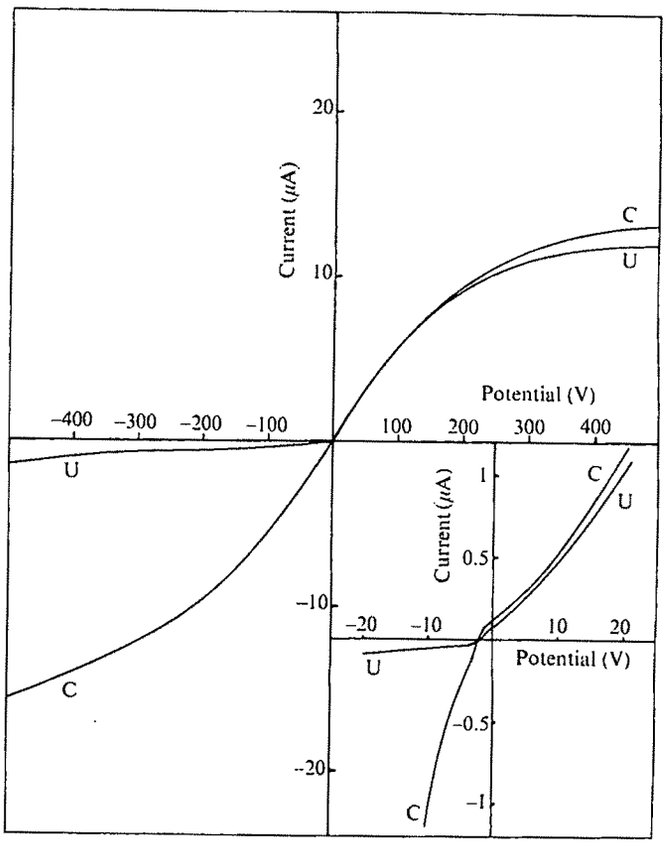


Fig. 2 Current/potential characteristics of the wire with respect to the burner, of coated (C) and uncoated (U) wires in mid-flame.

We do not suggest that the entire subject of sooting in flames can be accounted for in terms of free electrons and we think it very likely that the underlying chemical processes retain the importance that has been attributed to them. But it does seem possible to account for the behaviour of ionising additives entirely in terms of the electrons they produce as regards both suppression and promotion, the effect of the amount of additive, the site of its introduction and the effect of ion size.

What is probably much more important is that we can produce all the observed effects by controlling the emission of free charges electrically, using coated surfaces maintained hot by the flame, and connected to a source of electrical potential—without introducing additives into the fuel and, thereafter, into the atmosphere by way of exhaust gases. The power that would be involved in injecting electrons in this manner would, of course, be quite small, 1 mA being sufficient to provide 6×10^{15} particles s^{-1}.

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1 Plaee, E. R., and Weinberg, F. J., *Proc. R. Soc.*, **A289**, 192–205 (1965).
2 Lawton, J., and Weinberg, F. J., *Electrical Aspects of Combustion* (Clarendon Press, Oxford 1969).
3 Mayo, P. J., and Weinberg, F. J., *Proc. R. Soc.*, **A319**, 351–371 (1970).

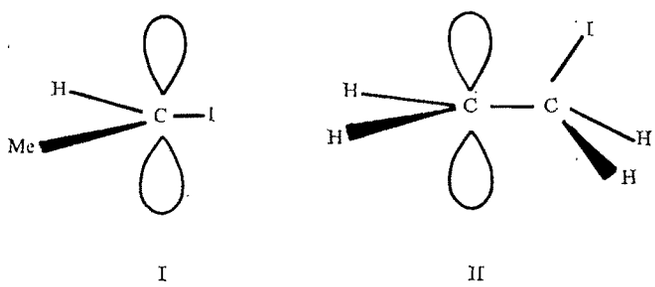
[4] Addecott, K. S. B., and Nutt, C. W., *Am. Chem. Soc. Div. Petrochem.*, **14** (4), A69–80 (1969).
[5] Cotton, D. H., Friswell, N. J., and Jenkins, D. R., *Combustion and Flame*, **17**, 87–98 (1971).
[6] Salooja, K. C., *Nature*, **240**, 350–351 (1972).
[7] Salooja, K. C., *Combustion Institute European Symposium* (edit. by Weinberg, F. J.) 400–405 (Academic Press, London, 1973).
[8] Feugier, A., *ibid*, 406–411.

Radicals in irradiated ethyl iodide and related compounds

IN studies of the radiolysis of various alkyl halides, particular attention has been focused on a multiline electron spin resonance (ESR) spectrum obtained from ethyl iodide at 77 K (ref. 1). Despite the use of a wide range of isotopically enriched materials, however, there has been no satisfactory interpretation of these complex ESR spectra and this remains one of the unsolved problems of ESR spectroscopy[2].

The spectrum for normal CH_3CH_2I comprises sets of 'parallel' and 'perpendicular' type features covering a total range of about 1,000 gauss which seem, in the outer regions of the spectra, to comprise sextets with $a \approx 22$ gauss. These are centred close to the free-spin g value (see Figures in ref. 1).

Studies of other alkyl halides[3–5], indicate that the most likely candidates are the α– or β–iodo alkyl radicals (Fig. 1, I and II). Radical I is, however, expected to exhibit a very marked g-value variation which would lead to considerable spectral asymmetry,



I II

Fig. 1

with strong features in the region of low-field, and even if these radicals were librating or rotating the resulting g_{av} value would be $>> 2.0023$. This is not observed, and indeed, the spectra are completely different from that obtained from irradiated α–iodo acetamide, which we assign to $HCI(CONH_2)$ radicals (G. W. Neilson, and S. P. Mishra, unpublished results).

Again, the β–iodo radical, II, is expected to have a far larger hyperfine coupling to ^{127}I than is possible for the ethyl iodide radical, and the α–proton coupling should give rise to a triplet having $a \leqslant 20$ gauss. (Coupling to the two β–protons should be small.)

Thus I and II can be firmly rejected. The radical $CH_3CH_2 - - - I^-$ is, however, a likely candidate. Methyl bromide in a methyl cyanide matrix gives a species $CH_3 - - - Br^-$, which has the normal magnetic properties for methyl radicals, but with each feature split into a pair of quartets by hyperfine coupling to ^{79}Br and ^{81}Br (ref. 6). We have extended this work to include methyl iodide, which again gave a spectrum characteristic of methyl, with a weak hyperfine coupling to iodine[7] (Table 1).

We find that the spectra published by Willard *et al.*[1], and ourselves for normal CH_3CH_2I can be quite satisfactorily analysed on this hypothesis, using the parameters given in Table 1. In particular, the 22-gauss sextets have their $\pm 5/2$ components broadened, as is usually the case for ethyl radicals in rigid media, and therefore could be mistaken for quartets (1 : 3 : 3 : 1), thus closely resembling the features for $CH_3CD_2 - - - I^-$, as noted by Willard *et al.*[1].

Comparable species were not obtained from methyl iodide or from solutions of ethyl iodide in CD_3OD. A very similar species, $(Me)_3C\cdot - - - I^-$, was, however, obtained from t-butyliodide (Table 1).

Table 1 ESR parameters for $CH_3CH_2 - - - I^-$ and related anions

Medium	Radical	Hyperfine coupling (gauss) *, † ^1H	^{127}I
CD_3OD‡	$CH_3 - - - I^-$	21 (∥)78	(⊥)56
C_2H_5I	$CH_3CH_2 - - - I^-$	22 (∥)168	(⊥)126
Me_3CI	$Me_3C - - - I^-$	22 (∥)> 53.5	(⊥)53.5

*, 1 gauss = 10^{-4} T;
†, all g values close to 2.0023;
‡, ref. 6.

We conclude that the process involved is

$$RI + e^- \rightarrow R\cdot - - - I^-$$

in which the radical, R·, has its normal planar geometry, but, under favourable circumstances, exhibits a very weak charge-transfer interaction with iodide. We suggest that the occurrence of this interaction, and its magnitude, is governed primarily by the crystal structure of the host compound and therefore by the mobility of the alkyl radical. The results for CD_3OD solution show that the interaction is lost when iodide can be solvated, even at 77 K.

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Received October 8, 1973.

[1] Egland, R. J., Ogren, P. J., and Willard, E. E., *J. phys. Chem.*, **75**, 467 (1971) and references therein.
[2] *Electron Spin Resonance, Vol. 1* (edit. by Norman, R. O. C.) Chemical Society Specialist Report, 130 (London, 1973).
[3] Kispert, L. D., and Myers, F., *J. chem. Phys.*, **56**, 2623 (1972).
[4] Mishra, S. P., Neilson, G. W., and Symons, M. C. R., *J. Am. chem. Soc.*, **95**, 605 (1973).
[5] Lyons, A. R., and Symons, M. C. R., *J. Am. chem. Soc.*, **93**, 7330 (1971).
[6] Sprague, E. D., and Williams, F., *J. chem. Phys.*, **54**, 5425 (1971).
[7] Mishra, S. P., and Symons, M. C. R., *J. chem. Soc., Perkin II*, 391 (1973).

Lyoluminescence of trehalose dihydrate

LYOLUMINESCENCE of trehalose.$2H_2O$ in water has been observed over a wide range of radiation doses. It has been possible to cover linearly the range from 10 rad up to 150 krad. With some samples the lower limit was 100 mrad.

The emission of light when irradiated saccharides are dissolved in water has been described[1,2]. Saccharides were chosen because of their good solubility in water and because they are quite good soft tissue equivalent materials. Free radicals which formed during irradiation of the solid probably cause the light emission when they are set free to react in solution[3].

There is evidence from our experiments that the diffusion controlled reactions involved in the process of light emission are of the type:

$$R^{\cdot} + R^{\cdot} \rightarrow R\text{–}R \quad (1)$$

$$R^{\cdot} + O_2 \rightarrow RO_2^{\cdot} \quad (2)$$

$$2RO_2^{\cdot} \rightarrow R\text{–}R + (O_2)_2^{*} \quad (3)$$

This is based on the several observations.

(1) The emission spectrum of all saccharides (for example, trehalose.$2H_2O$, glucose, mannose) has a maximum at approximately 4,900 Å as determined by interference filters. This coincides with the absorption peaks of the S→T transition of $(O_2)_2$ associate[4].

(2) When irradiated saccharides are annealed at different temperatures, they tend to exhibit more lyoluminescence. This is a result of the temperature-assisted diffusion of O_2 during annealing.

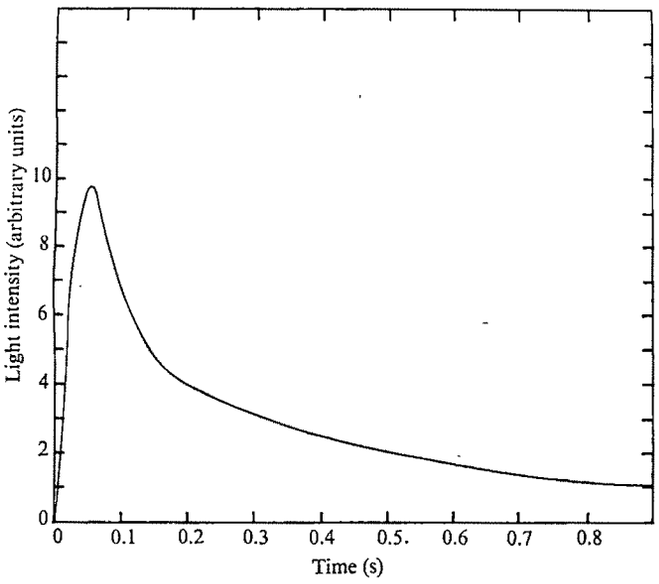


Fig. 1 Lyoluminescence decay curve of trehalose.$2H_2O$ in water.

(3) The addition of $\approx 10^{-4}$ M concentration of Cu^+, Cu^{2+}, $Mn\,O_4^-$ and $Fe(CN)^{3-}$ quenches the light to 31%, 14%, 4% and 1.5%, respectively, of the value for pure water. Addition of fluorescein has no effect, whereas water saturated with O_2 enhances the light emission. The quenching effect of these ions is because of the tendency of radicals to react preferentially with them instead of with oxygen. The failure of fluorescein to enhance the light, suggests that no energy transfer occurs between the produced R–R and fluorescein molecules.

(4) Reaction (2) has to compete with reaction (1). At higher concentrations of radicals, that is, at high doses, reaction (1) becomes more frequent as the distance between radicals decreases. This explains the saturation and the decrease in the light yield over 150 krad, which is common for all saccharides (Fig. 3). The actual saturation level in solid saccharides, as determined by ESR, is over 100 Mrad (ref. 5).

When luminol solution was used instead of water the enhancement of light emission was a million times in the case of glucose and twenty times for trehalose.$2H_2O$. The light emission accompanying the oxidation of luminol strongly suggests that $RO_2^{\cdot}$, with its oxidising properties, is involved in the process.

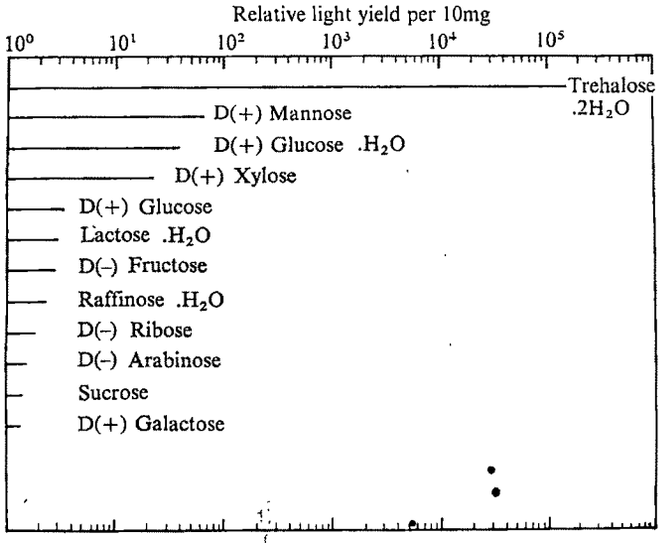


Fig. 2 Lyoluminescence response of saccharides to radiation. All given the same dose (67 krad) using a ^{60}Co γ source.

Figure 1 shows the lyoluminescence decay curve of trehalose .$2H_2O$ in water. The light which can be seen easily with the naked eye, was integrated for a period of 3 s, which adequately covers the light output. The relative lyoluminescent sensitivity of various saccharides is presented in Figure 2.

The relationship between the radiation dose (using a ^{60}Co γ source) and the integrated light intensity is shown in Figure 3. The curve is linear between 100 mrad and 150 krad. The lower limit is mostly controlled by the dark current of the photomultiplier tube and can be improved by one order of magnitude if a cooled tube is used. It also depends on the lyoluminescence response of trehalose which varies for samples of different origin.

The lyoluminescence stability of trehalose.$2H_2O$ after irradiation was revealed by the isothermal annealing after

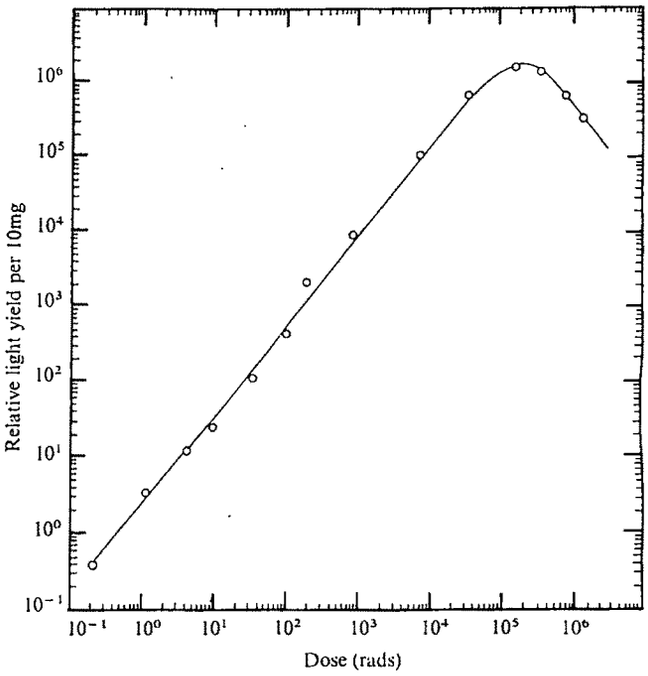


Fig. 3 Light yield against radiation dose for trehalose.$2H_2O$ dissolved in water.

irradiation. A sample heated to 80° C for 2 h showed no change in the light output and heating to 60° C for 60 h caused a decrease of only 18%. These results are consistent with the measurements of Löfroth *et al.*[6], who found that irradiated trehalose.$2H_2O$ loses only 10% of its ESR signal after storage for six months.

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[1] Atari, N. A., Ettinger, K. V. and Fremlin, J. H., *Radiat Effects*, **17**, 45 (1973).
[2] Atari, N. A., and Ettinger, K. V., *Radiat Effects*, **20**, 135 (1973).
[3] Ahnström G., and Ehrenstein, G., *Acta chem. scand.*, **13**, 199 (1959).
[4] Stauff, J., Schmidkunz, H., and Hartmann, G., *Nature*, **198**, 281 (1963).
[5] Löfroth, G., *Acta chem. scand.*, **21**, 1997 (1967).
[6] Löfroth, G., Ehrenberg, L., and Ehrenberg, A., *Proceedings of the Fifth International Symposium on Free Radicals, Stockholm*, 1961 (Almqvist and Wiksell, Stockholm, 1962).

BIOLOGICAL SCIENCES

SV40 nucleoprotein complex activity unwinds superhelical turns in SV40 DNA

THE replication of closed circular superhelical DNA of simian virus 40 (SV40) has been shown to proceed through replicative intermediates that are composed of newly synthesised linear polynucleotide chains hydrogen bonded to closed circular template (parental) strands[1–3]. To replicate these molecules in a semiconservative fashion a swivel, about the phosphodiester backbone of the template strand of DNA, required during polynucleotide chain propagation[2,3]. Such a swivel could be obtained by a nuclease which introduces a single-stranded break in the template strand, thereby allowing free rotation about the phosphodiester bond opposite the nick and relaxing the superhelical turns in the DNA molecule. This type of single-stranded break must, however, be transient and rapidly ligased closed in the cell because breaks in the template strands of replicative intermediates have not been detected[1–3]. Alternatively, an activity has been described in *E. coli*[4,5] and mouse cells[6], that can relax superhelical turns in closed circular DNAs without introducing either single or double-stranded chain scissions in the DNA product of this reaction. This 'untwisting activity' is also a potential candidate for the swivel required during SV40 DNA replication.

The replicative intermediates of SV40 DNA have most often been characterised[1,2,7] after lysing SV40-infected cells with sodium dodecyl sulfate (SDS) in the presence of 1 M NaCl[8]. This results in the isolation of viral DNA molecules that contain little or no protein associated with the DNA[7]. When polyoma[9] or SV40-infected[10,11] cells are lysed instead with the nonionic detergent Triton X-100 in the presence of 0.2 M NaCl, all the viral DNA is recovered in a rapidly sedimenting nucleoprotein complex (44–53S). This complex contains both mature viral DNA as well as the SV40 replicative intermediates[10,13], associated with histone-like proteins and at least two of the viral coat proeins coded for by the *ts*B8 and *ts*B11 loci of SV40[11,12]. The complex containing replicative intermediates sediments faster than that composed solely of mature viral DNA[10,13].

Here we report that the SV40 nucleoprotein complex has associated with it an untwisting activity that reduces the number of superhelical turns in exogenously added SV40 DNA. The underwound product of this reaction remains closed and circular as detected in the ethidum bromide (EtBr)–caesium chloride (CsCl) dye-density gradient. This activity has been shown to be associated with the nucleoprotein complex that contains either mature or replicative intermediates of viral DNA.

SV40-infected African Green Monkey Kidney cells were labelled with ^{3}H-thymidine for 20 h, starting 16 h post-infection[7]. The SV40 nucleoprotein complex was isolated as described in Fig. 1. After concentrating the preparation and dialysis, the complex was incubated at 37°C for 2 h in the presence or absence of exogenous purified closed circular superhelical (Form I) SV40 DNA[7]. At the end of this incubation period, ^{32}P-labelled Form I SV40 DNA was added (at 0°C) to the sample incubated in the absence of exogenous SV40 DNA. Each of these reaction mixtures was then analysed in an EtBr-CsCl equilibrium gradient to determine the amount of Form I and II DNAs present in each sample and to monitor the superhelical density of the endogenous DNA (^{3}H-labelled) in the nucleoprotein complex and exogenously added ^{32}P-labelled DNA.

The results of the EtBr-CsCl gradients are presented in Fig. 1. Two conclusions can be drawn from these data: First, incubation at 37°C for 2 h of 50S complex alone or this complex with exogenously added Form I SV40 DNA resulted in no detectable endonuclease activity that generated relaxed (Form II or III) SV40 DNA molecules (Table I) under these assay conditions. When the 50S nucleoprotein complex was incubated with ^{32}P-labelled exogenous SV40 Form I DNA, however, the superhelical density of this DNA was decreased as seen by a shift of the ^{32}P-labelled viral DNA to a heavier density position in dye-density gradients (Fig. 1*b*). The superhelical density of Form I DNA in the complex (endogenous; ^{3}H-labelled) may also show a small reduction in the number of superhelical turns (Fig. 1*a*, *b*).

To determine if this untwisting activity was preferentially associated with the nucleoprotein complex containing mature DNA or replicating intermedates, the experiment described above was repeated. A second neutral sucrose gradient was used to separate the faster sedimenting component of the nucleoprotein complex that contains mainly replicating DNA from the complex containing mostly mature viral DNA. The untwisting activity was found to be associated with both species of this complex.

These experiments demonstrate the presence of an untwisting activity, associated with 50S nucleoprotein complex, that could act as a swivel during replication of

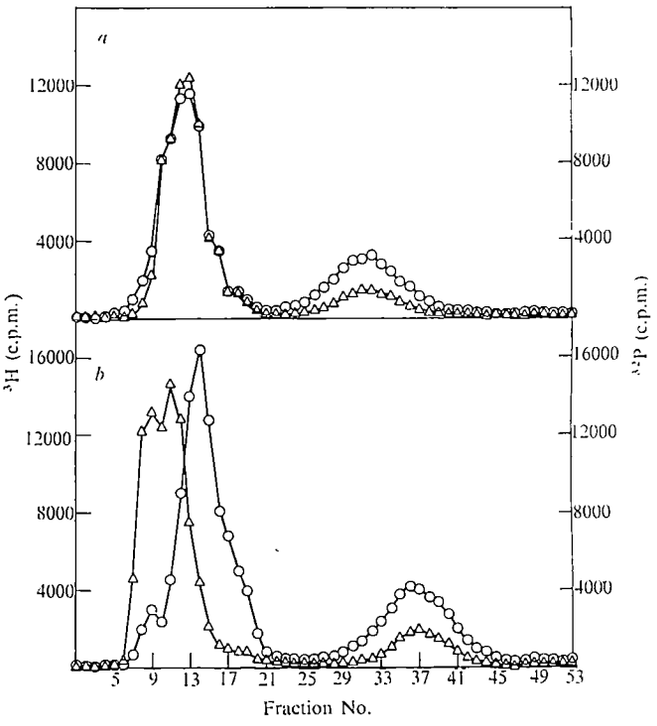


Fig. 1 EtBr-CsCl equilibrium gradients of SV40 DNA incubated with the SV40 nucleoprotein complex. SV40-infected AGMK cells were labelled with ^{3}H-thymidine 16–36 h after infections. The cells were lysed as described previously and the 50S complex was isolated from a sucrose gradient (ref. 9 and A. S., R. Hancock, and A. J. L., unpublished). The complex was then concentrated (5–8 fold) by dialysis against glycerol followed by dialysis against 10 mM Tris buffer (*p*H 7.4), 0.2 M NaCl, 4 mM EDTA and 1 mM β-mercaptoethanol. The ^{3}H-labelled (thymidine) nucleoprotein complex was incubated at 37°C for 2 h with or without exogenously added ^{32}P-labelled purified Form I SV40 DNA (ref. 7). The reaction mixture contained 0.5 μg Form I viral DNA (1.5×10^5 c.p.m. μg^{-1}), 10 mM Tris buffer (*p*H 7.9), 0.1 m NaCl, 2 mM EDTA. The reaction was stopped by adding 1 M NaCl and 1.598 g ml^{-1} of CsCl ($\eta = 1.3900$). 100 μg ml^{-1} of EtBr was added (final concentration) and this mixture (6 ml total) was spun to equilibrium at 32,000 r.p.m. for 90 h in a fixed angle 50 rotor. Samples were collected and counted as described previously[7]. ○, ^{3}H c.p.m. endogenous complex SV40 DNA; △, ^{32}P c.p.m. exogenously added SV40 DNA.

Table 1 Test for nuclease activity in the 50S complex

Procedure	Endogenous DNA Form I	Endogenous DNA Form II	Exogenous DNA Form I	Exogenous DNA Form II
No incubation	90%	10%	76%	24%
Incubation: 2 h at 37°C (50S complex alone)	88%	12%	—	—
Incubation: 2 h 37°C (50S complex plus exogenous DNA)	94%	6%	72%	28%

The experimental details are presented in the legend to Fig. 1. The percentage of Form I and II DNA observed after each incubation produce was calculated from the experimental points in Fig. 1.

superhelical SV40 DNA. The untwisting activity cosediments with the 50S complex upon repeated centrifugation, and is not found sedimenting at the 50S position of a sucrose gradient when uninfected cells are lysed under similar conditions, but it is not clear whether this activity is associated with nucleoprotein complex *in vivo*. Experiments are in progress to characterise conditions where the untwisting activity will act as efficiently upon endogenous viral DNA in the nucleoprotein complex as on exogenously added purified Form I DNA. When such conditions are determined, the nucleoprotein complexes containing replicating viral DNA would then become useful templates for an *in vitro* DNA replication system.

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1 Jaenisch, R., Mayer, A., and Levine, A. J., *Nature new Biol.*,
2 Sebring, E. D., Kelly, T. J., Thoren, M. M., and Selzman, N. P., *J. Virol.*, **8**, 478 (1971).
3 Mayer, A., and Levine, A. J., *Virology*, **50**, 328 (1972).
4 Wang, J. C., *J. molec. Biol.*, **43**, 236 (1969).
5 Wang, J. C., *J. molec. Biol.*, **55**, 523 (1971).
6 Champoux, J. J., and Dulbecco, R., *Proc. natn. Acad. Sci., U.S.A.*, **69**, 143 (1972).
7 Levine, A. J., Kang, H. S., and Billheimer, F. E., *J. molec. Biol.*, **50**, 549 (1970).
8 Hirt, B., *J. molec. Biol.*, 26, 365 (1970).
9 Green, M. H., Miller, H. J., and Hendler, S., *Proc. natn. Acad. Sci., U.S.A.*, **68**, 1032 (1971).
10 Eeason, M., and White, R., *J. Virol.*, **8**, 363 (1971).
11 Sen, A., Hancock, R., Levine, A. J., *Virology* (in press).
12 Tentmayer, P., and Ozer, H. L., *Virol.*, **9**, 516 (1971).
13 Mayer, A., thesis, Princeton Univ., (1972).

Enzymatic aziridine synthesis from β aminoalcohols—a new example of endogenous carcinogen formation

BETA aminoalcohols are not only used as drugs, such as ephedrine, chloramphenicol or pronethalol, but also occur naturally in animals and man, as adrenaline and noradrenaline, for example. It would be interesting to know whether these β aminoalcohols can be metabolised to aziridines, a class of carcinogenic compounds. Howe[1] described the carcinogenicity of pronethalol (I) and supposed that the aziridine (II) could be the ultimate carcinogen (Fig. 1).

In vitro formation of aziridines from β aminoalcohols only occurs through a less stable intermediate, for example by the sulphate ester in the Wenker synthesis[2]. On the other hand, the enzymatic formation of sulphate esters is catalysed by various sulphotransferases and requires phosphoadenosinephosphosulphate (PAPS) as a sulphate donor[3]. For practical purposes PAPS can be substituted by a mixture of ATP and SO_4^{2-}, which is transformed partially into PAPS by enzymes, contained in the supernatant fraction of liver homogenates[4]. It should be noted, however, that the carcinogenic 2-acetylaminofluorene is also metabolised through a sulphate ester to the ultimate carcinogen[5]. We supposed that the hydroxyl group of β aminoalcohols also could react enzymatically in special conditions to form O sulphates. These sulphates can then be transformed into aziridines in a slightly alkaline medium. To confirm this hypothesis we investigated the enzymatic formation of 1, 2-dimethyl-2-phenylaziridine[6] (III) from the β aminoalcohol[6] (IV), an isomer of ephedrine (Fig. 1):

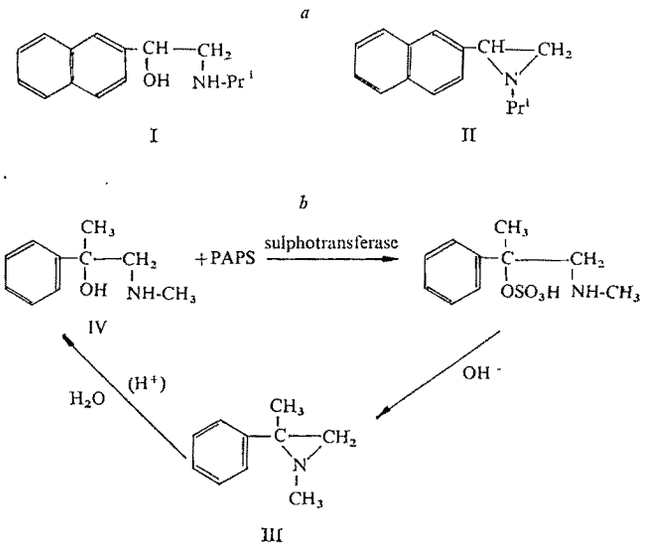


Fig. 1 *a*, Pronethalol (I) and its aziridine (II); *b*, enzymatic formation of 1, 2-dimethyl, 2-phenylaziridine from the β aminoalcohol.

A liver homogenate of a female Hauben rat (20% in 0.1 M potassium phosphate buffer, *p*H 7) was prepared by means of a Potter homogeniser and centrifuged at 100,000*g* for 1 h. To 5 ml of the supernatant, 75 μmol **IV**, dissolved in 0.05 ml ethanol, 50 μmol ATP, 50 μmol K_2SO_4 and 50 μmol $MgCl_2$ were added. After 2.5 h incubation at 37° C the reaction was terminated by the addition of 10 ml of ethanol and the precipitate removed by centrifugation. The supernatant was adjusted to approximately *p*H 8 by 1N NaOH and incubated at 37° C for 1 h. After evaporating to 5 ml under reduced pressure, the alkaline solution was extracted with ether. Basic compounds were separated from the ether solution by extraction with 2N H_2SO_4. The acid solution was alkalised immediately to avoid opening the ring of the aziridine formed. Repeated extraction with ether and evaporation to about 0.5 ml yielded a concentrate, which was investigated by means of TLC on silica gel. The solvent systems used were ethyl acetate–ammonia–methanol (85 : 5 : 10) and benzene–acetone (1 : 1), respectively.

For detecting aziridines, the colour reaction of 4-(4-nitrobenzyl-)pyridine perchlorate (NBP-ClO_4) with alkylating agents[7] was modified. The plate was sprayed with 0.5% NBP-ClO_4 in 1, 2-dimethoxyethane, incubated at 100° C for 30 min and, after cooling, sprayed with 25% aqueous ammonia. The aziridine (III) appeared blue with R_f values of 0.80 and 0.32, respectively, identical with those of authentic samples in both systems. After standing overnight in 2N H_2SO_4, **III** could not be detected in the extract, indicating ring-opening to **IV**. When SO_4^{2-} and ATP were not added to the reaction mixture, aziridine (III) was not formed. We therefore suppose that the aziridine is formed enzymatically from the β aminoalcohol according to the pathway shown in Fig. 1.

These investigations were made from the point of view that aziridines could be synthesised enzymatically not only from various drugs, but also from biogenic β aminoalcohols. This

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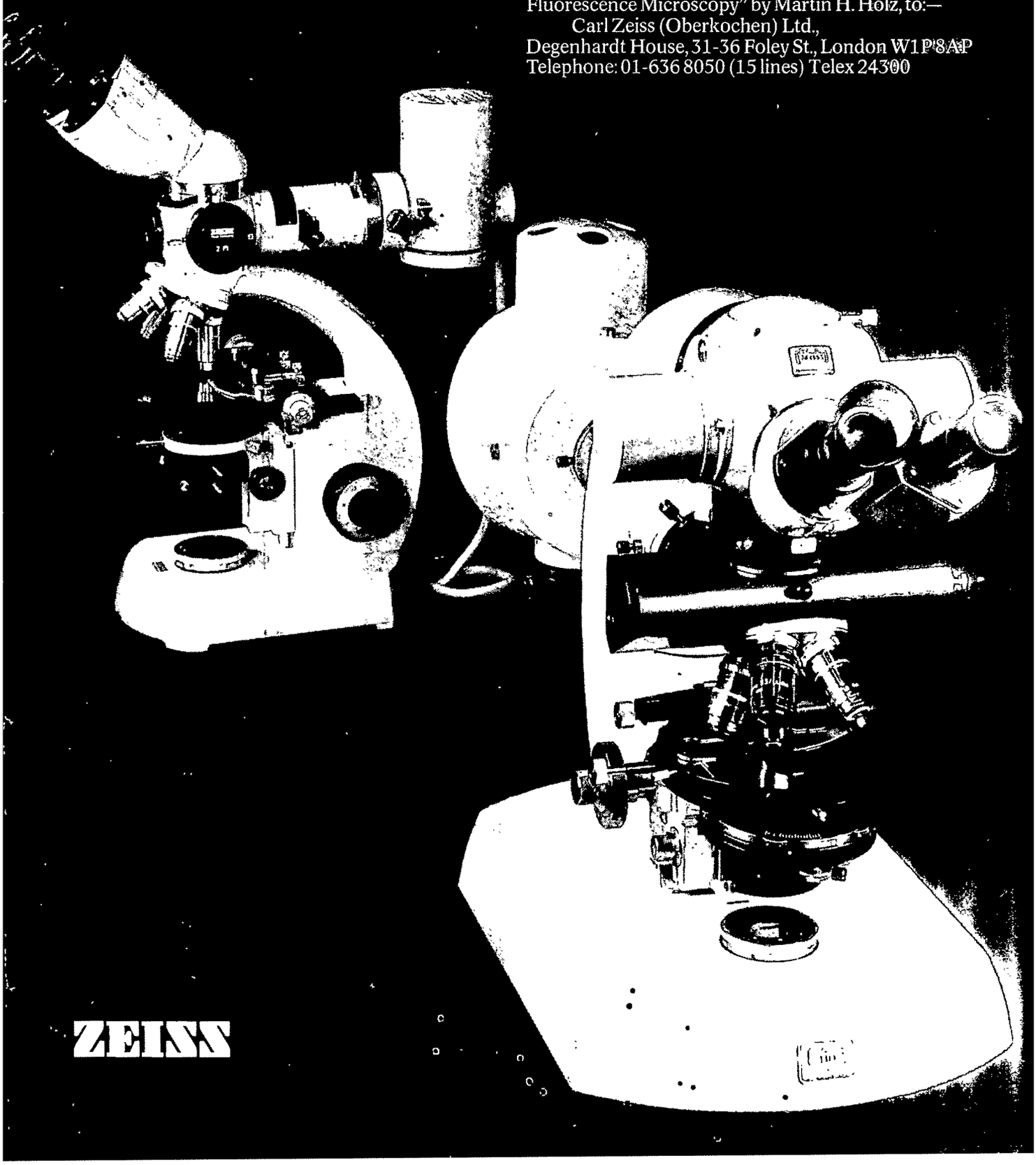
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aziridine synthesis might be one of the causes of tumours in animals and men[1].

Further investigations on the formation of aziridines from the above mentioned β aminoalcohols and on the carcinogenicity of these compounds are in progress.

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[1] Howe, R., *Nature*, **207**, 594 (1965).
[2] Wenker, H., *J. Am. chem. Soc.*, **57**, 2328 (1935).
[3] Williams, R. T., in *Biogenesis of Natural Compounds*, second ed. (edit. by Bernfeld, P.) 589 (Pergamon Press, New York, 1967).
[4] De Meio, R. H., and Lewycka, C., *Endocrinology*, **56**, 489 (1955).
[5] De Baun, J. R., Miller, E. C., and Miller, J. A., *Cancer Res.*, **30**, 577 (1970).
[6] Bicker, U., Dissertation, German Academy of Science, (Berlin, 1972).
[7] Preussmann, R., Schneider, H., and Epple, F., *Azrneimittel-Forsch*, **19**, 1059 (1969).

Intragenic regulation of the synthesis of ΦX174 gene *A* proteins

ONLY one phage gene, *A*, has been shown to be necessary for the replication of ΦX174 double-stranded replicative form (RF) DNA[1–3]. The host genes required have yet to be fully determined although one host mutation, *rep*$^-$$_3$, prevents progeny RF synthesis[4]. Dissection of the characteristics of gene *A* has shown that it is (*a*) *cis* acting[5]; (*b*) necessary for the detection of a *cis*-related viral strand specific discontinuity in the parental RF[6,7]; (*c*) the region of the genome which contains the origin of DNA replication[8], and (*d*) a gene from which two proteins, A and A′ (molecular weights 35,000 and 60-65,000, respectively) are synthesised[9,10].

A model for the synthesis of two proteins from one gene is shown in Fig. 1. The synthesis of the smaller protein, A, is initiated internal to gene *A*. Amber mutants mapping from N14 to R7 synthesise only the A molecule, while amber mutants mapping from H90 to S29 do not synthesise either molecule[9]. The tryptic peptides of A′ have been shown to include the tryptic peptides of A in addition to unshared peptides[10].

While trying to understand how two proteins could be synthesised from one gene, we noticed that more of the smaller molecule, A, was synthesised than the larger A′. If a single mRNA served as a template for both proteins of gene *A*, a simple explanation for this observation could be that A′ is translated only from the new mRNA while A is synthesised from old mRNA. This model would predict that synthesis of A′ but not A would be prevented by preventing phage mRNA synthesis.

Preliminary experiments with cells irradiated with ultraviolet light before infection (to eliminate host protein synthesis[11,12]) indicated that A′ synthesis was selectively prevented when rifampicin of 200 μg ml^{-1} was added various times after infection (1, 5, 10 and 15 min). To verify that this effect was associated with gene *A* function, DNA replication experiments were planned. Therefore, the rifampicin effect was then examined in mitomycin C-pre-

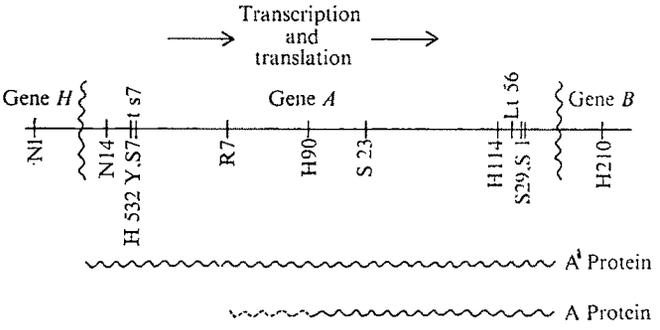


Fig. 1 A model representing synthesis of two proteins from gene *A* of ΦX174. The presumed internal initiator would be between the R7 and H90 positions. Amber *A* mutants N14, S7, H532y and R7 synthesise a stable A molecule while amber *A* mutants H90, S23, H114, S29, and S1 do not synthesise stable A or A′ molecules. Lt 56 and t's-7 are temperature-sensitive gene *A* mutants. The transcriptional and translational directions were derived from previous studies[25–28]. The map of gene *A* was constructed by Marie N. Hayashi.

treated cells (to eliminate host DNA synthesis[2]) using two hosts: *E. coli* HF4704 Rif^r, a mutant resistant to rifampicin in RNA polymerase, and *E. coli* HF4704, a rifampicin-sensitive host. Figure 2*a* shows the effect of adding 200 μg ml^{-1} of rifampicin on ^{3}H-uridine incorporation with mitomycin C-pretreated, ΦX174-infected HF4704 15 min after infection. Figure 2*b* illustrates the same with the rifampicin-resistant host, HF4704 Rif^r. Rifampicin selectively inhibits RNA synthesis in HF4704 cells but not in HF4704 Rif^r cells.

The same protocol was used for labelling ΦX174-specific proteins. Rifampicin (200 μg ml^{-1}) was added 15 min after infection and ^{3}H-lysine was added 1 min later. The infection was allowed to proceed for 15 min and then the protein was isolated and run on sodium dodecyl sulphate (SDS) polyacrylamide gels[11]. Control cultures without rifampicin were labelled with ^{14}C-lysine from 16 to 30 min after infection (with no mitomycin C pretreatment of cells, phage protein is primarily synthesised 15 min after infection (E. Linney, unpublished observation)). Figure 3*a* shows the gel patterns of protein from the infected rifampicin-resistant host. The patterns with or without rifampicin are identical. Figure 3*b* illustrates that with the rifampicin sensitive host A′ is clearly not synthesised when rifampicin is added. All the other phage proteins, previously identified by this laboratory[9,11], are synthesised.

If A is translated from old mRNA, the ratio of A/A′ synthesised might be expected to increase as the infection proceeds. Table 1 summarises results which illustrate this point. *E. coli* HF4704 was preirradiated with ultraviolet light to eliminate host protein synthesis. Cells were then infected with an amber mutant, N11, in the lysis gene *E*. Protein was labelled with ^{3}H-lysine from 0–10, 10–20 and 30–40 min after infection. The protein was isolated and separated on SDS polyacrylamide gels[9,11]. The ratio of A/A′ was calculated from their regions in the gels. In addition, a 0–10 min labelled culture was washed and chased 10–30 min after infection with cold lysine. Similarly, a 10–20 min culture was washed and chased 20–40 min after infection with cold lysine. The results in Table 1 support the view that more A is synthesised than A′ as the infection proceeds. Additionally the chase data support the view that A′ does not chase into the smaller protein A.

Because of the many conflicting reports on the molecular weight of gene *A* products[9,11,13–15], this effect of rifampicin on the synthesis of A′ was examined to see whether it correlated with gene *A* function. To examine this, mitomycin C-pretreated HF4704 cells were infected in the pre-

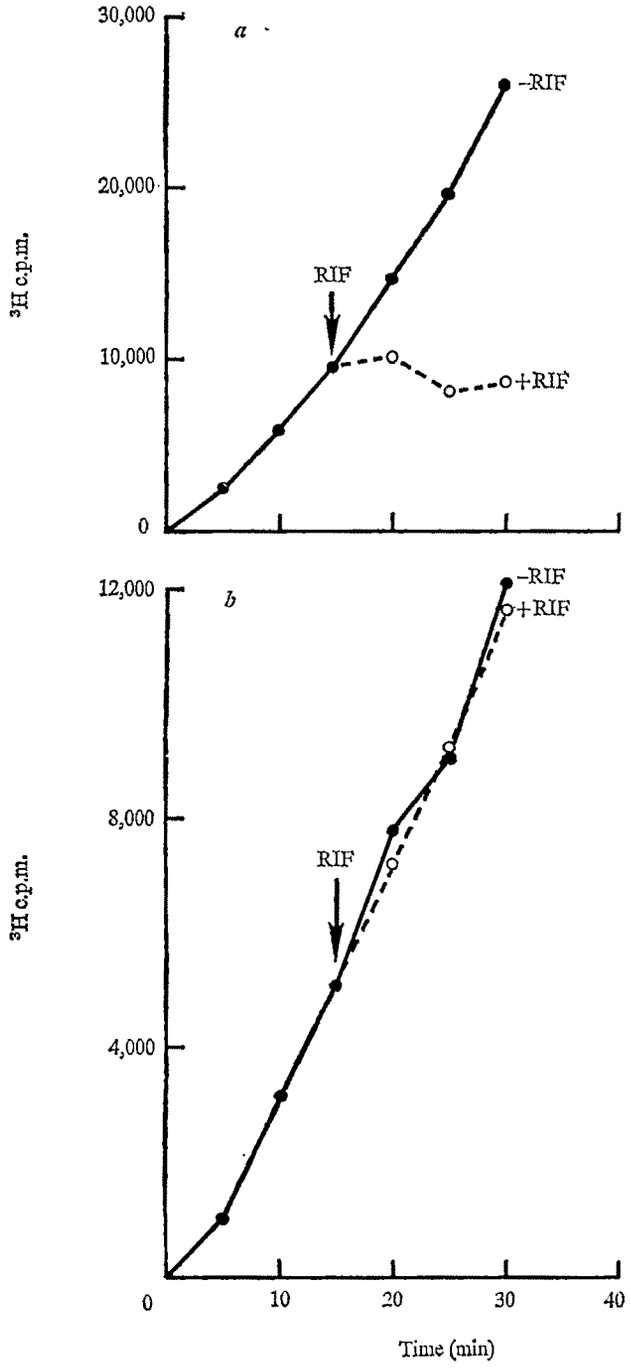


Fig. 2 Incorporation of ^{3}H-uridine into mitomycin C-pretreated, ΦX174-infected cells. *E. coli* HF4704 Rif^r is a spontaneuos mutant of *E. coli* HF4704 selected on rifampicin plates by Marie N. Hayashi. It was found to be rifampicin-resistant in RNA poly erase. HFC and HFB medium have been described previously[11]. Cells were pretreated with mitomycin C at 37° C at a cell concentration of 5×10^8 ml^{-1} in HFC for 30 min with no agitation[2]. Cells were then sedimented and washed once in cold HFB. Phages were adsorbed for 5 min in HFB at a cell concentration of 5×10^9 ml^{-1} at 37° C before starting the infection by diluting ten-fold with HFC. RNA was labelled by adding 20 μCi ml^{-1} ^{3}H-uridine (New England Nuclear Corp., 26 Ci mmol^{-1}) to cultures containing 2 μg ml^{-1} uridine. Fractions (200 μl) were taken for incorporation experiments, precipitated with trichloroacelic acid (TCA) and collected on Whatman GF/C filters for scintillation counting. *a*, Host cell, HF4704; no rifampicin (●—●) 200 μg ml^{-1} rifampicin added 15 min after infection (O- - -O). *b*, Host cell rifampicin-resistant, HF4704 Rif^r; no rifampicin (●—●); 200 μg ml^{-1} rifampicin added 15 min after infection (O- - -O).

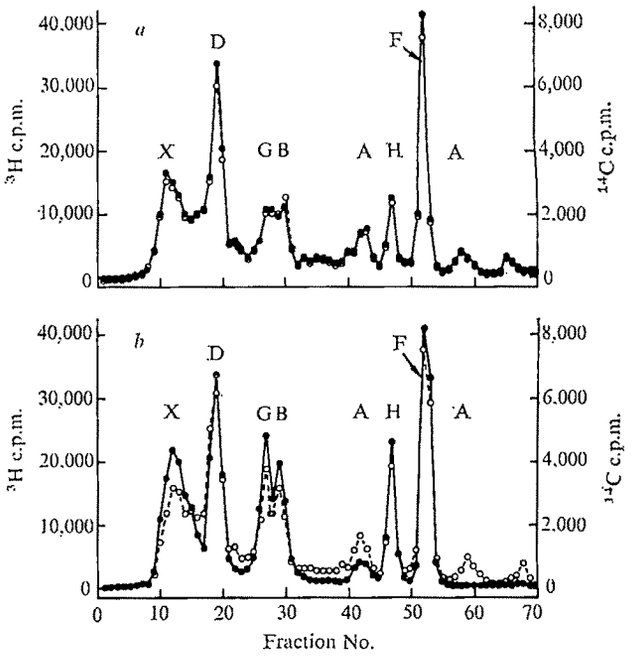


Fig. 3 SDS polyacrylamide gel of ^{3}H and ^{14}C-lysine of ΦX174 total protein from mitomycin C-pretreated cells labelled 16-30 min after infection. Decreasing molecular weight is to the left. The letters represent the gene products previously identified in this laboratory[9,11]. The electrophoresis methods have been described previously[9]. For labelling protein 20 μg ml^{-1} of ^{3}H-lysine (Amersham-Searle, 5.3 6 mmol^{-1}) was added at the given time or ^{14}C-lysine (Amersham-Searle, 342 MCi mmol^{-1}) at an equivalent lysine concentration. *a*, Protein from ΦX174-infected HF4704 Rif^r: 200 μg ml^{-1} rifampicin, 15 min after infection, ^{3}H-lysine, 16-30 min after infection (●—●); no rifampicin, ^{14}C-lysine 16-30 min after infection (O- - -O). *b*, Protein from ΦX174-infected HF4704: 200 μg ml^{-1} rifampicin 15 min after infection (——); ^{3}H-lysine, 16-30 min after infection (●—●); no rifampicin, ^{14}C-lysine, 16-30 min after infection (O- - -O).

sence of 200 μg ml^{-1} chloramphenicol to allow parental RF synthesis[1,16] and mRNA synthesis (unpublished observations of M.H.) but to inhibit protein synthesis. After 10 min of infection the chloramphenicol was washed out in the presence and in the absence of 200 μg ml^{-1} of rifampicin. After washing, the infection was allowed to proceed in the presence or absence of 200 μg ml^{-1} of rifampicin, respectively. Thus, the rifampicin culture would have to rely on old mRNA for gene *A* function. Figure 4 illustrates ^{3}H-thymidine incorporation under these conditions. In the presence of chloramphenicol for 30 min, DNA replication was inhibited while if the chloramphenicol was removed in the absence of rifampicin DNA replication proceeded. If chloramphenicol was replaced with rifampicin, DNA replication remains inhibited.

Thus, phage DNA replication is prevented when it is made dependent on old phage mRNA. That this effect is related to the absence of A′ synthesis and not the absence of a necessary newly synthesised host protein is suggested by previous findings that in cells preirradiated with ultraviolet light to eliminate host protein synthesis, phage DNA replication occurs[11].

The results indicate that A′ is necessary for gene *A* function. The tight coupling between translation and transcription could be responsible for the *cis* effect of gene *A*. Since both A and A′ have been shown to bind tightly to DNA cellulose columns[9], if A′ is synthesised at the DNA template as the tight coupling suggests, this affinity might increase the possibility of A′ remaining there and not diffusing to another phage DNA molecule.

Because the smaller gene *A* protein, A, and the other identified phage proteins are synthesised in the presence of rifampicin the results suggest that the 5′ end of the gene *A* message is unique. A promoter is presumed to be located at the beginning of gene *A*[17,18] because the observed gene *F* to gene *H* polarity has not been found to extend to gene *A*. Although ΦX174 mRNA has been found to be structurally stable in a macromolecular sense[17], it has been shown[19] that the functional half life of gene *F* mRNA from the related phage S13 follows a two-component curve: about 70% of functional message decays with a half life ($t_{1/2}$) of approximately 1.4 min and 30% with a $t_{1/2}$ of approximately 14 min. Since a promoter site is presumed to be at the beginning of gene *F* (ref. 18), the $t_{1/2}$ of approximately 1.4 min was suggested to be caused by rapid degradation of the 5′ end of the gene *F* message[19]. This model of degradation from the promoter end of phage mRNA[19] is consistent with our results for A′ synthesis. Quantitation of A′ synthesis immediately after the addition of rifampicin is impossible at this point in time because it is a minor peak in the total gel profile. When rifampicin and ^{3}H-lysine are added together, 15 min after infection, no detectable A′ is synthesised. The possibility remains that a small base-pairing region at the 5′ end of gene *A* message rather than selective degradation at the end could explain this phenomenon. However, the decrease in A synthesis after the addition of rifampicin (see Fig. 3*b*) supports the degradation model (Fig. 5). It is still not known whether there exists a promoter internal to gene A that might also explain the synthesis of the smaller gene *A* protein. If this were the correct explanation there would still be different mRNA stabilities for the A′ and A mRNAs.

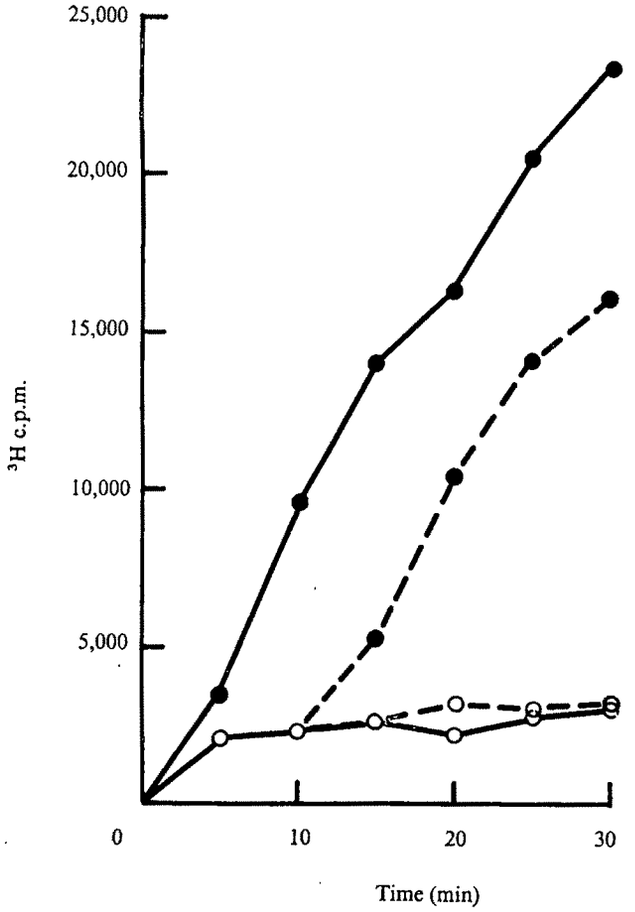


Fig. 4 ^{3}H-thymidine incorporation into mitomycin C-pretreated HF4704, ΦX174-infected cultures. Chloramphenicol (a gift from Parke-Davis Co.) was used at a concentration of 200 μg ml^{-1}. Cells were washed twice with HFC with ^{3}H-thymidine plus or minus rifampicin to remove the chloramphenicol. Rifampicin (Calbiochem) dissolved in dimethyl sulphoxide (20 mg ml^{-1}) was used at a concentration of 200 μg ml^{-1}. DNA was labelled by adding 20μCi ml^{-1} of ^{3}H-thymidine (New England Nuclear Corp., 6.7 Ci mmol^{-1} was added to the media containing 5 μg ml^{-1} thymine. No chloramphenicol (●—●); 200 μg ml^{-1} chloramphenicol from 0 to 30 min after infection (O—O); chloramphenicol present from 0 to 10 min after infection and then washed out (●···●); chloramphenicol washed out in the presence of 200 μg ml^{-1} of rifampicin 10 min after infection and the infection then allowed to proceed in the presence of rifampicin (O···O)

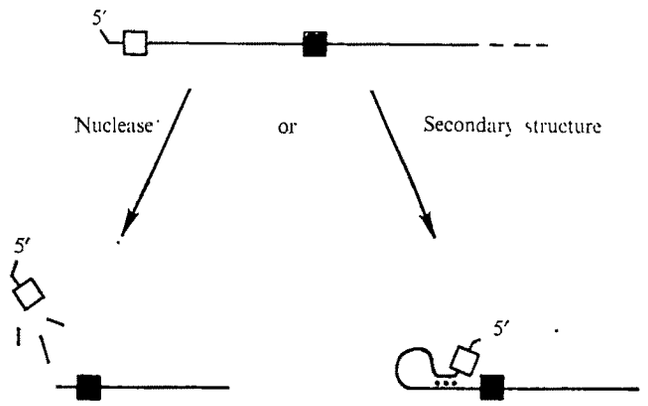


Fig. 5 Two models for gene *A* on RNA. □ A′ translational initiation site; A translational initiation site.

Table 1 Ratio of ^{3}H-lysine c.p.m. in A and A′ from *E. coli* HF4704 preirradiated with ultraviolet light to eliminate host protein synthesis[11,12] and then infected with the ΦX174 lysis mutant, N11

Conditions	A c.p.m./A′ c.p.m.
0-10 min pulse	1.9
10-30 min chase	1.9
10-20 min pulse	2.5
20-40 min chase	2.6
30-40 min pulse	2.8

The effect of rifampicin on ΦX174 DNA replication has been described before. Although it does not prevent complementary strand synthesis on the incoming single-stranded DNA molecule[20], as has been found for M13 (ref. 21), it has been shown to inhibit phage DNA replication when added before infection[22]. Addition of rifampicin later in infection allowed replication to occur. The assumption made in ref. 22 was that gene *A* mRNA was long lived and sufficient mRNA was available for gene *A* to function after the addition of rifampicin. The results are consistent with ours but our interpretation would be that once gene *A* is allowed to function, new A′ is not necessary for continued phage DNA replication.

Although the results suggest that the larger of the two gene *A* proteins is necessary for gene *A* function, they provide no explanation for the smaller protein, A. For reasons we cannot explain this molecule is not clearly separated from gene *H* protein when *bis* acrylamide is used as a cross-linker in SDS polyacrylamide gels. Since *bis* acrylamide has been commonly used by the other laboratories, our use of ethylene diacrylate as a cross-linker which clearly separates A and H (see Fig. 3*a* and *b*) explains why no other laboratory has associated a 35,000 molecular weight protein with gene *A*. No function has been established for A, though a minor virion component (approximately one molecule per particle[13]) described previously[13,23,24] has been

found to coelectrophorese with A isolated from single-stranded DNA cellulose columns.

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1 Tessman, E. S., *J. molec. Biol.*, **17**, 218 (1966).
2 Lindqvist, B. H., and Sinsheimer, R. L., *J. molec. Biol.*, **30**, 69 (1967).
3 Iwaya, M., and Denhardt, D. T., *J. molec. Biol.*, **57**, 159 (1971).
4 Denhardt, D. T., Dressler, D. H., and Hathaway, A., *Proc. natn. Acad. Sci. U.S.A.*, **57**, 803 (1967).
5 Tessman, E. S., *Virology*, **25**, 303 (1965).
6 Francke, B., and Ray, D. S., *J. molec. Biol.*, **61**, 565 (1971).
7 Francke, B., and Ray, D. S., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 475 (1972).
8 Baas, D. D., and Jansz, H. S., *J. molec. Biol.*, **63**, 569 (1972).
9 Linney, E. A., Hayashi, M. N., and Hayashi, M., *Virology*, **50**, 381 (1972).
10 Linney, E., and Hayashi, M., *Nature new Biol.*, **245**, 6 (1973).
11 Gelfand, D. H., and Hayashi, M., *J. molec. Biol.*, **44**, 501 (1969).
12 Burgess, A. B., and Denhardt, D. T., *J. molec. Biol.*, **44**, 377 (1969).
13 Godson, G. N., *J. molec. Biol.*, **57**, 541 (1971).
14 Benbow, R. M., Mayol, R. F., Picchi, J. C., and Sinsheimer, R. L., *J. Virol.*, **10**, 99 (1972).
15 Van der Mei, D., Zandberg, J., and Jansz, H. S., *Biochim. biophys. Acta*, **287**, 312 (1972).
16 Sinsheimer, R. L., Hutchison, C. A., and Lindqvist, B., in *Molecular biology of viruses*, 175 (Academic Press, New York 1967).
17 Hayashi, Y., and Hayashi, M., *Cold Spring Harbor Symp. quant. Biol.*, **35**, 171 (1970).
18 Vanderbilt, A. S., Borrás, M. T., Germeraad, S., Tessman, I., and Tessman, E. S., *Virology*, **50**, 171 (1972).
19 Puga, A., Borrás, M. T., Tessman, E. S., and Tessman, I., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2171 (1973).
20 Silverstein, S., and Billew, D., *Biochim. biophys. Acta*, **247**, 383 (1971).
21 Brutlag, D., Schekman, R. W., and Kornberg, A., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2826 (1971).
22 Van der Mei, D., Brons, J. Th., and Jaisz, H. S., *Biochim. biophys. Acta*, **262**, 463 (1972).
23 Suruda, A. J., and Poljak, R. J., *Virology*, **46**, 164 (1971).
24 Zuccarelli, A., Benbow, R. M., and Sinsheimer, R. L., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1905 (1972).
25 Hayashi, M., Hayashi, M. N., and Spiegelman, S., *Proc. natn. Acad. Sci. U.S.A.*, **50**, 664 (1963).
26 Vanderbilt, A. S., and Tessman, I., *Nature*, **228**, 54 (1971).
27 Tessman, I., Kumar, S., and Tessman, E. S., *Science*, **158**, 267 (1967).
28 Vanderbilt, A. S., Borrás, M., and Tessman, E. S., *Virology*, **43**, 352 (1971).

Requirement for either DNA polymerase I or DNA polymerase III in post-replication repair in excision-proficient *Escherichia coli*

WHEN *Escherichia coli* bacteria deficient in excision repair (*uvr*) replicate their DNA after ultraviolet irradiation the newly-synthesised DNA contains gaps which are rejoined during subsequent incubation[1] by a postreplication repair process believed to be recombinational in nature[2]. Double mutants (*uvr recA*) deficient in both excision and post-replication repair are up to 25 times more sensitive to ultraviolet than those deficient in excision alone[3]. Bacteria deficient in excision repair and DNA polymerase I (*uvr polA*) in contrast are no more ultraviolet-sensitive than those deficient solely in excision[4], which might suggest that DNA polymerase I is not required for successful post-replication repair. The fact that DNA polymerase III seems to be able to substitute for DNA polymerase I in the repair of excision gaps[5], and type II DNA strand breaks[6] after X irradiation, however, indicates that DNA polymerase I cannot be excluded from involvement in post-replication repair if similar substitution by DNA polymerase III could occur there.

We have therefore examined the filling and joining of daughter-strand gaps in DNA synthesised after exposure to ultraviolet of bacteria deficient in DNA polymerase I (*polA*) and possessing temperature-sensitive DNA polymerase III activity (*dnaE*). The results are consistent with a requirement for either DNA polymerase I or DNA polymerase III in the gap-filling process.

We used *E.coli* BT1026 *dnaE polA*-1 *thy* (ref. 7) and 1026-1 which is a *polA*$^+$ MMS-resistant revertant isolated by W. Goebel. Some experiments were also carried out with a related strain BT1126 *dnaE polA*-1 *thy* (ref. 8) which carries a different *dnaE* mutation. All these strains are deficient in endonuclease I. The bacteria were grown at 33° C (permissive temperature) to about 2×10^8 ml^{-1} in M9 minimal medium supplemented with 1% casein hydrolysate and 2 μg ml^{-1} thymine and exposed to ultraviolet. They were then pulse labelled at 33° C by the addition of 100 μCi (^{3}H-methyl) thymidine ml^{-1} for a period of 7.5 to 12.5 min, filtered on membrane filters, washed with ten volumes of prewarmed buffer, and resuspended in unlabelled medium. The length of the pulse was adjusted to ensure that label uptake was not too dissimilar in irradiated and unirradiated cultures to minimise end-error artefact[9]. The molecular weight of the DNA synthesised during the pulse was examined by centrifugation through an alkaline sucrose density gradient immediately after the pulse and at various times during subsequent incubation of the bacteria at 33° C or 44° C (restrictive temperature for *dnaE*).

Strain 1026-1 is phenotypically PolA$^+$ DnaE$^+$ at 33° C and, as expected, filled gaps in daughter-strand DNA efficiently within 20 min at that temperature (Fig. 1*b*, *c*). At 44° C, daughter-strand gaps were also joined within 20 min. (Fig. 1*d*) indicating that postreplication repair can occur with no noticeable loss of efficiency in the absence of DNA polymerase III activity. It also shows that continued normal semiconservative replication is not necessary for post-replication gap filling.

As found with other *polA* bacteria[10], DNA synthesised in BT1026 at 33° C did not reach normal size as fast as in *polA*$^+$

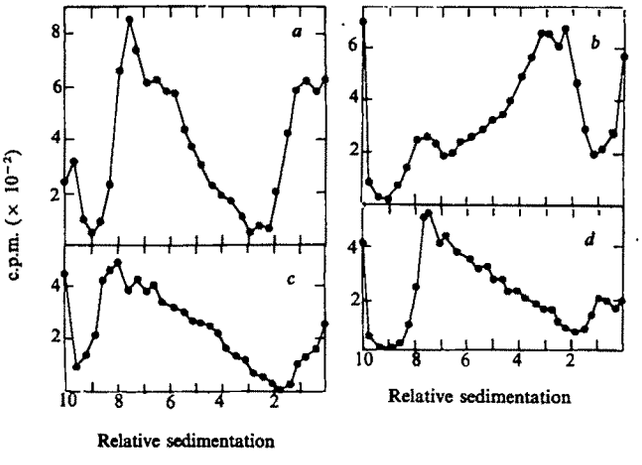


Fig. 1 Alkaline sucrose gradient sedimentation profiles of newly-synthesised DNA from *E. coli* 1026-1. *a* No ultraviolet, 7.5 min ^{3}H-thymidine pulse; *b* 100 ergs mm^{-2} ultraviolet, 10 min ^{3}H-thymidine pulse; *c*, 100 ergs mm^{-2} ultraviolet, 10 min ^{3}H-thymidine pulse, 20 min incubation at 33° C; *d*, 100 ergs mm^{-2} ultraviolet, 10 min ^{3}H-thymidine pulse, 20 min incubation at 44° C.

bacteria and about 20 min of subsequent incubation were needed before the normal profile was obtained (Fig. 2*a*). Further incubation did not result in any further increase in size. DNA synthesised in irradiated BT1026 was of smaller size. After 80 min incubation at 33° C, however, the DNA had been joined together to form pieces of normal size (Fig. 2*c*). Thus at 33° C, where BT1026 is phenotypically PolA$^-$ DnaE+, daughter-strand gaps could be filled indicating that a deficiency of DNA polymerase I does not prevent postreplication gap-filling although the process is slower in this strain.

In BT1126 there was postreplication repair during 30 min incubation at 33° C (Fig. 3*c*) indicating that in this strain the polymerase I deficiency did not markedly slow down the rate of gap filling. Other experiments have shown that *polA dnaE*$^+$ bacteria are able to carry out gap filling at 44° C (data not shown).

In contrast, in both BT1026 and BT1126 at 44° C, where the bacteria were phenotypically PolA DnaE$^-$, DNA that had been synthesised on an irradiated template (Fig. 3*b*) never increased to normal size and was degraded (Figs 2*d* and 3*d*). Degradation was faster in BT1126, the smaller pieces being completely acid-soluble within 15 min. Thus where both polymerase I and polymerase III were inactive there was no detectable filling of daughter-strand gaps. If there was any residual repair (perhaps involving DNA polymerase II) it was very limited in extent and did not give rise to DNA of normal size.

An interesting observation with BT1026 was that when unirradiated bacteria were transferred to 44° C after being pulse labelled at 33° C, the initially small DNA increased in

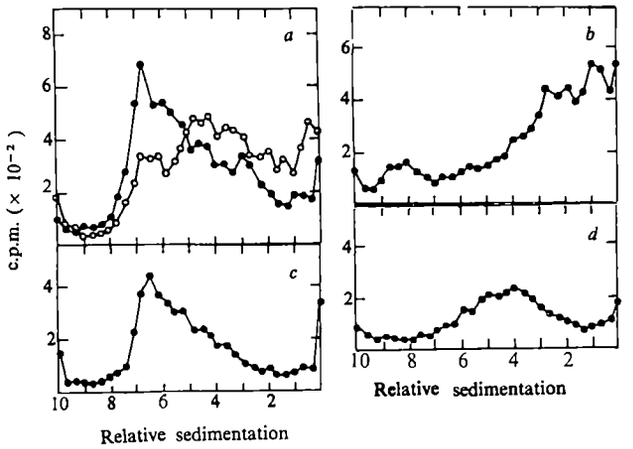


Fig. 2 Alkaline sucrose gradient sedimentation profiles of newly-synthesised DNA from *E. coli* 1026. *a*, No ultraviolet, 7.5 min ^{3}H-thymidine pulse with no (●) or 20 min (O) incubation at 33°; *b*, no ultraviolet, 7.5 min ^{3}H-thymidine pulse, with 20 min (●) or 80 min (O) incubation at 44° C; *c*, 100 ergs mm^{-2} ultraviolet, 12.5 min ^{3}H-thymidine pulse, no (●), 20 min (O) or 80 min (▲) incubation at 33°; *d*, 100 ergs mm^{-2} ultraviolet, 12.5 min ^{3}H-thymidine pulse, 20 min (●) or 80 min (O) incubation at 44° C.

size and sedimented at essentially the normal molecular weight after 20 min incubation but further incubation to 80 min resulted in a decrease in size without any loss of counts (Fig. 2*b*). We have no explanation for this observation.

We conclude that ultraviolet-irradiated bacteria deficient in either DNA polymerase I or DNA polymerase III are able to carry out postreplication gap filling. Bacteria deficient in both polymerases show little or no postreplication gap filling and tend to degrade newly-synthesised DNA. The results are consistent with the hypothesis that the two polymerases can substitute one for the other in daughter-strand gap filling, as in repair replication after excision repair[5]. The data do not give any indication as to which is the normal polymerase in conditions in which both enzymes are present and potentially active.

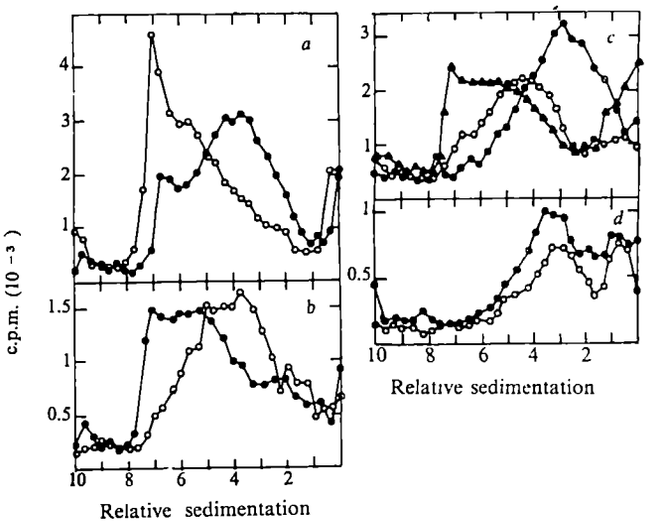


Fig. 3 Alkaline sucrose gradient sedimentation profiles of newly-synthesised DNA from *E. coli* BT1126. *a*, No ultraviolet, 7.5 min ^{3}H-thymidine pulse with no (●) or 30 min (O) incubation at 33 C; *b* 100 ergs mm^{-2} ultraviolet, 10 min ^{3}H-thymidine pulse; *c*, 100 ergs mm^{-2} ultraviolet, 10 min ^{3}H-thymidine pulse, 30 min incubation at 33° C; *d*, 100 ergs mm^{-2} ultraviolet 10 min ^{3}H thymidine pulse, 45 min incubation at 44° C.

It should be emphasised that these results have been obtained with excision-proficient strains. Their postreplication repair process may conceivably be different from that in excision-deficient strains. It is possible, for example, to formulate a model for excision-proficient bacteria in which photoproducts are excised from recently replicated parental DNA using the homologous parental strand transiently rehybridised with it as the template for repair replication. Such a process might be expected to require both excision and recombination genes. Circumstantial evidence for a process with such requirements has been recently reported[11].

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[1] Rupp, W. D., and Howard-Flanders, P., *J. molec. Biol.*, **31**, 291 (1968).
[2] Rupp, W. D., Wilde, E. E., Reno, D. L., and Howard-Flanders, P., *J. molec. Biol.*, **61**, 25 (1971).
[3] Howard-Flanders, P., and Boyce, R. P., *Radiation Res.*, Supplement, **6**, 156 (1966).
[4] Monk, M., Peacy, M., and Gross, J. D., *J. molec. Biol.*, **58**, 623 (1971).
[5] Youngs, D. A., and Smith, K. C., *Nature new Biol.*, **244**, 241 (1973).
[6] Youngs, D. A., and Smith, K. C., *J. Bact.*, **114**, 851 (1973).
[7] Gefter, M., Hirsta, Y., Kornberg, T., Barnoux, C., and Weschler, J. A., *Proc. natn. Acad. Sci., U.S.A.*, **68**, 3150 (1971).
[8] Weschler, J. A., Nusslein, V., Otto, B., Klein, A., Bonhoeffer, F., Herrman, R., Gloger, L., and Schaller, H., *J. Bact.*, **113**, 1381 (1973).
[9] Lehmann, A. R., and Ormerod, M. G., *Nature*, **221**, 1053 (1969).
[10] Okazaki, R., Arisawa, M., and Sugino, A., *Proc. natn. Acad. Sci., U.S.A.*, **68**, 2754 (1971).
[11] Bridges, B. A., *Nature new Biol.*, **240**, 52 (1972).

Is the cytoplasm a site of nuclear DNA synthesis?

OBSERVATIONS which do not harmonise with the original self-replicative scheme of DNA synthesis have received little attention. In particular, interest in cytoplasmic DNA synthesis in eukaryotic cells has been limited to semiautonomous structures which contain DNA templates. But it was pointed out[1] as early as 1958 that transfer of genetic information between DNA, RNA and protein often can proceed in either direction. It is possible that an initial step of DNA synthesis, corresponding for example to RNA-primed synthesis of Okazaki fragments[2,3], may generally occur out of cell nuclei and without direct participation of a DNA template.

Table 1 Incorporation of ^{14}C-thymidine into nuclear, organellar and cytoplasmic DNA fractions of germinating wheat embryos

Germination time (h)	DNA amount and radioactivity (per 100 embryos)					
	Nuclear		Organellar		Cytoplasmic	
	(μg)	(d.p.m.)	(μg)	(d.p.m.)	(μg)	(d.p.m.)
3	125	<10	15	<10	<5	<10
6	120	250	20	<10	<5	220
12	140	640	25	40	<5	210
24	170	1,350	20	150	<5	120

A sample of 350 wheat grains was surface sterilised with 2% NaClO and left at 2° C for 8 h with 10 ml of ^{14}C-thymidine solution (1.0 μCi ml^{-1}, 53 μC-μmol^{-1}). The prelabelled sample was washed with water and allowed to germinate at 22° C for the indicated time. Embryos were then separated and ground in a mortar with 10 ml of ice-cold 0.25 M sucrose containing 15 mM potassium phosphate (*p*H 7.3) and 10 mM $MgCl_2$. The homogenate was filtered through a nylon tissue and fractionated by centrifugation. Nuclear (1,000*g* pellet), organellar (18,000*g* pellet) and cytoplasmic (18,000*g* supernatant) fractions were obtained and washed with 0.3 M $HClO_4$ containing 1 mM thymidine. RNA was removed by an alkaline hydrolysis (0.3 M KOH, 37° C, 15 h) and DNA fraction obtained as an acid hydrolysate (0.5 M HCl, 100° C, 30 min). The acid hydrolysate was applied to paper chromatography in a water-saturated butan-1-ol solvent system[10]. Adenine and guanine were eluted from the corresponding spots and determined spectrophotometrically to calculate the DNA content. The origin, containing a mixture of pyrimidine nucleotides resulting from the acid hydrolysis of DNA, was used for the measurement of radioactivity in a Packard liquid scintillation counter.

We tested for a prenuclear stage of DNA formation in germinating wheat embryo, in which biosynthetic events triggered after the breaking of dormancy are naturally synchronised[4]. It has been observed[5] that incorporation of thymidine into DNA of germinating wheat seeds begins only after some preliminary activation of RNA synthesis is completed, implying an RNA-dependent DNA synthesis in this system. To check whether the cytoplasm may contribute to the early stage of DNA synthesis, wheat grains were prelabelled with ^{14}C-thymidine and germinated for various periods. Total amounts and radioactivities of nuclear, organellar and cytoplasmic DNA of the embryo were then determined (Table 1).

I found that after 6 h germination radioactive DNA appeared in the nuclear and soluble cytoplasmic fractions (Table 1). Specific radioactivity of the cytoplasmic DNA was, however, at least 20-fold higher than that of the nuclear DNA. As germination proceeded further the radioactivity of the cytoplasmic DNA decreased considerably and that of the nuclear DNA rose correspondingly. These observations are consistent with the hypothesis of a precursor–product relationship between the cytoplasmic and nuclear DNA. Rapid activation of cytoplasmic DNA synthesis in an early response to proliferative stimuli has been observed in other eukaryotic cells[6-8]. The experimental data do not exclude the possibility of an independent synthesis of the cytoplasmic and nuclear DNA. But the only explicable reason for the synthesis of deoxyribopolynucleotides in the supernatant fraction of the cytoplasm, which is virtually free of mature DNA molecules, is to supply them to the nucleus and other DNA-containing structures.

Another explanation is the transport of informational DNA from nucleus to cytoplasm, which has been reported for cultured animal cells[9]. This explanation, however, can be hardly extended to the germinating wheat embryo, where radioactivity of the cytoplasmic DNA decreases after the nuclear DNA becomes highly radioactive. The same observation seems to exclude the possibility of a significant contamination of the cytoplasmic fraction by nuclear DNA during the homogenate fractionation. I have therefore come to the conclusion that the cytoplasmic DNA synthesis may represent a prenuclear stage of the genome replication.

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[1] Chargaff, E., in *The Origin of Life on the Earth* (edit. by Oparin, A. I.), 297 (Pergamon Press, London, 1959).
[2] Okazaki, R., Okazaki, T., Sakebe, K., Sugimoto, K., Sugino, A., and Iwatsuki, N., *Cold Spring Harbor Symp. quant. Biol.*, **33**, 129 (1968).
[3] Okazaki, R., Sugino, A., Hirose, S., and Tamanoi, F., *Ninth Int. Congress of Biochem.*, Stockholm, 126 (1973).
[4] Dobrzanska, M., Tomaszewski, M., Grzelczak, Z., Rejman, E., and Buchowicz, J., *Nature*, **244**, 507 (1973).
[5] Rejman, E., and Buchowicz, J., *Phytochemistry*, **10**, 2951 (1971).
[6] Hanson, K. P., Ivanova, L. V., Nikitina, Z. S., Shutko, A. N., and Komar, V. E., *Biokhimiya*, **35**, 635 (1970).
[7] Chang, L. M. S., and Bollum, F. J., *J. biol. Chem.*, **247**, 7958 (1972).
[8] Chang, L. M. S., Brown, M. K., and Bollum, F. J., *J. molec. Biol.*, **74**, 1 (1973).
[9] Koch, J., and Pfeil, H. V., *FEBS Lett.*, **24**, 53 (1972).
[10] Buchanan, J. G., *Nature*, **168**, 1091 (1951).

Effect of 5-bromodeoxyuridine on the size distribution of DNAs isolated from sea urchin embryos

5-BROMODEOXYURIDINE (BUdR), an analogue of thymidine, has been shown to selectively inhibit cell differentiation in various cell types and development of embryos, without affecting cell maintenance capabilities[1]. Gontcharoff and Mazia[2] demonstrated that exposure of newly fertilised sea urchin eggs or early cleavage stage embryos to BUdR resulted in embryos which did not gastrulate or produce echinochrome pigment. From this laboratory evidence has been reported[3] for a BUdR-induced modification of DNA replication in developing sea urchin embyros. Pulse-chase experiments in sea urchin gastrulae demonstrated that newly synthesised low molecular weight (8-15S) nuclear DNA labelled with ^{3}H-BUdR was not readily chased by unlabelled BUdR into higher molecular weight DNA. In contrast, 8-15S DNA labelled with ^{3}H-thymidine was converted into higher molecular weight DNA during a relatively short chase period with unlabelled thymidine. This data suggested the incorporation of BUdR into newly synthesised low

molecular weight DNA molecules in embryos might prevent or slow the rate of normal linkage of such molecules into higher molecular weight DNA.

We report here that morula and blastula stage sea urchin embryos grown continuously from fertilisation in the presence of BUdR accumulate DNA molecules (30-60S) which are not integrated into higher molecular weight bulk DNA.

The effect of BUdR on the size distribution of isolated long term ^{32}P-labelled DNA from embyos was determined on neutral sucrose gradients. A control culture of morula stage embryos grown continuously from fertilisation in the presence of ^{3}H-thymidine was collected and mixed with a culture of morula stage embryos similarly labelled with ^{32}P but grown continuously in the presence of either 50 μg ml^{-1} BUdR or 50 μg ml^{-1} thymidine (TdR). DNA was coisolated from mixtures of ^{3}H- and ^{32}P-labelled embryos and centrifuged through a neutral sucrose gradient as described in the legend to Fig. 1. ^{32}P-labelled morula stage DNA isolated from embryos grown in the presence of 50 μg ml^{-1} TdR sedimented with a distribution similar to ^{3}H-labelled morula stage DNA coisolated from embryos grown without added nucleoside (Fig. 1*a*). In contrast, a portion of ^{32}P-labelled morula stage DNA isolated from embryos grown in the presence of 50 μg ml^{-1} BUdR sedimented more slowly than ^{3}H-labelled morula DNA coisolated from the control culture (Fig. 1*b*). Similar differences in sedimentation distribution of control DNAs and DNAs isolated from embryos grown in the presence of BUdR were obtained from blastula stage embryos.

A summary of data demonstrating the effect of BUdR on the size distribution of ^{32}P-labelled morula and blastula stage DNAs on neutral sucrose gradients is presented in Table 1. In addition to the coisolation and sedimentation of BUdR and control DNAs as shown in Fig. 1, long term ^{32}P-labelled DNAs were isolated separately and consedimented with marker ^{3}H-DNA from bacteriophage Φ80. This facilitated a determination of the proportion of different size ranges of sea urchin DNA compared with the position of the marker DNA. A portion of the ^{32}P-labelled DNAs isolated from both morula and blastula stage embryos grown in the presence of BUdR sediments more slowly than ^{32}P-labelled DNA isolated from control cultures. The difference is represented by a decrease in the proportion of DNA sedimenting at greater than 60S and a corresponding increase of 8–10% in the morula and 3–5% in the blastula stage DNA sedimenting in a range from 30–60S. That this difference in sedimentation distribution is specifically the result of the presence of BUdR during embryogenesis rather than excess deoxyribonucleosides in the culture medium is demonstrated by the close similarity between the sedimentation distribution of DNA isolated from control cultures and those in which the embryos were grown in 50 μg ml^{-1} TdR (Fig. 1*a* and Table 1).

To ensure that the difference in sedimentation of ^{32}P-labelled DNAs obtained from embryos grown in the presence of BUdR (compared with control DNAs) was not the result of ^{32}P-containing molecules other than DNA, samples of the sea urchin DNA preparations labelled with ^{32}P were subjected to enzymatic and alkaline treatment. More than 90% of the ^{32}P was resistant to a mixture of pancreeatic and T_1 RNases; more than 95% of the radioactivity was DNase-sensitive; more than 85% of the ^{32}P was resistant to 0.2 M NaOH at 60° C for 1 h. Thus at least 85% of the ^{32}P recovered was in DNA. More importantly, ^{32}P-labelled morula stage DNA isolated from embryos grown in the presence of BUdR was incubated with or without the addition of DNase and then centrifuged through neutral sucrose gradients (data not shown). The small percentage of DNase-resistant ^{32}P was found near the top of the gradient. Therefore, we believe that the shift in distribution of ^{32}P observed in the sedimentation profiles of DNAs isolated from embryos grown in the presence of BUdR (Fig. 1*b* and Table 1) represents ^{32}P-containing DNA.

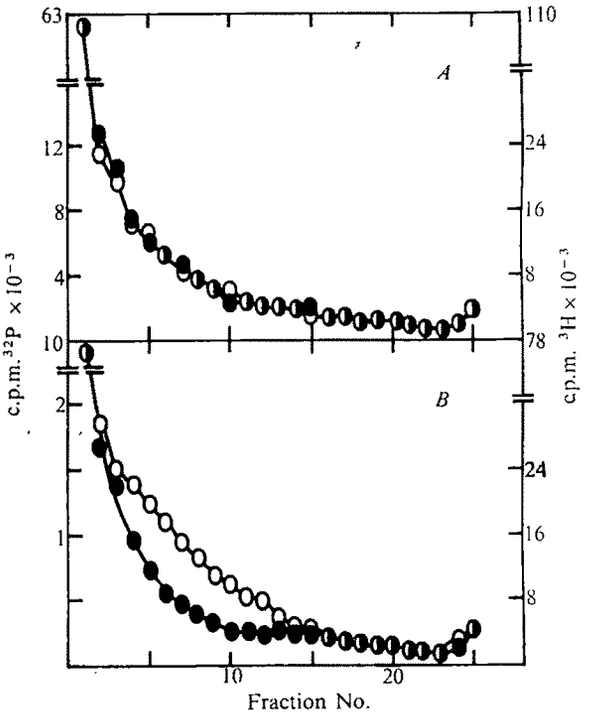


Fig. 1 Comparison on neutral sucrose gradients of profiles of control DNAs and DNAs synthesised in the presence of BUdR. *Strongylocentrotus purpuratus* embryos were cultured[3] in artificial seawater[11] containing penicillin and streptomycin to the morula stage of development: *A*, DNA coisolated from embryos grown under normal conditions in the presence of 2 μCi ml^{-1} ^{3}H-methyl-thymidine (20 Ci mmol^{-1}) and then mixed after growth with embryos grown in the presence of 50 μg ml^{-1} TdR with 1 μCi ml^{-1} ^{32}P; *B*, DNA coisolated from control embryos grown under normal conditions in the presence of ^{3}H-TdR and then mixed with embryos grown in the presence of 50 μg ml^{-1} BUdR and 1 μCi ml^{-1} ^{32}P. In all comparative experiments cultures were derived from a single batch of eggs pooled from several females. Nucleosides were added immediately after fertilisation and radioisotopes were added 10 min after fertilisation, all being present until the embryos were collected. Each 100 ml culture of embryos was killed by the addition of an equal volume of 20% phenol (freshly redistilled, saturated with 50 mM sodium phosphate buffer, *p*H 7.0) in –20° C ethanol. The embryos were pelleted from the alcohol-phenol solution by centrifugation at 10,000 *g* for 15 min and resuspended in 100 ml of 100% ethanol. After an additional centrifugation, embryos were resuspended and combined in a total of 10 ml of buffer which was 50 mM Tris, 50 mM EDTA, *p*H 8.5 at 37° C. Cells were lysed by the addition of sodium dodecylsulphate to 0.5%. Pronase (predigested 1.5 h at 37° C in Tris-EDTA buffer) was added to 1 mg ml^{-1} and the lysate was kept overnight at 37° C. α-Amylase (Worthington, twice crystallised) was added to 100 μg ml^{-1} and pancreatic RNase (Worthington, stock solution is 2 mg ml^{-1} in 0.15 M NaCl, heated to 85° C for 20 min) to 20 μg ml^{-1}; the mixture was then incubated at 37° C for 2 h. Predigested pronase was then added to 100 μg ml^{-1} and the DNA was held at 37° C overnight. The resulting DNA solution was extracted three times with phenol and extensively dialysed against 1 × SSC (SSC is 0.15 M NaCl, 0.015 M sodium citrate). Approximately 45 μg of the ^{3}H- and ^{32}P-labelled coisolated DNA was brought to a final volume of 1.0 ml in 1 × SSC and layered onto a 30 ml 5-20% sucrose gradient containing 1 M NaCl, 20 mM Tris-HCl, 1 mM EDTA, *p*H 7.3. Centrifugation was in a Beckman SW 25.1 rotor for 10 h at 24,000 r.p.m. at 20° C. Fractions were collected from the bottom of the gradient and precipitated at 4° C by the addition of 2 volumes of 10% trichloroacetic acid and 25 μg bovine serum albumen. Fractions were collected on Whatman GF/A filters, dried, and counted in toluene-based scintillation fluid. O, ^{32}P; ●, ^{3}H.

It should be noted that the portion of isolated bulk DNA which contains radioactivity represents only nuclear DNA, for mitochondrial DNA replication does not occur in sea urchin embryos until after gastrulation[4,5].

Table 1 Summary of data demonstrating an effect by BUdR on sedimentation in neutral sucrose gradients of ^{32}P-labelled DNAs isolated from sea urchins embryos.

$S_{20,w}$ distribution*	Calculated† molecular weight $\times 10^{-6}$	% of total counts Morula Control	Morula BUdR	Morula TdR	Blastula Control	Blastula BUdR	Blastula TdR
>60	>154	47.9	38.7	50.4	70.3	64.9	70.9
30—60	20.7—154	39.1	46.5	36.3	23.8	27.3	22.1
0—30	0—20.7	13.0	14.8	11.4	5.9	7.8	7.0

DNA was isolated and centrifuged as described in Fig. 1, except that ^{32}P-labelled DNA was not coisolated with ^{3}H-labelled DNA. The DNAs were extracted from embryos grown without added nucleoside or with the addition of BUdR or TdR to 50 μg ml^{-1}.
* $S_{20,w}$ values were determined by the relative sedimentation distance of freshly prepared ^{3}H-TdR-labelled φ80 DNA[12] cosedimented in each gradient.
† Molecular weight was calculated from $S_{20,w}$ values using the equations of Studier[13]. Native φ80 phage DNA has a calculated $S_{20,w}$ value of 33.4 based on a molecular weight of 28.6×10^6 (ref. 14).

Thus it seems that the ^{32}P-labelled nuclear DNA isolated from early stage sea urchin embryos allowed to develop in the presence of BUdR contains an increased proportion of molecules in the 30–60S range, compared with DNA isolated from embryos grown in the absence of BUdR or in the presence of TdR. This shift in the size distribution of DNA could result from at least one of several mechanisms: (1) certain sequences in higher molecular weight DNA synthesised in the presence of BUdR may be sensitive to excision *in vivo* or breakage during the isolation of the DNA; (2) BUdR may induce the amplification of some episomal DNA; (3) certain newly synthesised DNA molecules may not be able to integrate into bulk DNA when substituted with BUdR. Though the data presented do not distinguish between the above possibilities, certain indirect evidence may favour the latter mechanism.

(1) Birnboim *et al.*[6] have reported the presence of long (greater than 4S) pyrimidine stretches which remain after hydrolysis of HeLa cell DNA in the presence of formic acid and diphenylamine. If the incorporation of BUdR renders DNA more sensitive to excision *in vivo* or breakage during isolation, one might expect incorporation of BUdR to change the size distribution of such long pyrimidine lengths. A comparison of ^{32}P-labelled pyrimidine runs obtained from DNAs isolated from embryos grown in the presence or absence of BUdR demonstrated no difference in length distributions (S.T.C., unpublished observation). This might be taken as evidence against excision or breakage of BUdR-substituted DNA within long pyrimidine runs, though such an occurrence in a small proportion of the pyrimidine lengths or in short oligopyrimidines would not be detected.

(2) Kotzin and Baker[3] reported that 8–15S (alkaline sucrose gradient) newly synthesised sea urchin DNA (apparently occurring as a normal intermediate in DNA synthesis[7]), pulse-labelled with ^{3}H-BUdR, could not be chased into higher molecular weight DNA with unlabelled BUdR during a 20 min chase period. These experiments suggest that a portion of newly synthesised short DNA pieces either cannot integrate with existing higher molecular weight DNA, or the rate of integration is retarded by the BUdR. In the results shown in Fig. 1 and Table 1, exposure of embryos to BUdR over a long period results in accumulation of pieces longer than the 8–15S size range seen at the end of the relatively short pulse-chase period previously described[3]. Thus, it seems possible that BUdR retards rather than prevents the integration of short pieces of certain nuclear DNAs. The 30–60S pices accumulated in embryos continuously grown in BUdR may, however, represent a maximum size beyond which certain DNA sequences cannot increase by acquiring smaller DNA pieces or by integrating into existing longer pieces.

There is some support for the possibility that particular nuclear DNA sequences might incorporate BUdR preferentially. Walther *et al.*[8] report that in the developing pancreas there is a low molecular weight, double-stranded, nuclear DNA fraction (detected with ^{3}H-thymidine) which normally does not incorporate into higher molecular weight DNA. This DNA fraction preferentially incorporates BUdR relative to bulk DNA[1]. Reassociation studies demonstrate that this 15–25S fraction of DNA is not a random sample of chromosomal sequences and does not contain highly repetitive sequences[1].

It is perhaps interesting to note that the size of the DNA molecules which seem to be affected by exposure to BUdR during replication (30–60S in the work reported here) are within the size range reported for the replicons of several higher cell types[9,10]. This suggests a possible mechanism for the selective effect of BUdR on certain specialised functions associated with differentiation. Substitution of BUdR for thymidine could preferentially prevent or retard integration, into higher molecular weight DNA, of those replicons containing sequences coding for or regulating products related to specialised functions associated with differentiation. If such integration is necessary for regulation of gene expression it would be possible for this thymidine analogue to inhibit differentiation without affecting cell viability and proliferation.

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1 Rutter, W. J., Pictet, R. L., and Morris, P. W., *A. Rev. Biochem.*, **42**, 601–646 (1973).
2 Gon'charoff, M., and Mazia, D., *Expl Cell Res.*, **46**, 315–327 (1967).
3 Kotzin, B. L., and Baker, R. F., *J. Cell Biol.*, **55**, 74–81 (1972).
4 Piko, L., Tyler, A., and Vinograd, J., *Biol. Bull.*, **132**, 68–90 (1967).
5 Piko, L., *Am. Zool.*, **9**, 1118 (1969).
6 Birnboim, H. C., Mitchel, E. J., and Straus, N. A., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2189-2192 (1973).
7 Baker, R. F., *Biochem. biophys. Res. Commun.*, **43**, 1415-1420 (1971).
8 Walther, B. T., David, J. D., Levine, S., Pictet, R., and Rutter, W. J., *Fedn Proc.*, **30**, 1128 (1971).
9 Callan, H. G., *Proc. R. Soc.* **B/181**, 19-41 (1972).
10 Taylor, J. H., Adams, A. G., and Kurek, M.P., *Chromosoma*, **41**, 361-384 (1973).
11 Tyler, A., *Biol. Bull.*, **104**, 224-229 (1953).
12 Thomas, jun., C. A., and Abelson, J., in *Procedures in Nucleic Acid Research* (edit. by Cantoni, G. L. and Davies, D. R.), 553-561 (Harper and Row, New York, 1966).
13 Studier, F. W., *J. molec. Biol.*, **11**, 373-390 (1965).
14 Davidson, N., and Szybalski, W., in *The Bacteriophage Lambda* (edit. by Hershey, A. D.), 48 (Cold Spring Harbor Laboratory, New York, 1971).

Are 'N bands' selective staining of specific heterochromatin?

BY *in situ* DNA–RNA-hybridisation and the association patterns in meiotic and mitotic chromosomes, the nucleolus organiser regions (NOR) have been localised on specific chromosomal segments of some mammalian species[1–4]. Matsui and Sasaki[5] described a staining technique ('N bands') which was assumed to stain the NOR of mammalian chromosomes with high specificity.

This assumption was based on the following findings. First, the satellites of human acrocentric chromosomes are exclusively stained but not the centromeres, short arms or stalks of satellites. From their interpretation of the literature[1,2], they believe that the NOR is located in the satellites of human D and G group chromosomes. Second, N bands appear in the secondary constriction of the rat kangaroo (*Potorous tridactylis*) X chromosome to which the NOR is assigned[6,7]. Third, N bands are visible in the presumed nucleolus organiser regions of several other species (*Rattus norvegicus, Crisetulus griseus, Muntiacus muntiac, Macaca fasicularis* and *Mus musculus*).

We have checked these hypotheses using essentially the same technique[5] with chromosome preparations of human lymphocytes, fibroblasts of the vole species *Microtus oeconomus* and *Microtus ochrogaster*, and the dormouse *Eliomys quercinus*.

In accordance with the results of Matsui and Sasaki, on human metaphase plates we found distinctive red spots on the satellites of acrocentric chromosomes (Fig. 1). Yet, not all satellites stain with this technique as should be the case if this technique gives a differential staining of the NOR. In contrast to the statements of these authors we observed an additional differential staining of the short arm of one D group chromosomes (Fig. 1). No staining of the stalks of satellites could be achieved in all metaphases analysed.

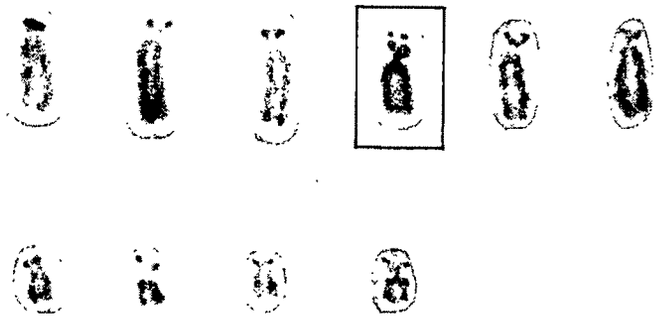


Fig. 1 D and G group chromosomes of a human lymphocyte in metaphase with N bands. Note the polymorphism of the N bands on the short arm and satellites in the marked D chromosome.

As the nucleolus organiser regions in human chromosomes have been assigned to the secondary constrictions of the acrocentric chromosomes[1,2,4], it has to be concluded that the N-band technique does not give a differential staining of the human NOR.

In metaphase chromosomes of *M. oeconomus*[13] N bands were visible at the short arms of the acrocentric chromosomes 13 and 14. The unpublished results of W. Engel, H. Hoehn and W. Krone have shown that the short arm and the secondary constriction of the long arm of chromosome 14 are involved in 'satellite associations'.

Accordingly, in the long arm of chromosome 14 the NOR shows no N band and the N band of chromosome 13 is not correspondent to a NOR. These findings are evidence that the N-band positive sites do not coincide with NORs also in this species. We also treated metaphase plates of *M. ochrogaster* and *Eliomys quercinus* with the N band technique. It was not possible to detect any N bands in either species. As these species are expected to possess NOR, it is obvious that N bands do not represent a differential staining of the NOR.

In many of the species investigated, heterochromatic regions are located adjacent to the NORs. This is true for man[9], mouse[12,11], Indian muntjac[3,8], and the short arm of chromosome 14 in *M. oeconomus*, for example. In a species without heterochromatin (*Eliomys quercinus*, our unpublished results) no N bands can be found. Therefore we conclude that N bands correspond to a specific kind of heterochromatin which is situated adjacent to the NOR in most instances.

We have circumstantial evidence[10] that the production of G bands is determined by qualitative differences of nonhistone proteins along the chromosomes. Matsui and Sasaki[5] give evidence that the specific nonhistone proteins are stained with the N-band technique. Therefore one may assume that N bands reflect nonhistone proteins corresponding to a special kind of heterochromatin. Though N bands apparently do not stain the NORs, they are useful markers in human cytogenetics like other special staining methods.

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1 Henderson, A. S., Warburton, P., and Atwood, K. C., *Proc. natn. Acad. Sci., U.S.A.*, **69**, 3394–3398 (1972).
2 Bross, K., and Krone, W., *Humangenetik*, **14**, 137–141 (1972).
3 Comings, D. E., *Expl Cell Res.*, **67**, 441–460 (1971).
4 Ohno, S., Trujillo, J. M., Kaplan, W. D., and Kinoshita, R., *Lancet*, ii, 123–126 (1961).
5 Matsui, S.-J., and Sasaki, M., *Nature*, **246**, 148–150 (1973).
6 Hsu, T. C., Brinkley, B. R., and Arrighi, F. E., *Chromosoma*, **23**, 137–153 (1967).
7 Ohnuki, Y., Olson, R. S., and Rounds, D. E., *Jap. J. Genet.*, **47**, 133 (1972).
8 Hsu, T. C., and Arrighi, F., *Chromosoma*, **34**, 243–253 (1971).
9 *Paris Conf. (1971). Standardization Human Cytogenet., Birth Defects* (original article Series), **VIII**, 7 (The National Foundation, New York, 1972).
10 Vogel, W., Faust, J., Schmid, M., and Siebers, J.-W., *Humangenetik*, **21**, 227–236 (1974).
11 Levan, A., Hsu, T. C., and Stich, H. F., *Hereditas*, **48**, 677–687 (1962).
12 Hsu, T. C., Cooper, J. E., Mace, M. L., jun., and Brinkley, B. R., *Chromosoma*, **34**, 73–87 (1971).
13 *An Atlas of Mammalian Chromosomes* (edit. by Hsu, T. C., and Benirschke, K.) (Springer-Verlag, Berlin, Heidelberg and New York, 1967).

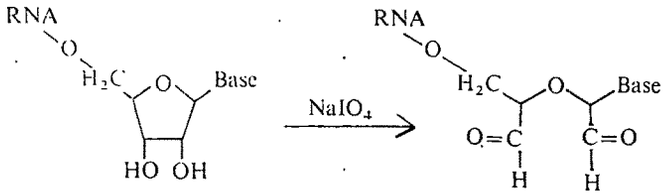
Messenger and aminoacylation functions of brome mosaic virus RNA after chemical modification of 3′ terminus

THE ability of RNA from several plant viruses to bind a specific amino acid in a manner like tRNA is now well established. All members of the tymovirus group so far examined contain RNA which can bind valine [1-3]; all members of the bromovirus group bind tyrosine (ref. 4 and T.C.H., in preparation), and tobacco mosaic virus (TMV) RNA binds histidine[5]. The ubiquity of the reaction, and the similarity of the esterification to that of aminoacylation of tRNA indicates that a significant biological function for the property exists. A tRNA-like function such as that of a virus-specific tRNA, or a mis-sense tRNA seems plausible. Although there is evidence that turnip yellow mosaic virus (TYMV) RNA, or a fragment containing the 3′ terminus of TYMV RNA, may donate valine to nascent polypeptides[6], it has been established that tyrosine, bound to brome mosaic virus (BMV) RNA, is not donated to peptidyl material in a cell-free system from wheat embryo when directed by viral, plant, or synthetic messenger RNA (ref. 7).

Of the several viral RNAs capable of aminoacylation, only BMV RNA has been shown to function as an efficient messenger,

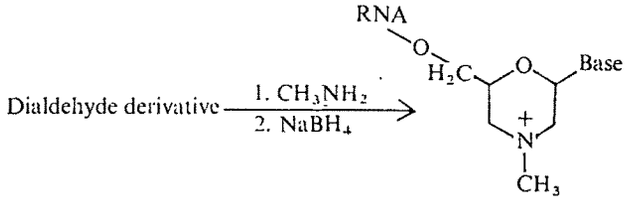
capable of directing the synthesis of authentic viral proteins in a cell-free system[8]. Therefore, with BMV RNA, a unique opportunity exists for determining the inter-relationship between aminoacylation activity and messenger function of viral RNA. A useful approach to understanding this inter-relationship is to test retention of these activities following chemical modification of the viral RNA. A definitive modification should be that of oxidation of the 3′ terminus as this is known to prevent aminoacylation of tRNA (ref. 9).

Two procedures were adopted for RNA modification[10]. First, the 3′ end of BMV RNA was oxidised to the dialdehyde derivative with periodate according to the reaction:



Dialdehyde derivative

It is known, however, that the oxidised 3′ end of the RNA can be removed by an amine-catalysed β-elimination reaction[11], and therefore, the possibility existed that under our *in vitro* reaction conditions replacement of the terminal pA_{OH} could occur, thereby restoring the RNA to its original form. To prevent such repair of the 3′ terminus the second modification procedure used was the preparation of the morpholine derivative according to the reaction:



Morpholine derivative

Both modification procedures resulted in the loss of the ability of the BMV RNA to accept an amino acid but no quantitative difference in messenger function was observed (Table 1).

To determine whether qualitative differences exist in the pattern of proteins synthesised under the direction of the modified RNA, the products synthesised *in vitro* were subjected to sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis. Protein synthesised using unmodified BMV RNA (comprised of components 1,2,3, and 4) (ref. 12) as messenger consists mostly of BMV coat protein, together with a small amount of a protein designated protein 3a and some uncompleted proteins (Fig. 1*a*). The electrophoretic profiles of proteins synthesised under the direction of the dialdehyde derivative, and of the morpholine derivative, appear in Fig. 1*b* and *c*, respectively. The pattern of radioactivity obtained for products of the reaction directed by the dialdehyde derivative (Fig. 1*b*) was identical to that of the unmodified BMV RNA products (Fig. 1*a*), confirming that normal products can be synthesised by viral RNA lacking an aminoacylatable 3′ terminus. Analysis of the products of the reaction directed by the morpholine derivative (Fig. 1*c*) also showed the presence of coat protein and protein 3a. In this case, however, a relatively higher proportion of protein 3a, and a correspondingly lower proportion of coat protein was synthesised. The reason for this difference is unknown. A likely possibility is that configurational changes

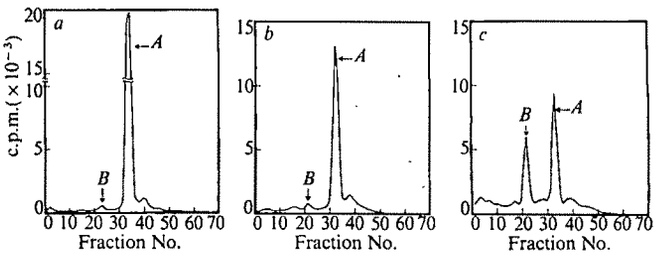


Fig. 1 Polyacrylamide gel patterns of products synthesised *in vitro* under the direction of modified and unmodified BMV RNA. Conditions for *in vitro* protein synthesis and gel electrophoresis were as previously described[8]. ^{14}C-leucine was used as label. The products synthesised under the direction of unmodified RNA (control), the dialdehyde derivative of the RNA, and the morpholine derivative of the RNA are shown in (*a*), (*b*), and (*c*), respectively. Electrophoresis was from left to right. *A*, Coat protein; *B*, protein 3a.

occurred in the BMV RNA molecules after methylamine and sodium borohydride treatment, causing alterations in the affinities of ribosomes for the different cistrons. While this in itself is an interesting finding, the fact that both products synthesised under the direction of the modified RNAs are similar to proteins obtained using native BMV RNA as messenger strongly suggests that aminoacylation of BMV RNA is not an obligatory step for translation of this viral RNA.

Although the apparent tRNA-like structure of BMV RNA, TYMV RNA, TMV RNA, and other plant virus RNAs makes a tRNA-like function an attractive possibility, the present data greatly detract from the proposition that the biological role of aminoacylation of plant virus RNA is directly related to the donation of an amino acid to a viral peptide. The present results do not eliminate the possibility of such a role as a helpful, but not obligatory, step in viral growth. Nevertheless, it now seems more likely that binding of an amino acid to the virus RNA could be involved as a recognition signal for the replicase as proposed by Litvak and coworkers[13], or could function as a regulatory system such as that proposed by Kolakofsky and Weissmann for phage Qβ (ref. 14).

Table 1 Effect of modification of 3′ terminus of BMV RNA on its tyrosine binding ability and on its activity in stimulating amino acid incorporation.

RNA added	Tyrosine binding (c.p.m.)	Amino acid incorporation (c.p.m.)
Control	10,239	13,641
Periodate derivative	254	13,000
Morpholine derivative	495	13,882

BMV RNA was modified according to the procedures described by Fahnestock and Nomura[10] for modification of *Escherichia coli* 5S rRNA. For preparing the control RNA, all the manipulations for periodate oxidation and methylamine-borohydride derivatisation were carried out except that the modifying agents were omitted. Conditions for BMV RNA aminoacylation were as previously described[4], each reaction (0.1 ml) containing 15 μg of RNA. A 20 μl sample was assayed for ^{3}H-tyrosine binding to virus RNA after 30 min incubation at 30°C. Amino acid incorporation conditions were as previously described[8]. Each incorporation mixture (175 μl) contained 30 μg of RNA and 0.5 μCi of a mixture of ^{14}C-labelled alanine, arginine, leucine, and lysine. Aliquots of 50 μl were counted to determine amino acid incorporation into protein.

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[1] Litvak, S., Carré, D. S., and Chapeville, F., *FEBS lett.*, **11**, 316 (1970).
[2] Pinck, M., Yot, P., Chapeville, F., and Duranton, H. M., *Nature*, **226**, 954 (1970).
[3] Pinck, M., Chan, S-K., Genevaux, M., Hirth, L., and Duranton, H., *Biochimie*, **54**, 1093 (1972).
[4] Hall, T. C., Shih, D. S., and Kaesberg, P., *Biochem. J.*, **129**, 969 (1972).
[5] Oberg, B., and Philipson, L., *Biochem. biophys. Res. Commun.*, **48**, 927 (1972).
[6] Haenni, A. L., Prochiantz, A., Bernard, O., and Chapeville, F., *Nature new Biol.*, **241**, 166 (1973).
[7] Chen, J. M., and Hall, T. C., *Biochemistry* **12**, 4570 (1973).
[8] Shih, D. S., and Kaesberg, P., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1799 (1973).
[9] Deutscher, M. P., in *Progress in Nucleic Acid Research and Molecular Biology* (edit. by Davidson, J. N., and Cohn, W. E.) **13**, 51 (Academic Press, New York, 1973).
[10] Fahnestock, S. R., and Nomura, M., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 363 (1972).
[11] Neu, H. C. and Heppel, L. A., *J. biol. Chem.*, **239**, 2927 (1964).
[12] Lane, L. C., and Kaesberg, P., *Nature new Biol.*, **232**, 40 (1971).
[13] Litvak, S., Tarragó, A., Tarragó-Litvak, L., and Allende, J. E., *Nature new Biol.*, **241**, 88 (1973).
[14] Kolakofsky, D., and Weissmann, C., *Nature new Biol.*, **231**, 42 (1971).

Virus-induced cell fusion enhanced by phytohaemagglutinin

ONCOGENIC transformation by viruses or chemicals[1–5], treatment with proteolytic enzymes[1,6] and infection with nononcogenic enveloped viruses[7,8], produce cell surface alterations that increase cellular susceptibility to agglutination by plant lectins. More recently, lectins have been used to modify the replication and cytopathogenicity of enveloped viruses grown in normal cells[9,10]. It has been shown that treatment of cells with con A inhibits paramyxovirus-induced cell fusion.[11] In contrast, the results reported here indicate that another plant lectin, phytohaemagglutinin (PHA), can enhance the fusion of cells infected with the paramyxovirus Newcastle disease virus (NDV).

Freeze-dried PHA (Wellcome Reagents Ltd., Beckenham, Kent) was reconstituted in phosphate-buffered saline (PBS) and cells were treated with 1.0 ml diluted in maintenance medium at the concentrations described below. The properties of the NDV strains, cell culture methods, infection of cell cultures and measurement of the extent of cell fusion have been described[12–14]. All cell cultures were grown on coverslips in Leighton tubes at 37° C.

To demonstrate the effect of different concentrations of PHA on NDV-induced cell fusion from within[15], virus was removed 1 h after infection and replaced with 1.0 ml of medium containing different dilutions of PHA, incubated for a further 8 h at 37° C in the presence of PHA, finally fixed and stained and the extent of cell fusion measured. Fusion of BHK 21 cells infected with NDV strain Herts was enhanced in the presence of PHA (Fig. 1). Incubation of PHA with 0.5 M N-acetyl-galactosamine, a haptenic inhibitor of PHA, for 1 h at 37° C before addition to infected cell cultures eliminated its ability to enhance fusion

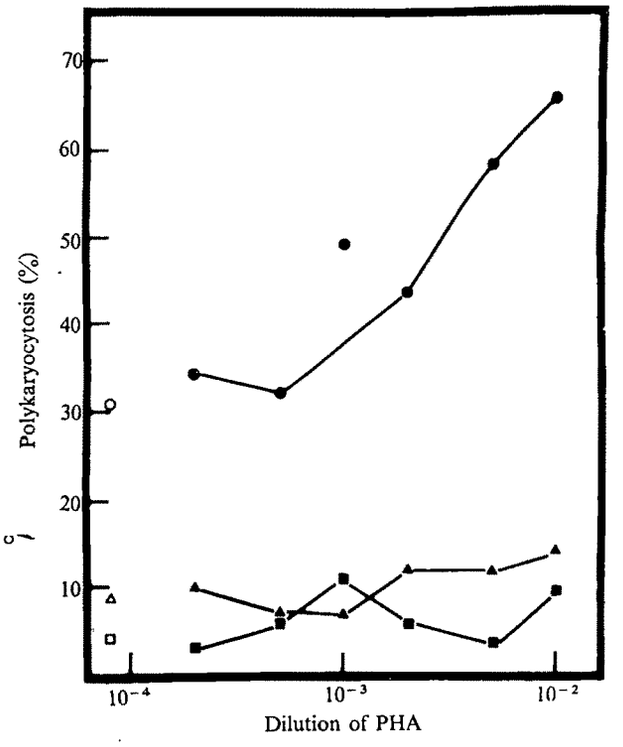


Fig. 1 Effect of different concentrations of PHA on fusion of BHK-21 cells infected with 25 EID_{50}/cell NDV-Herts (●), 50 EID_{50}/cell NDV-Queensland (■), and uninfected control cells (▲). At 1 h after infection, virus was replaced with PHA diluted in maintenance medium. Polykaryocytosis was estimated 8 h after infection. O, □, △, Fusion in the absence of PHA.

from within by strain Herts. PHA had no effect, however, on the fusion of cells infected with NDV strain Queensland (a non-fusing strain) or uninfected control cells. The time of addition of PHA after infection was important in determining the degree of enhancement of cell fusion (Fig. 2). The length of time for which PHA is in contact with the cells is not important, however, as similar enhancement of fusion from within was found after exposure to PHA for 90 min or 8 h with PHA added 1 h after infection. It is more likely that the decrease in enhancement is related to the progression of virus replication and fusion (Fig. 2), that is, once a fusion event has taken place there can be no enhancement of that event.

Fusion from without (ref. 15) was also enhanced by pretreatment with PHA. Maximum enhancement was recorded if cell cultures were treated with PHA (10^{-2} dilution) for 1.5 h or more before infection. Such treatment before infection with 1600 EID_{50}/cell NDV-Herts induced 57.5% polykaryocytosis compared to 37.2% in untreated and 4.1% in uninfected cell cultures. Experiments using PHA-Sepharose 4B conjugate gave similar results and showed that it was not necessary for PHA to enter the cell to cause enhancement. Cells were pretreated for 1.5 h with PHA-Sepharose, which had been washed extensively to remove any free PHA and diluted to give similar activity (as titrated by haemagglutination) to the unbound PHA used, and then infected with 1600 EID_{50}/cell NDV-Herts. Three hours after infection, cells treated with PHA-Sepharose showed 57.5% polykaryocytosis compared to 37.2% in infected, untreated cells and 3.9% in uninfected cells.

The results presented here indicate that PHA has a marked enhancing effect on cell fusion by NDV and that PHA acts rapidly on a prefusion event and does not require the presence of virus products at the cell surface for the initial interaction. The results obtained with PHA-Sepharose indicate that, as with lymphocyte

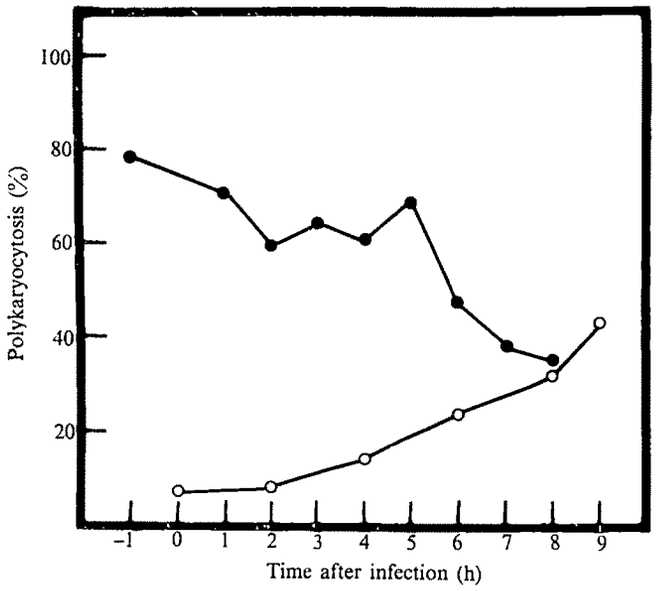


Fig. 2 Effect of PHA (10^{-2} dilution) added at different times on cell fusion of BHK-21 cells at 9 h after infection with 25 EID_{50}/cell NDV-Herts (●). Fusion in untreated cells at different times after infection with NDV-Herts is also shown (○).

activation[16], PHA does not have to enter the cell to be effective.

Further work is required to determine the mode of action of PHA. It may be significant, however, that other studies have indicated that lysosome fragility follows stimulation of lymphocytes with PHA[17] and we have previously suggested the possible role of lysosomal enzymes in virus-induced cell fusion[18].

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1 Inbar, M., and Sachs, L., *Proc. natn. Acad. Sci., U.S.A.*, **63**, 1418 (1969).
2 Inbar, M., and Sachs, L., *Nature*, **223**, 710 (1969).
3 Aub, J. C., Sanford, B. H., and Cote, M. N., *Proc. natn. Acad. Sci., U.S.A.*, **54**, 396 (1965).
4 Burger, M. M., and Goldberg, A. R., *Proc. natn. Acad. Sci., U.S.A.*, **57**, 359 (1970).
5 Cline, M. J., and Livingston, D. C., *Nature new Biol.* **232**, 155 (1971).
6 Burger, M., *Proc. natn. Acad. Sci., U.S.A.*, **62**, 994 (1969).
7 Becht, H., Rott, R., and Klenk, H-D., *J. gen. Virol.* **14**, 1 (1972).
8 Poste, G., Reeve, P., Alexander, D. J., and Terry, G., *J. gen. Virol.*, **17**, 81 (1972).
9 Rott, R., Becht, H., Klenk, H-D., and Scholtissek, C., *Z. Naturforsch.* **27**, 227 (1972).
10 Reeve, P., Poste, G., and Alexander, D. J., in *Negative Strand Viruses* (edit. by Mahy, B. W. J., and Barry, R.) (Academic Press, London, in the press).
11 Poste, G., Alexander, D. J., Hewlett, G., and Reeve, P., *J. gen. Virol.* (in the press).
12 Alexander, D. J., Reeve, P., and Allan, W. H., *Microbios*, **2**, 155 (1970).
13 Reeve, P., and Poste, G., *J. gen. Virol.*, **11**, 17 (1971).
14 Poste, G., Waterson, A. P., Terry, G., Alexander, D. J., and Reeve, P., *J. gen. Virol.*, **16**, 95 (1972).
15 Bratt, M. A., and Gallaher, W. R., *Proc. natn. Acad. Sci., U.S.A.*, **64**, 536 (1969).
16 Greaves, M. F., and Bauminger, S., *Nature new Biol.*, **235**, 67 (1972).
17 Konig, E., Brittinger, G., and Cohnen, G., *Nature new Biol.*, **244**, 247 (1973).
18 Reeve, P., Poste, G., Alexander, D. J., and Pope, G., *J. gen. Virol.*, **15**, 219 (1972).

Thymus involvement in female sexual maturation

It is now established that the thymus plays a critical role in a variety of immunological functions. Evidence that the thymus can also function as an endocrine gland has mainly been derived from immunological investigations[1,2]. Because endocrine glands do not function independently, it is likely that the thymus is involved in a number of endocrine functions and some experimental data support such an assumption. Thus, hypothyroidism and persistence of the foetal zone in the adrenals is observed in congenitally athymic nude mice[3] and degranulation of pituitary eosinophil cells occurred following neonatal thymectomy in mice[4].

Here we present experimental evidence that the thymus also influences the maturation of female sexual function during a critical period of development.

Conventional female Charles River mice were surgically thymectomised or sham-operated when 2 or 10 d old. Mice thymectomised on day 2 were implanted on day 3 with 1–2 thymus glands obtained from male or female animals of the same age and other groups of 3 d old were injected intraperitoneally with 5×10^6 thymocytes from adult mice (2–3 months old). Among the parameters studied in the different experimental groups were vaginal opening time (VOT) (the day of complete vaginal opening with full differentiation of the external sexual structures); the sexual cycle (based on daily cytological studies of the vaginal contents), and the morphology of the ovary and uterus (based on gross inspection and conventional histology). The criterion for defining puberty was the vaginal opening time followed by the first oestrus appearing within 4 d.

On completion of the various experiments, all mice were killed for gross morphological and histological inspection of the sexual organs. Any animals found to be incompletely thymectomised were discarded.

It can be seen in Table 1 that there was a marked delay in VOT in animals that had been thymectomised on day 2 (VOT 50.5 ± 1.4 d) compared with the untreated (VOT 35.1 ± 0.8 d) and sham-operated mice (VOT 34.8 ± 0.9 d). It is especially noteworthy that when thymectomy was performed on day 10, there was no delay in VOT (35.4 ± 1.4 d). Grafting the thymus into mice 3 d old and thymectomised 1 d earlier, counteracted the delay in the VOT (37.8 ± 1.5). In contrast to an intact thymus, the administration of 5×10^6 thymocytes did not influence the delay in the VOT in thymectomised 2-d-old mice (47.8 ± 2.8 d).

The sexual cycle, as reflected by vaginal cytology, showed that irrespective of thymic presence or lack of it, once the vagina had opened, the first oestrus appeared in all animals within 4 d. The cycles were entirely normal during the 15 d of observation.

Morphological studies in neonatally thymectomised mice revealed that before the delayed vaginal opening occurs, the ovaries and uterus are very small and display the typical

Table 1 Delay in vaginal opening time in Charles River mice after neonatal thymectomy and restoration by early thymus grafting

Treatment	Number of animals	Vaginal opening time (d) < 40	> 40 < 55	> 55	$\chi^2 \pm$ s.e.m.
Controls (normal)	33	33	—	—	35.1 ± 0.8
Controls (sham-operated)	14	14	—	—	34.8 ± 0.9
Thymectomised on day 2	33	6	20	7	50.5 ± 1.4
Thymectomised on day 10	5	5	—	—	35.4 ± 1.4
Thymectomised on day 2; grafted with thymus on day 3*	11	8	2	1	37.8 ± 1.5
Thymectomised on day 2; injected with 5×10^6 thymocytes on day 3	8	1	5	2	47.8 ± 2.8

* Syngeneic thymus graft obtained from male or female 3-d-old Charles River mice.

histological pattern characteristic of prepubertal immaturity.

What we have said of the thymectomised mice is also true of congenitally athymic female nude mice, the behaviour of which prompted us to investigate the thymectomised animals. Nude mice have a thymic anlage in the foetus but on day 14 of pregnancy it is already abnormal. The animals have a scarcity of lymphocytes in the paracortical area of lymph nodes and fail to reject allogeneic skin grafts[5].

Congenitally athymic female nude mice (*nu/nu* BALB/c) and their normal thymus-bearing female litter mates (BALB/c) were kept in specific pathogen-free (SPF) conditions until transfer to this laboratory. Some of the animals were bred and kept in germ-free conditions.

Data on athymic nude mice are given in Table 2. Vaginal opening time was also delayed in germ-free conditions.

The great difference in the size of the uterus and ovary between a 43-d-old athymic nude and a normal mouse is clearly shown in Fig. 1. Especially striking is the normalisation of these organs following thymus transplantation on day 2. By 10 d after VOT, the ovaries and uterus had already developed and presented an entirely normal appear- by increasing their residence times. Also that any appreance histologically.

To determine whether the thymus involvement in sexual maturation is dependent on its immunological function, newborn nude mice were injected intraperitoneally with 15×10^6 each of thymocytes (four animals); spleen cells (three animals); and lymph node cells (two animals) from adult BALB/c mice 3 months old.

Vaginal opening time was found to be delayed (VOT 49.1 ± 0.4 d). 1–2 weeks after VOT, the animals were transplanted with skin from C57 black mice. After 2 weeks, six out of nine nude mice injected with cells had rejected the skin grafts. Thus, it seems that whereas the injected lymphoid cells can establish cell-mediated immunity in the nudes, they cannot normalise sexual maturation.

Our findings indicate that the absence of the thymus in the early stages of life, as in Charles River mice thymectomised on day 2 and in congenitally athymic nude mice, causes a significant delay in puberty (as reflected in vaginal opening time and first oestrus) and a delay in the maturation of the ovaries and uterus. A few days after attainment of the VOT, a normal sexual cycle and normal uterus and ovary morphology are established. The fact that the same delay in sexual maturation occured in germ-free nude mice rules out the likelihood that in these immunodeficient animals, infection is involved in this phenomenon. Thymus grafts were able to speed up the onset of puberty provided they were implanted on the second or third day of life. Postponement of thymus implantation to day 10 no longer effected an acceleration of the sexual function. Furthermore, the injection of lymphoid cells into newborn nudes does not normalise sexual maturation. All these findings indicate a causal relationship between a functioning thymus and the normal development of sexual function in the female during the early stages of postnatal life.

Pfeiffer[6], showed that the pattern of sexual functions is established during the perinatal period. The presence or absence of the testicular hormone determines the future male or female rhythm of gonadotrophin release by the hypophysis (tonic or cyclic pattern). Current concepts[7,8] consider sexual hormones as at least partially responsible for the organisation of some of the central nervous system (CNS) control mechanisms which determine the activation of the sexual endocrine system in the adult.

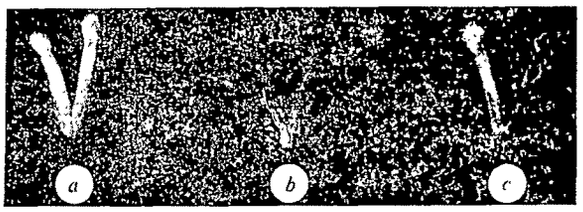


Fig. 1 Normalisation of sexual organ development by thymus. Gross appearance of uterus and ovaries of 43-d-old BALB/c mice (*a*), athymic nude mice (*b*), and athymic nude mice with a thymus graft (*c*). The thymus graft was made on animals 2 d old.

As the thymus has now been shown to influence the onset of the sexual functions, and as this effect is observed during the perinatal period, in which the CNS is being programmed, it is a reasonable assumption that the thymus is likewise involved in this process. The onset of puberty is a neuroendocrine phenomenon[9]; for this reason one can conceive of the thymus exerting its influence by the elaboration of hormones that could either act directly on the CNS or indirectly through endocrine glands. These hormonal influences of the thymus may be greatest during the perinatal period, when this organ is known to be more crucial than in later life.

Table 2 Delay in vaginal opening time in congenitally athymic nude mice (*nu/nu* BALB/c) and restoration by early thymus grafting

Treatment	Number of animals	Vaginal opening time (d) < 35	> 35 < 50	> 50	$\chi^2 \pm$ s.e.m.
Controls (normal BALB/c*)	55	55	—	—	31.3 ± 0.4
Athymic nude (conventional)	39	4	28	7	45.8 ± 0.9
Athymic nude (germ-free)	8	—	6	2	48.0 ± 1.6
Athymic nude grafted with thymus on day 2†	11	10	1	—	33.2 ± 0.9
Athymic nude grafted with thymus on day 10‡	7	1	5	1	46.9 ± 1.5
Athymic nude with thymocytes injected on day 2§	7	1	5	1	47.1 ± 1.6

* BALB/c mice: normal brothers of the nudes.
† Thymus from male and female 2-d-old BALB/c mice.
‡ Thymus from male or female 10-d-old BALB/c mice.
§ Thymocytes from male and female adult BALB/c mice 3 months old.

The delay in puberty in mice without thymus glands can provide an explanation for the persistence of the reticular zone of the adrenals in nude mice[3] because this zone tends to disappear after puberty.

Nishizuka and Sakakura[10–12] have observed earlier ovary dysgenesis (lack of follicles and of corpora lutea) and hyperplasia of the interstitial cells in about 50% of neonatally thymectomised female mice. This work, however, was not concerned with sexual development, because, depending on the strain studied, the dysgenesis was not observable before 60–120 d of age, a time when puberty had already occurred and normal sexual cycles were well established. This ovarian dysgenesis was prevented by spleen or thymus grafts and by lymphoid cell injection given during the first days of life. These experiments were performed in animals maintained in conventional conditions. As the administration of lymphoid cells and thymus or spleen grafts (which can restore immune functions) prevented the ovarian dysgenesis, the results obtained by these authors could have been influenced by intercurrent infection and might reflect an early manifestation of wasting disease or express some lymphoid cell function. Whichever of these possibilities apply, this situation can hardly involve an endocrine thymic function.

It has been demonstrated that the thymus is involved in the complex endocrine mechanisms controlling sexual development during a critical period of life, presumably by an elaboration of hormonal factor, as immunocompetent cells can not compensate for thymus absence, as far as sexual maturation is concerned. Such thymus involvement in sexual function has been suspected earlier as reviewed by Hammar[13]. In as much as the thymus is involved in the onset of puberty, and thymus involution also begins at this time, this would seem to indicate that a feedback mechanism is in operation.

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1 *Thymic Hormones* (edit. by Luckey, T. D.) (Urban and Schwarzenberg, München, Berlin and Wien, 1973).
2 Stutman, W., and Good, R. A., in *Contemporary Topics in Immunebiology* (edit. by Davies, A. J. S., and Carter, R.L.) **2**, 299 (Plenum Press, New York and London, 1973).
3 Pierpaoli, W., and Sorkin, E., *Nature new Biol.*, **238**, 282 (1972).
4 Pierpaoli, W., Fabris, N., and Sorkin, E., in *Hormones and the Immune Response*, Ciba Foundation Study Group No. 36 (edit. by Wolstenholme, G. E., and Knight, Julie) 126 (J. & A. Churchill, London, 1970).
5 Pantelouris, E. M., *Immunology*, **20**, 247 (1971).
6 Pfeiffer, C. A., *Am. J. Anat.*, **58**, 195 (1936).
7 Harris, G. W., *Endocrinology*, **75**, 627 (1964).
8 Levine S., and Mullins, R. F., jun., *Science*, **152**, 1585 (1966).
9 Donovan, B. T., and Van der Werff ten Bosch, J., *Physiology of Puberty* (Williams and Wilkins, Baltimore, 1965).
10 Nishizuka, Y., and Sakakura, T., *Science*, **166**, 753 (1969).
11 Nishizuka, Y., and Sakakura, T., *Endocrinology*, **89**, 886 (1971).
12 Nishizuka, Y., and Sakakura, T., *Endocrinology*, **89**, 902 (1971).
13 Hammar, J. A., *Die normal-morphologische Thymusforschung Analyse und Synthese* (Barth, J. H., Leipzig, 1936).

Evidence for non-identity of T killer and T helper cells sensitised to allogeneic cell antigens

Bone marrow-derived lymphocytes (B cells) can be triggered for antibody synthesis by a stimulus from immunocompentent allogeneic[1] or xenogeneic[2] lymphoid cells. The stimulus was shown to act directly on the B cell, since a response may occur in the absence of syngeneic thymus-derived lymphocytes (T cells)[1] and is possible even to a hapten[2]. This observation has led to the hypothesis that cooperating T cells (T-helper) are identical to cytotoxic T cells (T-killer) and act on B cells by surveying them for immunogen bound to their cell surface[3]. The T cells recognise the cell-bound immunogen and emit a sublethal signal to the B cells, triggering them to produce antibody. But the question arises, whether this is the way triggering of B cells usually occurs in the immune response. Recent experiments have shown that T cells sensitised to foreign erythrocyte antigens are able to cooperate with B cells to induce humoral antibody synthesis, but are not able to perform cytotoxic lysis of erythrocyte target cells[4,5]. T helper cells therefore need not be cytotoxic to the sensitising antigen to trigger B cells. This finding led to more extensive experiments concerning the question of whether T killer and T helper functions are performed by the same cell or not.

I shall show here that T helper and T killer cells sensitised to the same immunogen are not likely to be identical, because they are found to be mutually exclusive under certain immunisation conditions.

The DBA/2J mastocytoma P185 injected into C57BL/6J mice has been shown to induce cytotoxic effector cells as well as humoral antibody synthesis[6,7]. For both of these responses, T cells are necessary: T killer cells act as cytotoxic effectors[6] and T helper cells trigger B cells for antibody synthesis. This model therefore seemed to be suitable to explore the relation of T helper and T killer cells. I set up experiments to find immunisation procedures which favour the appearance of either T killer or T helper activity in the spleen. Since it had previously been shown that treatment of erythrocytes with formaldehyde or glutaraldehyde inhibits the humoral immune response to such treated immunogen, without affecting the priming of T helper cells[8], P815 cells were fixed in the same way. P815 cells treated with formaldehyde or glutaraldehyde (F-P815 and G-P815) were injected into C57BL/6J mice and their spleens assayed for cytotoxicity after 9 d. It is seen from Table 1 that F-P815 and G-P815 cells are not able to induce cytoxicity in C57BL/6J splens, while normal P815 cells do so.

To find out whether one or the other of such immunised spleen cell preparations contain T helper cells, the same spleen cell preparations were incubated in Mishell Dutton type cultures[9] together with trinitrophenyl-coupled[10] P815 cells (TNP-P815) as antigen. Table 1 shows that while F-P815 and G-P815 primed spleens vigorously stimulate the TNP response, cytotoxic P815 primed spleens do not. This result could have various explanations: it may be that P815 primed spleen cells are not able to respond to *in vitro* antigen stimulation. But in other experiments it was shown that P815 primed spleen cells respond as well as, or even better than, normal or F-P815 primed spleen cells to sheep red blood cell antigens *in vitro* (unpublished results).

Another possibility could be that P815 primed spleen cells do contain P815 specific T helper cells but these helper cells do not display their activity, maybe because they are not at the proper concentration in these spleens. To check this, cytotoxic P815 primed spleen cells were mixed in varying proportions with normal spleen cells and assayed for carrier priming. Table 2 demonstrates that also under these conditions no helper function can be detected in cytotoxic spleens. Cytotoxic spleens therefore seem to contain no

Table 1 Induction of cell-mediated cytotoxicity and helper activity by normal and fixed P815 cells

In vivo immunogen	*In vitro* cytotoxicity assay % cytotoxicity at 1 h	2.5 h	*In vitro* helper activity assay 10^6 TNP-P815 cells per culture	P.F.C. per 10^6 on TNP-BRBC
Experiment 1:				
10^7 P815	15	33	—	69
10^7 P815	—	—	+	3
10^7 F-P815	1	1	—	80
10^7 F-P815	—	—	+	540
10^7 G′ 0.25 P815	0	1	—	42
10^7 G′ 0.25 P815	—	—	+	121
10^7 G′ 0.025 P815	0	0	—	62
10^7 G′ 0.025 P815	—	—	+	248
	2 h	4 h		
Experiment 2:				
—	—	—	—	16
—	—	—	+	8
10^7 P815	33	69	—	44
10^7 P815	—	—	+	1
2×10^7 F-P815	0	0	—	34
2×10^7 F-P815	—	—	+	196

C57BL/6J mice were injected with varying doses of normal and fixed P815 cells intraperitoneally (i.p.) on day −11. DBA-2 mastocytoma cells P815 were grown in Dulbecco's modified MEM supplemented with 10% calf serum and fixed with 1.5% formaldehyde, 0.25% or 0.025% glutaraldehyde exactly as previously described for erythrocytes[8]. *In vitro* cytotoxicity assay: spleen cells of immunised mice were assayed for cytoxicity using ^{51}Cr-labelled P815 target cells as described previously[5,6]. Attacker to target cell ratio was 100:1. Percentage cytotoxicity (over spontaneous release) is calculated from the ratio of released counts to that released after three times freeze thawing[5]. *In vitro* helper activity assay: spleen cells were incubated in Mishell Dutton type cultures[9] with 10^6 TNP-P815 cells per culture. TNP-P815 cells were prepared as described for erythrocytes by Rittenberg and Pratt[10] and subsequently irradiated with 5,000 r. The TNP response was assayed on day 5 on TNP-BRBC[9,10].

helper activity as assayed with TNP-P815 antigen *in vitro*. This finding is somewhat surprising since it had previously been shown that in mice injected with P815 cells antibody production can be detected[7]. T helper cells therefore should at least at some time be operative during the *in vivo* immune response to P815.

Another possibility could be that the helper cells in cytotoxic spleens are not detected, because their activity is suppressed by a population of suppressor cells like those described by Jacobson and Herzenberg[11]. To find out, cytotoxic spleens and carrier-primed spleens were mixed and assayed for helper activity. It is seen in Table 3, experiment 1, that cells from cytotoxic spleens suppress the helper activity of F-P815 primed spleens, either partially (Table 3) or completely (unpublished results). It is therefore not surprising that helper activity cannot be detected in cytotoxic spleens using this assay. The question arises: why do live P815 cells prime for both killer and suppressor activity but not for helper activity, while fixed P815 cells prime only for helper but not for killer and suppressor activity? Though it is tempting to speculate that the cytotoxic T cells are the cells which suppress the function of the helper cells, this has to be shown by further experiments.

Carrier priming for erythrocyte antigens is known to be especially effective with low doses of antigen[8,12]. Since fixation of P815 cells with formaldehyde destroys about 95% of the H-2d antigenic determinants on P815 cells (R. Hyman and G. Dennert, unpublished results), it is possible that carrier priming with fixed P815 cells is in fact a low dose priming insufficient to prime T killer cells. If this reasoning is correct, then low doses of normal P815 cells should also prime for helper activity. Table 4 shows that this is the case. P815 cells were irradiated with 2,000 r. to inhibit multiplication and injected in various doses into animals. It is seen that helper activity is found in spleens injected with low doses (2×10^6 and 4×10^5 P815 cells per mouse) but not in mice injected with a higher dose (10^7) of P815 cells. The latter dose was also insufficient to induce cytotoxic T cells in these mice and it was subsequently found that ten times higher doses of irradiated cells have to be injected to prime for T killer cells (unpublished results). These experiments therefore seem to support the idea that carrier priming by fixed P815 cells is actually due to a low dose priming. Priming with fixed P815 cells however is usually superior to priming with low doses of normal P815 cells. This was also found in experiments where carrier priming to normal and fixed erythrocytes was studied[8].

Though it had previously been shown[6] that cytotoxicity of

Table 2 Failure to detect carrier priming in mixtures of P815 primed spleen cells and normal spleen cells

Spleen cells primed with	dose per culture	10^7 Normal spleen cells per culture	10^6 TNP-P815 per culture	P.F.C. per 10^6 on TNP-BRBC
—	—	+	—	26
—	—	+	+	32
F-P815	10^7	—	—	36
F-P815	10^7	—	+	352
P815	10^7	—	—	49
P815	10^7	—	+	48
P815	5×10^6	+	—	31
P815	5×10^6	+	+	33
P815	10^6	+	—	45
P815	10^6	+	+	8
P815	2.5×10^5	+	—	23
P815	2.5×10^5	+	+	3
P815	5×10^4	+	—	73
P815	5×10^4	+	+	3
P815	5×10^3	+	—	35
P815	5×10^3	+	+	27

C57BL/6J mice were immunised with 10^7 F-P815 or 10^7 P815 cells i.p. on day −11. Spleen cells were collected and tested for cytotoxicity. Spleens primed with P815 showed 62% cytotoxicity at $t=4$ h at an attacker to target cell ratio of 100:1, spleen cells primed with F-P815 had no detectable cytotoxicity. Aliquots of spleen cells were cultured with TNP-P815 cells as antigen. P815 primed spleen cells were also mixed in various ratios with normal spleen cells. The plaque assay was done on day 5.

Table 3 Inhibition of helper activity by cytotoxic spleen cells and anti-θ serum treatment

Spleen cells per culture	10^6 TNP-P815 per culture	P.F.C. per 10^6 on TNP-BRBC
Experiment 1:		
10^7 normal	—	12
10^7 normal	+	19
10^7 P815 immunised	—	8
10^7 P815 immunised	+	12
10^7 F-P815 immunised	—	19
10^7 F-P815 immunised	+	350
5×10^6 P815 immunised+	—	25
5×10^6 F-P815 immunised	+	63
Experiment 2:		
normal	—	<1
normal	+	<1
F-P815 immune untreated	—	4
F-P815 immune untreated	+	134
F-P815 immune+anti-θ+C′	+	<1
F-P815 immune+C′	+	132

Experiment 1: C57BL/6J mice were primed with 5×10^7 F-P815 cells or 10^7 P815 i.p. on day −9. Helper activity was assayed as in Table 1. Experiment 2: C57BL/6J mice were immunised with 5×10^7 F-P815 cells on day −9 and spleens asssayed for helper activity. For anti-θ serum treatment[13] 2×10^7 spleen cells were incubated in 1 ml BSS and 0.1 ml anti-θ serum for 30 min at 0° C. Cells were washed and resuspended in 1 ml BSS containing 0.15 ml guinea pig complement[5] and incubated 30 min at 37° C. Controls were incubated with complement only.

Table 4 Carrier priming by fixed and normal P815 cells

In vivo immunogen	10^6 TNP-P815 per culture	P.F.C. per 10^6 on TNP-BRBC
—	—	1
—	+	< 1
5×10^7 F-P815	—	35
5×10^7 F-P815	+	446
10^7 P815 2,000 r.	—	3
10^7 P815 2,000 r.	+	< 1
2×10^6 P815 2,000 r.	—	11
2×10^6 P815 2,000 r.	+	54
4×10^5 P815 2,000 r.	—	9
3×10^5 P815 2,000 r.	+	102

C57BL/6J mice were injected with varying doses of F-P815 and normal P815, irradiated with 2,000 r. On day 11, spleens were collected and tested for cytotoxicity and carrier priming. None of the groups showed any cytotoxic activity in their spleens.

P815 immunised C57BL/6J spleen cells is effected by thymus-derived lymphocytes, it had to be demonstrated that the helper activity observed in F-P815 primed spleen cells is also due to T cells. Treatment of F-P815 primed spleens with anti-θ serum[13] and complement completely abrogated the helper activity of these spleens, confirming that this activity is mediated by thymus-derived lymphocytes (Table 3, experiment 2).

In summary, these experiments show that under appropriate immunisation conditions either helper T cells or killer T cells can be found in sensitised spleens: spleens which show helper activity are not cytotoxic and spleens which are cytotoxic show no helper activity. Since it is found, however, that cytotoxic spleens inhibit the helper activity of F-P815 primed spleen cells it cannot be excluded that cytotoxic spleens contain T helper cells.

The experiments do not show that low doses of irradiated P815 or F-P815 prime directly for T helper cells for the following reason: the T helper assay *in vitro* takes 5 d of incubation, while the cytotoxic assay takes only a few hours. Thus, in culture maturation of a T helper precursor to T helper could occur. These experiments, however, strongly argue that the helper cell (or the precursor thereof) and the cytotoxic killer cell are likely to be two different subsets of T cells.

Recently other subsets of T cells with specific functions have been described[14]. Cantor and Asofsky demonstrated the requirement of two cells with different sensitivities to anti-thymocyte serum in a graft-*versus*-host reaction[15]. Bach *et al.*[16] demonstrated the existence of two populations of cells, one responding in the mixed lymphocyte reaction and the other effecting cell mediated lysis. It is therefore not surprising that T helper and T killer cells are found here to be different cell types. Further experiments must be directed toward determining the relationships between the various subsets of T cells so far described.

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1 Schimpl, A., and Wecker, E., *Eur. J. Immun.*, **1**, 304 (1971).
2 Dennert, G., and Lennox, E. S., *Nature new Biol.*, **243**, 214 (1973).
3 Kreth,, H. W., and Williamson, A. R., *Nature*, **234**, 454 (1971).
4 Dennert, G., and Lennox, E. S., *Nature new Biol.*, **238**, 114 (1972).
5 Dennert, G., and Lennox, E. S., *J. Immun.*, **111**, 1844 (1973).
6 Cerottini, J. C., Nordin, A. A., and Brunner, K. T., *Nature*, **228**, 1308 (1970).
7 Nordin, A. A., Cerottini, J. C., and Brunner, K. T., *Eur. J. Immun.*, **1**, 55 (1971).
8 Dennert, G., and Tucker, D. F., *J. exp. Med.*, **136**, 656 (1972).
9 Mishell, R. I., and Dutton, R. W., *J. exp. Med.*, **126**, 423 (1967).
10 Rittenberg, M. B., and Pratt, K. L., *Proc. Soc. exp. Biol. Med.*, **132**, 575 (1969).
11 Jacobson, E. B., and Herzenberg, L. A., *J. exp. Med.*, **135**, 1151 (1971).
12 Falkoff, R., and Kettman, J., *J. Immun.*, **108**, 54 (1972).
13 Reif, A. E., and Allen, J. M. V., *J. exp. Med.*, **120**, 413 (1964).
14 Raff, M. C., and Cantor, H., *Progr. Immun.*, **1**, 83 (1971).
15 Cantor, H., and Asofsky, R., *J. exp. Med.*, **135**, 764 (1972).
16 Bach, F. H., Segall, M., Stouber Zier, K., Sondel, P. M., Alter, B. J., and Bach, M. L., *Science*, **180**, 403 (1973).

Type-specific protein in herpes simplex virus envelope reacts with neutralising antibody

CERVICAL carcinoma has been correlated with previous infection with herpes simplex virus type 2 detected serologically[1-3]. These tests have, however, been confused by the fact that type 1 and type 2 herpes simplex viruses cross neutralise and it is therefore difficult to use the data to provide clear evidence for previous type 2 infection[4]. We have previously shown that antisera to types 1 and 2 viruses contain antibodies to a structural antigen, Band II (ref. 5), which neutralise both types of virus[6]. By indirect absorption methods, we have shown that they also contain antibodies to type-specific proteins which neutralise the homologous type only[6].

We now present more direct evidence for the existence of such proteins by showing that antiserum to a glycoprotein of enveloped type 1 virus particles neutralises type 1 but not type 2 virus. These studies indicate that it should be possible to prepare type-specific antigens which could be used in seroepidemiological surveys to provide conclusive evidence of previous type 2 infection.

Enveloped particles of herpes simplex virus type 1 were obtained by precipitation by polyethylene glycol of the virus released into the supernatant growth medium of HEP 2 cells infected with herpes simplex type 1 (strain HFEM). The virus was purified by two cycles of sedimentation in sucrose gradients. Figure 1 shows the pattern of polypeptides revealed by electrophoresis in SDS polyacrylamide gels of the purified virus after disruption with SDS and dithiothreitol. The region marked VP2/3 has been shown to correspond to glycosylated polypeptides not present in naked virus particles (K.L.P., and D.H.W., unpublished). A longitudinal slice was removed

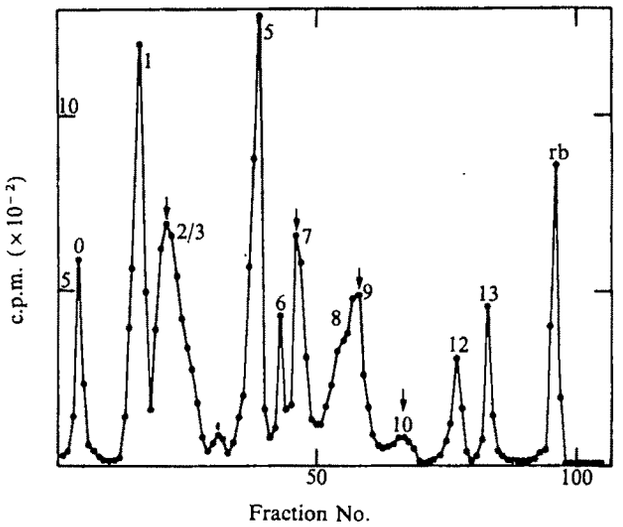


Fig. 1 Electrophoresis of disrupted enveloped Herpes simplex virus particles on SDS-polyacrylamide gel. Direction of migration from left to right (that is, higher molecular weight polypeptides to the left). Peaks identified as glycoproteins by glucosamine labelling are arrowed. Peak 1 is the major capsid polypeptide corresponding to VP5 of Spear and Roizman[8] and peak 2/3 (VP2/3) most likely corresponds to their broad glycoprotein peak VP7/8.

from other gels to identify the VP2/3 region and the appropriate segments removed from the remaining portion of the gels. The segments were macerated and injected with Freund's incomplete adjuvant into the footpads of rabbits. From the intensity of stain in the gel slices we estimate that about 40 μg protein was inoculated. A second injection was given 2 weeks later and the rabbits bled after a further 10 d.

The resulting antiserum was tested in neutralisation tests against herpes simplex type 1 strain HFEM and herpes simplex type 2 strain 3345 as previously described[6]. The neutralisation rate constant against type 1 virus was 0.167 (mean of seven determinations) and against type 2 virus was 0.001 (mean of nine determinations) which did not differ significantly from zero. In contrast, antiserum to Band II had neutralisation rate constants against type 1 virus of 0.18 and against type 2 virus of 0.06 (ref. 6). The neutralising activity of the antiserum to VP2/3 was unaffected by absorption with an extract of cells infected with herpes simplex virus type 2, but was completely abolished by absorption with an extract of cells infected with herpes simplex virus type 1. The neutralisation rate constant of antiserum to Band II antigen, however, was reduced by 90% after absorption with the same extract of cells infected with type 2 virus (Table 1).

Table 1 Proof of type specificity of antiserum to VP2/3 polypeptide by absorption tests.

Antiserum	Absorbed with	Neutralisation rate constant of absorbed serum for type 1 virus
Anti VP2/3	Extract of uninfected cells	0.120
Anti VP2/3	Extract of cells infected with type 1 virus	0
Anti VP2/3	Extract of cells infected with type 2 virus	0.132
Anti Band II	Extract of uninfected cells	0.644
Anti Band II	Extract of cells infected with type 1 virus	0
Anti Band II	Extract of cells infected with type 2 virus	0.052

The antiserum to VP2/3 had similar neutralisation rate constants in tests against two other type 1 strains but gave no significant neutralisation of two other type 2 strains. The type specificity was confirmed by complement fixation tests (Table 2).

Table 2 Type specificity of antiserum to VP2/3 polypeptide demonstrated by complement fixation

Antiserum	Complement fixing titre* with	
	Type 1 antigen	Type 2 antigen
Anti-VP2/3	1280	<40†
Anti-type 1 virus	2560	5120
Anti-type 2 virus	2560	2560

* Reciprocal of highest dilution fixing 1.5 units of complement using optimum dilutions of antigens.
† Antiserum had anticomplementary activity at lower dilutions.

An antiserum with similar neutralising properties was obtained by an alternative method. Antiserum to type 1 virus, rendered selective for type-specific antigens by absorption with an extract of type 2 virus-infected cells[6], was used to detect type-specific antigens in fractions obtained by preparative polyacrylamide gel electrophoresis (without SDS) of an infected cell extract[7]. Precipitin bands obtained in agar gel immunodiffusion of one type specific fraction and the antiserum, were injected into the footpad of rabbits. This antiserum and antiserum to VP2/3 gave single precipitin bands in agar gel immunodiffusion tests with extracts of cells infected with type 1 virus. The precipitin bands obtained with the two antisera showed reaction of identity. Neither antisera gave any reaction in immunodiffusion tests with extracts of cells infected with type 2 virus.

The results provide direct evidence for the presence of a type-specific antigen both in infected cells and as a glycoprotein on the surface of enveloped particles, whose interaction with its corresponding antibody leads to a loss of virus infectivity. They offer some hope that it may be possible to prepare a type-specific reagent for detection of previous type 2 infection in seroepidemiological surveys.

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Note added in proof: Since this work was completed we have heard that Dr R. J. Courtney (Baylor University College of Medicine, Houston, Texas) has prepared an antiserum with similar properties using extracts of cells infected with herpes simplex virus type 1. This antiserum also had type specific neutralising activity.

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1 Rawls, W. E., Laurel, D., Melnick, J. L., Glicksman, J. M., and Kaufman, R. H., *Am. J. Epidemiol.*, **87**, 647 (1968).
2 Nahmias, A. J., Josey, W. W., Naib, Z.M., Luce, C. F., and Duffey, A., *Am. J. Epidemiol.*, **91**, 539 (1970).
3 Skinner, G. R. B., Thouless, M. E., and Jordan, J. A., *J. Obst. Gynec.*, **78**, 1031 (1971).
4 Wildy, P., *Cancer Res.*, **33**, 1465 (1973).
5 Watson, D. H., and Wildy, P., *J. gen. Virol.*, **4**, 163 (1969).
6 Sim, C., and Watson, D. H., *J. gen. Virol.*, **19**, 217 (1973).
7 Watson, D. H., *J. gen. Virol.*, **4**, 151 (1969).
8 Spear, P., and Roizman, B., *J. Virol.*, **9**, 143 (1972).

In vitro generation of B lymphocytes in mouse foetal liver, a mammalian 'bursa equivalent'

THERE is good evidence that in birds a haemopoietic stem cells of yolk sac origin[1,2] differentiate into immunoglobulin-bearing lymphocytes in the bursa of Fabricius[3]. The differentiated lymphocytes then migrate to the peripheral lymphoid tissues[4,5], where they constitute the bursa-derived B lymphocyte population. The primary site(s) of B lymphocyte development in mammals, however, has remained a mystery, with gastrointestinal associated lymphoid tissues[6] (including appendix[7], tonsils[8] and Peyer's patches[9]) and haemopoietic tissues[10–12] generally considered the main candidates for mammalian 'bursa equivalent'. Here we report the development of IgM, IgG and probably IgA-bearing lymphocytes in organ cultures of mouse foetal liver removed at 14 d gestation, a time well before immunoglobulin-bearing cells or cells of lymphoid morphology could be detected in the intact animal. These studies indicate that foetal liver is a bursa equivalent in mice and that gastrointestinal lymphoid tissues are not a *sine qua non* for the initial development of B cells. In addition, they provide evidence that IgG and probablv IgA-bearing B lymphocytes can develop in the absence of T lymphocytes.

Foetal livers were removed from BALB/c embryos of 14 or 15 d gestation (the day of appearance of a vaginal plug being taken as day 0) and chopped with fine knives. Small explants (2–4 mm diameter) were placed on ultrathin Millipore filters (type TH), floating on 1 ml of RPMI 1640 medium containing 10% heat-inactivated foetal calf serum in the centre well of Falcon organ culture dishes (catalogue No. 3010), the outer trough being filled with distilled water. The dishes were incubated at 37°C in an atmosphere of 5% CO_2 in air. Foetal livers, spleens or cultured explants were

Table 1 Properties of fluorescein-conjugated antibodies

Antiserum	Abbreviation	Fraction used	F/P Ratio	Concentration used (mgml^{-1})	References	Proportion of cells labelled in adult spleen (%)
Rabbit anti-mouse immunoglobulin	Anti-MIg-fl	Purified antibody	1.5	2.0	14, 25	52.6*
Rabbit anti-mouse immunoglobulin	Fab Anti-MIg-fl	Fab IgG	2.1	10.0	25	40–55
Goat anti-mouse μ(IgM)	Anti-μ-fl	Purified antibody	3.0	0.5	21	30.6*
Goat anti-mouse γ_1(IgG$_1$)	Anti-γ_1-fl	,, ,,	3.2	0.5	21	0.3*
Goat anti-mouse γ_{2ab} (IgG$_{2a}$ and IgG$_{2b}$)	Anti-γ_{2ab}-fl	,, ,,	3.5	0.5	21	18.8*
Goat anti-mouse α(IgA)	Anti-α-fl	,, ,,	3.2	0.5	21	0.7*
Goat anti-human μ (IgM)	Anti-human μ-fl	,, ,,	3.2	0.5	16	0
Mouse anti-θC3H	Anti-θ-fl	IgG	0.8	10.0	15	25–40

The aggregate value for labelling with these reagents separately is 50.4%.
*Result of a single experiment where the proportion of cells stained with a mixture of fluorescein-fl-labelled anti-μ, anti-γ1, anti-γ2ab and anti-α was 47%.

Table 2 Proportion of Ig-bearing and θ-bearing cells in foetal liver and spleen*

		Liver						Spleen						
Day of gestation	No. of experiments	Ig.	μ	$\gamma/_1$	γ_2	α	θ	No. of experiments	Ig	μ	γ_1	γ_2	α	θ
12	1	$^0/10^4$	—	—	—	—	$^0/10^4$	0	—	—	—	—	—	—
13	1	$^0/1.2\times10^4$	—	—	—	—	$^0/2\times10^4$	0	—	—	—	—	—	—
14	7	$^0/1.6\times10^5$	—	—	—	—	$^0/2.7\times10^4$	2	$^0/600$	—	—	—	—	—
15	1	$^0/10^4$	—	—	—	—	—	0	—	—	—	—	—	—
16	2	$^0/10^4$	$^0/6\times10^4$	$^0/5\times10^4$	$^0/5\times10^4$	$^0/5\times10^4$	$^0/10^4$	2	—	$^0/5500$	—	$^0/200$	—	—
17	1	$^{11}/10^3$ (1.1%)	—	—	$^3/1.5\times10^4$ (0.02%)	$^0/10^4$	$^0/3\times10^3$	1	$^7/588$ (1.3%)	$^7/504$ (1.2%)	—	$^2/5000$ (0.04%)	$^0/12000$	—
18	2	$^5/10^3$ (0.5%)	$^{35}/6500$ (0.5%)	$^0/10^5$	$^5/10^4$ (0.05%)	$^0/10^4$	$^2/2\times10^4$ (0.01%)	2	$^6/500$ (1.2%)	$^{13}/313$ (4.2%)	—	$^7/2000$ (0.35%)	$^2/7500$ (0.03%)	$^5/2600$ (0.2%)
19	1	$^{23}/2400$ (1%)	$^{41}/3000$ (1.4%)	$^0/5000$	$^5/2500$ (0.2%)	$^0/5000$	—	1	$^{12}/401$ (3.9%)	$^3/306$ (2%)	$^0/5000$	$^3/1250$ (0.24%)	$^0/4000$	—
20	0	—	—	—	—	—	—	2	—	$^{16}/500$ (3.2%)	$^0/2000$	$^5/700$ (0.7%)	?	—

*Slides were scanned systematically using an oil-immersion objective and the total number of cells surveyed was estimated from the number of fields and number of cells per field examined.

Table 3 Development of Ig-bearing lymphocytes in foetal liver explant*

Experiment	Age of liver explanted (d)	Days in culture	Proportion of cells labelled with							
			Anti-MIg	Fab anti-MIg	Anti-μ	Anti-γ_1	Anti-γ_2	Anti-α	Anti-θ	Anti-human μ
1	14	2	$^0/7000$	—	—	—	—	—	$^0/2000$	—
2	14	4	$^9/2000$ (0.45%)	—	—	—	—	—	$^0/4000$	—
3	14	4	—	$^5/2500$ (0.2%)	$^7/3000$ (0.23%)	$^0/8000$	$^3/7300$ (0.04%)	$^0/4000$	$^0/8000$	—
4	15	3	$^2/2000$ (0.1%)	$^2/2000$ (0.1%)	—	—	—	—	—	—
5	14	5	—	$^{23}/1900$ (1.2%)	$^{18}/1280$ (1.4%)	—	$^5/3100$ (0.16%)	—	—	—
6	14	5	—	—	$^{23}/2500$ (0.92%)	$^0/3500$	$^3/3000$ (0.1%)	$^0/4000$	$^0/2000$	$^0/6000$
7	14	7	—	$^{12}/440$ (2.5%)	$^7/232$ (3%)	$^0/200$	$^3/200$ (1.5%)	$^2/4000$ (0.05%)	—	$^0/5000$

*See legend to Table 2.

taken at various times and teased in veronal-buffered saline containing 20% foetal calf serum and 0.2% sodium azide. Aliquots of cells were spun onto slides in a cytocentrifuge and stained with McNeal's tetrachrome. In addition, cells were incubated in suspension with a variety of fluorescein-conjugated antisera (see Table 1) at 0°C for 30 min, washed twice and examined for surface fluorescence using a modified Vicker's M40 microscope equipped for incident illumination as previously described[13]. Fluorescein-conjugated anti-immunoglobulin antibodies, polyspecific and class specific, were used to detect B lymphocytes[14] while a fluorescein-conjugated anti-θ serum was used to detect T cells[15]. All of these reagents have been used in previously reported experiments and their properties are outlined and referenced in Table 1. Usually the antibodies used were purified and absorbed using solid-phase immunoadsorbents. The proportion of cells labelled with the various reagents in adult BALB/c spleens is indicated in Table 1.

Differential cell counts on cytocentrifuge preparations from freshly teased (uncultured) tissues showed that most of the cells prepared from 12–19 d foetal liver belonged to the erythroid cell series. From 16 d, increasing numbers of granulocytic and monocytic cells were seen with the first lymphoid cells being detected at 17 d. We could distinguish lymphocytes from erythroid cells because lymphocytes have a thinner rim of cytoplasm which is paler staining than that of the erythroid series of cells. The picture in foetal spleen was similar to that in liver except that a greater proportion of granulocytic and monocytic cells were present at all stages. By fluoresence, immunoglobulin-bearing cells were first detected at 17 d gestation in both liver and spleen (Table 2) correlating well with the morphological studies. As has been shown in man[16], IgM and IgG-bearing cells developed before IgA-bearing lymphocytes. It seems that B cell development is relatively retarded in BALB/c mice compared to other strains where B cells have been detected

at 16 d gestation[11,17]. In agreement with this we have found that CBA mice have a higher proportion of B cells in their spleens at birth than do BALB/c mice.

Results of 14 d foetal liver organ culture experiments are outlined in Table 3. Although no cells of lymphocyte morphology were found in 14 d livers, after 4 d in culture approximately 0.5% of the nucleated cells were typical small lymphocytes on morphological criteria. With time in culture, erythopoiesis declined and an increasing percentage of the cells present were granulocytic, monocytic and lymphoid, with approximately 2–3% of the cells being lymphocytes after 7 d in culture. Correlating well with the morphological studies, 0.2–0.45% of cells from explants in culture for 4 d bore immunoglobulin, mostly IgM, with a smaller proportion of IgG_2-bearing cells. The proportion increased with time in culture, so that at 7 d between 2–3% of the cells were immunoglobulin-positive, and two cells (out of 4000 scanned) were found that stained distinctly, but faintly, with anti-α-fluorescein(fl). No cells labelled with anti-θ-fl, anti-γ_1-fl or anti-human-μ-fl after 4 or 5 d in culture, while an appropriate proportion labelled with Fab anti-MIg-fl, excluding the possibility that fluorescent labelling was related to the binding of the conjugates by Fc receptors of cells and indicating that θ-bearing T cells did not develop in these cultures.

It is highly unlikely that the immunoglobulin-bearing cells detected after 4 d in culture were present in the 14 d liver explants at the beginning of the culture period and then merely concentrated by selective cell survival, as no immunoglobulin-bearing cells were detected in scanning more than 0.4×10^6 cells from 14–16 d livers, including samples from all livers that were subsequently cultured. Although there was a selective loss of erythroid cells during culture, we were able to collect 3×10^5–6×10^6 cells following 4–7 d culture of 2 or 3 14 d livers (about 3×10^7 can be collected by teasing from this number of livers). Even with this cell loss, if the B cells which we detected after culture (up to 3% of the cells collected) were already present before culture, we would have detected them among the large number of 14 d foetal liver cells examined.

These experiments show that immunoglobulin-bearing B lymphocytes can differentiate within mammalian foetal liver, *in vitro*. On this basis mammalian foetal liver can be considered as a possible candidate for a mammalian 'bursal equivalent'. We believe that the liver provides a 'micro-environment' for B cell differentiation as we failed to find immunoglobulin-bearing cells in 5 d cultures of liver cells obtained from 14 day foetal liver either by trypsinisation or by gently teasing the tissue apart. The cells were cultured in Falcon flasks in exactly the same media and atmosphere as adopted for the whole organ culture. The possibility that B cells can also develop in other tissues, such as the appendix and Peyer's patches, has not been excluded, although the intestinal epithelial theory of B cell maturation[6,18,19] is no longer tenable in its original form. It will be of interest to investigate the capacity of other tissues, such as foetal spleen and bone marrow, to generate B cells *in vitro*, to determine if all haemopoietic tissues can serve this function, or whether foetal liver, which is largely derived from foregut epithelium, is the only bursa-equivalent organ. These experiments also demonstrate that IgM, IgG and probably IgA-bearing cells can develop in the foetal liver, in the absence of T cells, providing the most direct evidence thus far that T cells are not required for the postulated switch from IgM to IgG and/or to IgA in single B cells[20–23]. The failure of θ-bearing T lymphocytes to develop in 5d cultures is of interest in view of the recent evidence that agents such as poly(A)·poly (U), cyclic AMP and lipopolysaccharide can induce some cells in 14 d foetal liver to express θ (ref. 24). Our attempts, in collaboration with Peter Beverley, to induce the appearance of immunoglobulin-or θ-bearing cells during a 2 h incubation of 14 d BALB/c foetal liver cells with these agents have failed so far.

The development of B lymphocytes in 14 d foetal liver explants should provide a useful system for investigating the microenvironmental requirements for B cell differentiation, the putative immunoglobulin class switch, tolerance induction, and the generation of antibody diversity in the absence of T cells and in a relatively controlled antigenic environment. Elucidation of the bursa equivalent is of obvious importance to the understanding of a variety of immune deficiency diseases.

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1 Moore, M. A. S., and Owen, J. J. T., *Devl Biol.*, **14**, 40 (1966).
2 Moore, M. A. S., and Owen J. J. T., *Nature*, **215**, 1081 (1967).
3 Kincade, P. W., and Cooper, M. D., *J. Immun.*, **106**, 1421 (1971).
4 Cooper, M. D., Schwartz, M. M., and Good, R. A., *Science*, **151**, 471 (1966).
5 Durkin, H. G., Theis, G. A., and Thorbecke, G. J., *Morphological and Functional Aspects of Immunity* (edit. by Lindahl-Kiessling, K., Alm, G., and Hanna, M. G.) 119 (Plenum Press, New York, 1971).
6 Cooper, M. D., Perey, D. Y., McKneally, M. F., Gabrielson, A. E., Sutherland, D. E. R., and Good, R. A., *Lancet*, **i**, 1388 (1966).
7 Archer, O. K., Sutherland, D. E. R., and Good, R. A., *Nature*, **200**, 337 (1963).
8 Peterson, R. D., Cooper, M. D., and Good, R. A., *Am. J. Med.*, **38**, 579 (1965).
9 Perey, D. Y. E., Cooper, M. D., and Good, R. A., *Science*, **161**, 265 (1968).
10 Brahim, F., and Osmond, O. G., *Anat. Rec.*, **168**, 139 (1970).
11 Nossal, G. J. V., and Pike, B. L., *Immunology*, **25**, 33 (1973).
12 Abdou, N. I., and Richter, M., *Adv. Immun.*, **11**, 202 (1969).
13 Ashman, R. F., and Raff, M. C., *J. exp. Med.*, **137**, 69 (1973).
14 Raff, M. C., *Immunology*, **19**, 637 (1970).
15 Raff, M. C., *Nature new Biol.*, **246**, 350 (1973).
16 Lawton, A. R., Self, K. S., Royal, S. A., and Cooper, M. D., *Clin. Immunobiol., Immunopath.*, **1**, 104 (1972).
17 Spear, P. G., Wang, A., Rutishauser, U., and Eidelman, G. M., *J. exp. Med.*, **138**, 557 (1973).
18 Fichtelius, K. E., *Expl Cell. Res.*, **49**, 87 (1968).
19 Cooper, M. D., and Lawton, A. R., *Contemporary Topics in Immunobiology*, (edit. by Hanna, M. G.), **49**, (Plenum Press, New York, 1972).
20 Kincade, P. W., Lawton, A. R., Bockman, D. E., and Cooper, M. D., *Proc. natn. Acad. Sci., U.S.A.* **67**, 1918 (1970).
21 Lawton, A. R., Asofsky, R., Hylton, M. B., and Cooper, M. D., *J. exp. Med.*, **135**, 277 (1972).
22 Pernis, B., Forni, L., and Amante, L., *Ann. N.Y. Acad. Sci.*, **190**, 420 (1971).
23 Pink, R. Wang, A. C., and Fudenberg, H. H., *A. Rev. Med.*, **22**, 145 (1971).
24 Scheid. M. P., Hoffmann. M. K., Komur, K., Hammerling, U., Abbott, J., Boyse, E. A., Cohen, G. H., Hooper, J. A., Schulof, R. S., and Goldstein, A. L., *J. exp. Med.*, **138**, 1027 (1973).
25 Taylor, R. B., Duffus, W. P. H., Raff, M. C., and de Petris, S., *Nature new Biol.*, **233**, 225 (1971).

Expression of M locus differences by B cells but not T cells

Within or close to the major histocompatibility complex (MHC) of man and mouse there are genetic loci which control the production of lymphocyte-associated determinants capable of stimulating allogeneic lymphocytes to synthesise DNA in

Table 1 MLR between cells from MHC and M different mice

Responder	Stimulator					
	CBA/J spleen	CBA/J thymus	C3H/HeJ spleen	BALB/cJ spleen	DBA/2J spleen	DBA/2J thymus
CBA/J thymus	4,167 ± 503	—	4,251 ± 306	19,194 ± 2701	—	—
C3H/HeJ thymus	32,979 ± 1,269	2,143 ± 160	2,140 ± 310	—	—	—
BALB/cJ	—	13,802 ± 2,004	—	3,131 ± 460	41,103 ± 3,231	2,017 ± 126
DBA/2J thymus	33,683 ± 3,975	—	—	3,204 ± 706	3,918 ± 401	—

Numbers represent c.p.m. ± s.d.

mixed lymphocyte culture[1,2]. In the mouse the mixed lymphocyte reaction (MLR) is also controlled by an additional locus, the M locus, which is not linked to MHC[3]. These MLR loci seem to be distinct from the loci coding for serologically defined (SD) antigens[1,2,4]. Recent studies suggest that, in certain situations, a genetic difference between responder and stimulator cells is not an absolute requirement in MLR. Thus, Howe *et al.*[5] and Boehmer *et al.*[6,7] found that thymus cells from certain mouse strains produce high levels of thymidine incorporation when cultured with syngeneic B lymphocytes.

Lymphocytes which proliferate in response to cells differing at lymphocyte defined (LD) loci, but not at SD loci, are reported to be unable to develop into cytotoxic effector cells[1,2,4]. The nature of the LD stimuli which induce cell proliferation in this situation is unknown. This paper attempts to define the cells which carry these determinants.

We have employed Hannig's system of preparative cell electrophoresis[8] to prepare highly purified T and B cell populations for use as stimulating cells in MLR. BALB/c ($H2^d$, Mls^b), DBA/2 ($H2^d$, Mls^b), C3H ($H2^k$, Mls^c) and CBA/J ($H2^k$, Mls^d) mice were used in these studies. BALB/c and DBA/2 are MHC identical but differ at the M locus; a similar relationship exists between C3H and CBA/J.

Thymus cells, thoracic duct lymphocytes (TDL) and spleen cells were obtained by standard methods[9]. TDL were used as stimulating cells after separation by electrophoresis as described previously[10]. Thymocytes from 6-week-old mice served as responder cells. These cells were cultured with stimulator cells treated with mitomycin C[11] from thymus, spleen or TDL in Falcon plastic tubes 2005 (Gateway International, Los Angeles) at a total cell concentration of 5×10^6 per ml culture medium. With thymus cells as stimulators, the ratio of responding to stimulating cells was 3 : 1; with cells from spleen and TDL, a responder : stimulator ratio of 10 : 1 was chosen; in previous studies these ratios gave optimal stimulation with strain combinations differing at the MHC or M locus and reduced 'backstimulation' (see below) by the mitomycin C-treated cells (ref. 12 and unpublished observations). The culture medium used was RPMI 1640 supplemented with 10% foetal calf serum and mercaptoethanol[13]. The cells were cultured at 37° C in a humidified atmosphere of 10% CO_2 in air. ^{3}H-thymidine (Radiochemical Centre, Amersham, specific activity 25 Cimmol^{-1}) was added to the cultures at a final concentration of 1.3 μCiml^{-1} after 48 h and cells were collected after 60 h. Radioactivity in DNA extracted from the cells was measured as described previously[11]. Background thymidine incorporation was calculated from separate incubations of either 5×10^6 responder or stimulator cells.

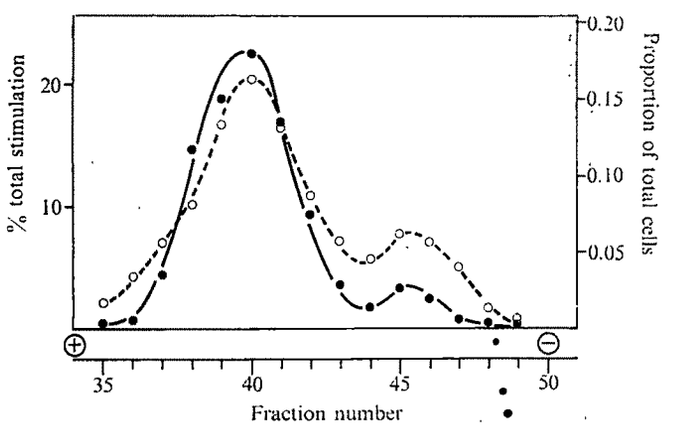


Fig. 1 Stimulating capacity of electrophoretically separated CBA TDL treated with mitomycin C cultured with BALB/c (MHC plus M different) thymus cells. Calculated background: 1,371 c.p.m. Peak stimulation with 5×10^5 stimulators: 103,428 ± 5,630 c.p.m. ●—●, Stimulation; ○--○, all cells.

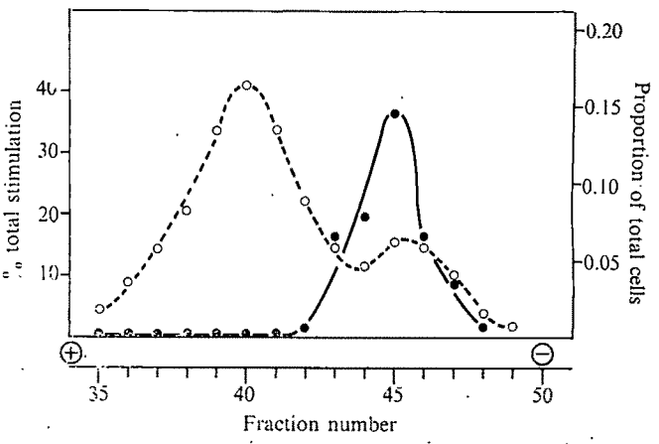


Fig. 2 Stimulating capacity of electrophoretically separated CBA TDL treated with mitomycin C cultured with C3H (M different) thymus cells. Calculated background: 2,528 c.p.m. Peak stimulation with 5×10^5 stimulators: 42,679 ± 3,926 c.p.m. ●—●, Stimulation; ○--○, all cells.

Table 1 shows DNA synthesis by thymus cells cultured with stimulating cells from various sources. It is apparent that there is undirectional stimulation of BALB/c thymus by DBA/2 spleen and of C3H thymus by CBA spleen. These findings are in agreement with the studies of Festenstein[14] and Salaman *et al.*[15]. They differ to some extent from the findings of Katz-Heber *et al*[16]. These workers found that while BALB/c cells responded strongly to DBA/2, a significant though weak response was also observed in the reverse direction. The authors used a low ratio of responder to stimulator cells in their cultures. Recent work of one of us (H.v.B.) suggests that, under these conditions, the response observed by Katz-Heber *et al.* when BALB/c cells were used as the stimulating population might have reflected 'backstimulation' of the DBA/2 cells. In this respect, significant DNA synthesis was observed when parental cells treated with mitomycin C were cultured at a low (but not a high) ratio with semiallogeneic F_1 hybrid cells. From additional evidence it was concluded that DNA synthesis in this situation represented backstimulation of F_1 cells responding to a 'recognition signal' from the T cells in the parental population treated with mitomycin C[17,18].

When thymus cells instead of spleen cells were used as stimulators, responses by thymus cells were significant when an MHC difference was involved but not significant when the cells differed at the M locus only (Table 1). The data in Table 1 suggest, therefore, that the lymphocyte-defined differences coded for by the M locus were carried by spleen cells but not

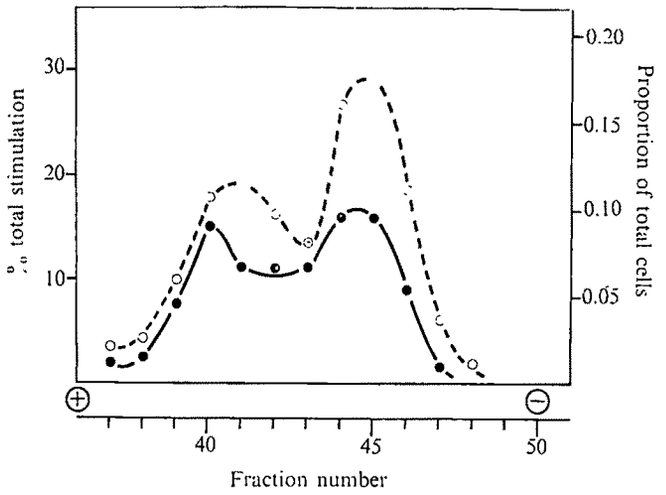


Fig. 3 Stimulating capacity of electrophoretically separated DBA/2 TDL treated with mitomycin C cultured with CBA/J (MHC plus M different) thymus cells. Calculated background: 3,049 c.p.m. Peak stimulation with 5×10^5 stimulators: 41,683 ± 1,050 c.p.m. (DBA/2 TDL were obtained 48 h after cannulation and have therefore a higher proportion of B lymphocytes.) ●—●, Stimulation; ○ – – ○, all cells.

by thymus cells. More precise information on this point was obtained by testing the stimulating capacity of purified populations of mature (small) T and B lymphocytes obtained from thoracic duct lymph.

TDL subjected to electrophoresis separate into two distinct cell populations which partly overlap[19]. Previous studies showed that T (θ positive) and B (immunoglobulin positive) lymphocytes were arranged in Gaussian distribution in the fast moving and slow moving cell fractions, respectively. Thus the fractions of highest electrophoretic mobility consisted of pure T cells and the fractions of lowest electrophoretic mobility of pure B cells; intermediate fractions contained both T and B lymphocytes.

Electrophoretically separated cell fractions of TDL from CBA mice were treated with mitomycin C and cultured separately at a ratio of 1:10 with thymus cells differing at the M locus (C3H) or at both MHC and M locus (BALB/c), The stimulation observed in these cultures is shown in Figs 1 and 2. As described elsewhere[18], the data are expressed as the proportional stimulation provided by each stimulator fraction. To make this calculation, the counts above background given by the aliquot (0.5×10^6 cells) of each fraction of stimulating cells was

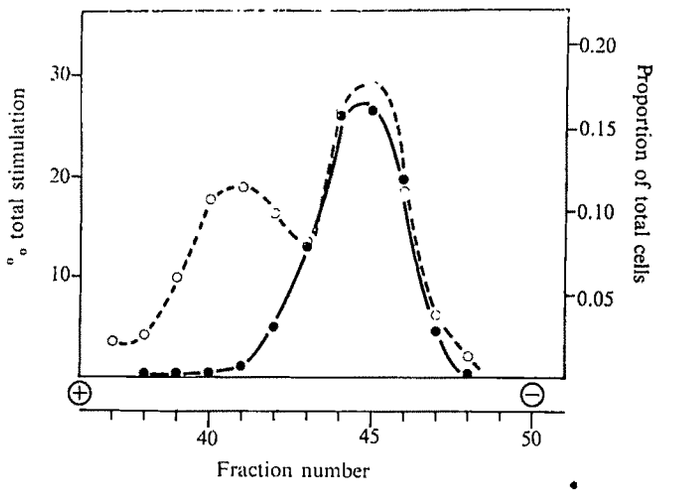


Fig. 4 Stimulating capacity of electrophoretically separated DBA/2 TDL treated with mitomycin C cultured with BALB/c (M different) thymus cells. Calculated background: 1,537 c.p.m. Peak stimulation with 5×10^5 stimulators: 66,003 ± 9,803 c.p.m. ●—●, Stimulation, ○ – – ○ all cells.

multiplied by the total number of cells in that fraction. Under the constant culture conditions used, addition of the values obtained for each cell fraction provided an estimate of the total stimulating capacity of the TDL populations as a whole. The proportional stimulation provided by each fraction was then calculated with reference to this combined value.

When the data are expressed in this fashion, it is evident that, with MHC differences, thymus cells responded to TDL fractions containing either T cells, B cells or mixtures of T and B cells to approximately the same extent. With a difference restricted to the M locus, by contrast, thymus cells responded only to cells from fractions containing B cells. Similarly, fractions B cell-depleted of DBA/2 TDL stimulated CBA (MHC plus M different) thymus but not BALB/c (M different) thymus (Figs 3 and 4).

The observation that T and B lymphocytes share the capacity to induce allogeneic (MHC different) T cells to synthesise DNA corroborates our previous findings[17,20]. A consensus of opinion has yet to be obtained on this point, however, since some workers have claimed that the allogeneic stimulus is carried predominantly by B cells[19], though others maintained it was only found on T cells[21]. The failure of these workers to use more precisely defined populations of T and B cells in their studies might have contributed to the disparity in their results.

We do not know the significance of the finding that, in the MLR, the stimulus to lymphocytes differing solely at the M locus is carried exclusively by B lymphocytes. As mentioned previously, B lymphocytes activated solely by M locus differences were reported to be unable to induce cell-mediated lympholysis (CML) (ref. 4). Phytohaemagglutinin (PHA)-transformed blasts were used as target cells for CML in this study. Our work questions the validity of this choice of target cells. Thus, in mice, PHA blasts are considered to be derived from T cells that is from cells which in our studies did not express detectable M locus differences. For this reason work is currently in progress in our laboratory to determine whether lymphocytes activated to LD differences can lyse B blast cells.

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1 Eijsvoogel, V. P., *et al.* in *Histocompatibility Testing* (edit. by Dausset, J., and Colombani, J.), 501 (Munksgaard, Copenhagen, 1972).
2 Bach, F. H., Widmer, M. B., Bach, M. L., and Klein, J., *J. exp. Med.*, **136**, 1430 (1972).
3 Festenstein, H., *Ann. N.Y. Acad. Sci.*, **921**, 567 (1966).
4 Abbasi, K., and Festenstein, H., *Eur. J. Immun.*, **3**, 430 (1973).
5 Howe, M. L., *J. Immun.*, **110**, 1040 (1973).
6 von Boehmer, H., and Byrd, W., *Nature new Biol.*, **235**, 50 (1972).,
7 von Boehmer, H., *Eur. J. Immun.* **4**, 105, (1974).
8 Hanning, K. *Free Flow Electrophoresis Handbook* (edit. by Norris, J. R., and Ribbons, D. W.), **513**, (Academic, London, 1971).
9 Sprent, J. *Cell. Immun.*, **7**, 10 (1973).
10 von Boehmer, H., Shortman, K., and Nossal, G. J. V., *Cell. Physiol.* (in the press).
11 Byrd, W., von Boehmer, H., and Rouse, B. T., *Cell. Immun.* **6**, 12 (1973).
12 von Boehmer, H., Shortman, K., and Adams, P. B., *J. exp. Med.* **136**, 1648 (1972).
13 Nabholz, M., Vives, J., Young, H. M., Meo, T., Miggiano, V., Rijnbeck, A., and Shreffler, D. C., *Eru. J. Immun.* (in the press).
14 Festenstein, H., Abbasi, K., Sachs, J. B., and Oliver, R. T. D., *Transplantn Proc.* **4**, 219 (1972).
15 Salaman, M. H., Wedderburn, N., Festenstein, H., and Huber, B., *Transplantation*, **16**, 29 (1973).
16 Katz-Heber, E , Peck, A B and Click, R. E., *Eru. J. Immun.*, **3**, 379 (1973).
17 von Boehmer, H., in *Proc. eighth Leucocyte Culture Conference, Uppsala* (edit by Fischer-Lindahl, K.) (1973).
18 von Boehmer, H., *J. Immun.* **112**, 70 (1974).
19 Plate, J. M. D., and McKenzie, I. F. C., *Nature new Biol.*, **245**, 247 (1973).
20 Sprent, J., and Miller, J. F. A. P., *Cell. Immun.*, **3**, 385 (1972).
21 MacLauren, B. P., *Clin. exp. Immun.*, **10**, 649 (1972).

Erythropoiesis in an avian thymus

As part of an ecological study of the red billed quelea, *Quelea quelea*, undertaken in Tanzania and Kenya between 1969 and 1972, the size and colour of the thymus glands in over 5,000 birds were recorded and subsequently correlated with the physiological state of the birds, such as the state and degree of moult and breeding condition. More than 200 lobes from these thymus glands were excised and examined by routine histological methods using impression smears, paraffin sections and araldite embedded sections prepared for light and electron microscopy. This work, carried out in collaboration with other workers, will be reported in full elsewhere and is briefly summarised here.

In *Quelea quelea*, as in other birds[1], the thymus consists of a number of lobes well separated from one another in two long chains one on each side of the neck. In embryos about to hatch each lobe was found to be small (less than 1 mm long) and white; but by the age of 10 d, 17 out of 60 individuals examined (28%) had pink, enlarged (over 4 mm long) thymi; and on becoming independent at 20-d-old 264 out of 275 (96%) had enlarged thymic lobes. At the commencement of the postjuvenile moult at about 9 weeks of age, 113 out of 140 (80%) young still had enlarged thymi and over the next 3 months, during the progress of this moult, the proportion fell to 54 out of 155 (35%). The fall apparently continued during the first prenuptial moult, though by this time it was difficult to distinguish first year from older adults.

Thus in queleas, as in most other animals[2,3], maximum thymus size is reached before the onset of sexual maturity. Thereafter the thymus is said to slowly involute with age, but this is not so in queleas where the thymus glands of adults undergo rapid cycles of enlargement and regression and there are probably several such cycles in one year.

The most remarkable changes occur in breeding adults. Queleas form highly synchronised breeding colonies, each colony lasting approximately 6 weeks (after which the adults may remain in breeding condition and move away to found other colonies[4]). As Fig. 1 shows for a colony sampled in Tanzania, the percentage of adults with enlarged thymi (there was no difference in thymus size between the sexes) was low at the start of the colony as mating and laying were under way and most individuals had regressed thymi. Then, during the incubation period, the precentage of individuals with enlarged thymi increased to a peak of 70% soon after hatching, most of the rest having thymi of intermediate size (either enlarging or regressing). Thymus activity declined markedly in the latter half of the rearing period. Similar results have been obtained from other colonies, though with less complete data. With the change from a regressed to an enlarged condition the thymic lobes also changed from white to pink or bright red, and the thymic arteries and veins became prominent.

In addition to the cycle of thymus enlargement and regression in breeding colonies enlarged thymic lobes were found in about 10% of the 919 adults collected while in prenuptial moult and in 27% of the 298 birds in postnuptial moult, though the percentages varied from year to year. When wild females were kept caged in Africa and fed food and water *ad libitum*, however only 1 out of 35 birds killed at various times during the postnuptial moult had enlarged thymi. We conclude from these results that thymus enlargement associated with moult is influenced by factors other than the moult itself.

These observations substantiate Hohn's findings[5,6] of thymus enlargement following the breeding season (and therefore during the postnuptial moult) in adult mallard ducks, American robins and house sparrows; and the observations of Ward and D'Cruz[7] and Anderson[8] in which thymus enlargement in a tropical bulbul and the pheasant respectively was associated with the postnuptial moult.

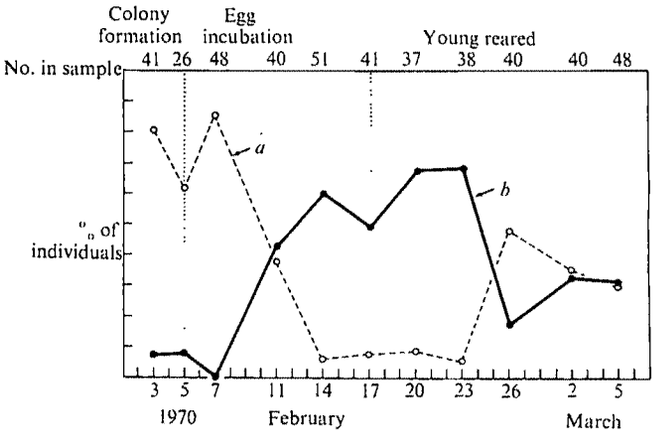


Fig. 1 Changes in the % of adult individuals with enlarged and regressed thymi in a synchronised breeding colony at Makuyuni, Tanzania. For each sample the remaining % represents birds with intermediate thymi (enlarging or regressing). The laying and hatching data are mean values for the colony. *a*, Regressed thymi; *b*, enlarged thymi.

Histologically, all except fully regressed lobes were composed of a peripheral cortex and a central medulla. The cortex of enlarging lobes contained mainly small lymphocytes (average 3–4 μm nuclear diameter) and displayed a high level of mitosis. In the enlarged lobe the typical cortical lymphocyte was in certain regions interspersed with lymphocyte-like cells[9], early erythroid cells (which were found by use of the analytical microscope EMMA-4 to contain iron), immature erythrocytes (polychromatophilic erythroblasts), mature erythrocytes or pyknotic cells. In the enlarged thymi of breeding adults the entire cortex of most lobes was packed with immature or mature erythrocytes with few, if any, thymic lymphocytes present; in some enlarged lobes the medulla also contained immature or mature erythrocytes. The presence of enormous numbers of erythrocytes imparted a bright red colour to the enlarged lobe which persisted in some lobes of intermediate size that we regarded as regressing. In regressing lobes, mitosis was not apparent, and the cortex contained very large numbers of erythrocytes which also filled all the blood vessels in the lobes. The medulla contained large Hassall's corpuscles which were not well developed in enlarging lobes. It seemed as though the efflux of erythrocytes from the enlarged lobes of breeding adults was sudden, for in some individuals collapsed lobes with little or no cortex were found.

Although invasion of the thymic lobes by immature erythrocytes produced elsewhere in the body cannot be entirely ruled out, the large numbers of these immature cells and the similarity between lymphocyte-like and early erythroid cells suggests that the erythrocytes were developing *in situ*. Terni[10] in 1924 considered the thymus of birds to be erythropoietic; and Albert, Wolf and Pryjma[11] presented evidence for erythropoiesis in the mouse thymus. Sealander and Bickerstaff[12] found a rise in red cell counts correlated with increased thymus weight in wild red-backed mice. Granulopoiesis has been demonstrated in the rat thymus[13–17] and we have found immature granulocytes (myelocytes) on electron micrographs of the *Quelea quelea* material. Pease[18] showed that the myeloblast is the primitive stem cell of the granulocytic and erythrocytic lines of cell development. Thus, erythropoiesis within the thymus is not an unprecedented concept. Identification of erythroid precursor cells is beyond the scope of this study but in view of the postulated role of the small lymphocyte in bone marrow erythropoiesis[9], the transformation of small lymphocytes under phytohaemagglutinin[9,20] and the similarity between the small lymphocytes of the marrow and thymus

in mice[21] the role of the small lymphocyte in the thymus needs investigating.

The significance of thymic erythropoiesis in the life of *Quelea quelea* is still a subject for speculation. The thymus enlarges while the young bird is growing rapidly and acquiring its first (juvenile) plumage and remains enlarged in most individuals well into the postjuvenile moult. In adults, it frequently becomes enlarged during moult and, in most if not all individuals, during the middle period of a breeding session. As an individual probably rears several broods in the course of a single breeding season, and moults both before and after the breeding season, we are left with the impression that a typical quelea may experience several complete cycles of thymus enlargement and recession each year of its adult life. There is reason to believe that the thymus enlarges whenever the need for extra erythrocytes is great, for example, during initial body growth and subsequently during each moult when large numbers of new feathers are growing and during the incubation period in a breeding colony when the adults are then recovering from a period of severe adverse nutritional balance that occurs after the start of each breeding session (ref. 22 and P.W., unpublished). Inanition (in this case manifested by a great fall in the bird's fat and protein reserves) is known to inhibit erythropoiesis[23,24], a massive production of erythrocytes following this period (that is, during the incubation period) seems quite feasible. At all these times the 14–16 lobes of the thymus may make a considerable contribution to the increased demand for red blood cells.

While the cyclic erythropoiesis that we have observed in the thymus of adult *Quelea quelea* has not previously been reported, this does not necessarily mean that it is exceptional for there has been no detailed study of the thymus of any other wild bird or mammal and for various reasons it may be expected not to occur in captive, domestic or laboratory animals.

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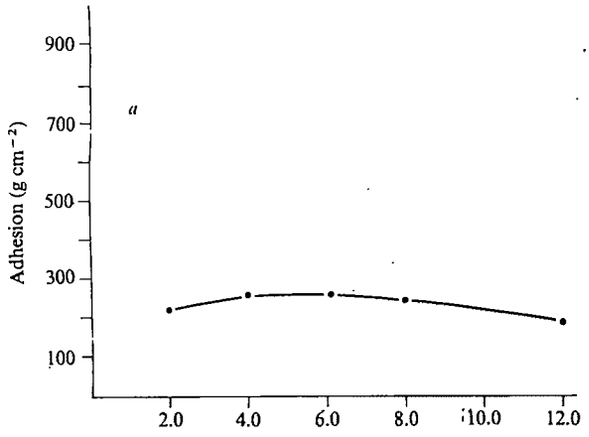
1 Romanoff, A. L., *The avian embryo. Structural and Functional Development* (Macmillan, New York, 1960).
2 Ham, A. W., *Histology* (Lippincott, Philadelphia and Toronto, 1969).
3 Romer, A. S., *The Vertebrate Body* (Saunders, Philadelphia and London, 1966).
4 Ward, P., *Ibis*, **113**, 275 (1971).
5 Hohn, E. O., *J. exp. Biol.*, **241**, 184 (1947).
6 Hohn, E. O., *Can. J. Biochem. Physiol.*, **34**, 90 (1956).
7 Ward, P., and D'Cruz, D., *Ibis*, **110**, 203 (1968).
8 Anderson, W. L., *Condor*, **72**, 205 (1970).
9 Yoffey, J. M., and Courtice, F. C., *Lymphatics, Lymph and the Lymphomyeloid Complex* (Academic Press, London, 1970).
10 Terni, T., *Arch. Ital. anat. embriol.*, **21**, 5 (1924).
11 Albert, S., Wolf, P., and Pryjma, I., *J. reticuloendothel. Soc.*, **2**, 30 (1965).
12 Seplander, J. A., and Bickerstaff, L. K., *Can. J. Zool.*, **45**, 253 (1967).
13 Weidenreich, F., *Münch. med. Wschr.*, **58**, 2601 (1912).
14 Weill, P., *Arch. mikr. Anat.*, **83**, 305 (1913).
15 Harland, M., *Anat. Rec.*, **77**, 247 (1940).
16 Sin, Y. M., and Sainte-Marie, G., *Br. J. Haemat.*, **11**, 613 (1965).
17 Sin, Y. M., and Sainte-Marie, G., *Br. J. Haemat.*, **11**, 624 (1965).
18 Pease, C. D., *Blood*, **11**, 501 (1956).
19 Carstairs, K., *Lancet*, **i**, 829 (1962).
20 McKinney, A. A., Stohlmann, F., and Brecher, G., *Blood*, **19**, 349 (1962).
21 Abe, K., Sasaki, K., and Ito, T., *J. Anat.*, **115**, 393 (1973).
22 Ward, P., *Ibis*, **114**, 444 (1972).
23 Doan, C. A., Cunningham, R. S., and Sabin, F. R., *Contr. Embryol.*, **16**, 163 (1925).
24 Weinberg, S. R., Siegel, C. D., and Gordon, A. S., *Anat. Rec.*, **175**, 7 (1972).

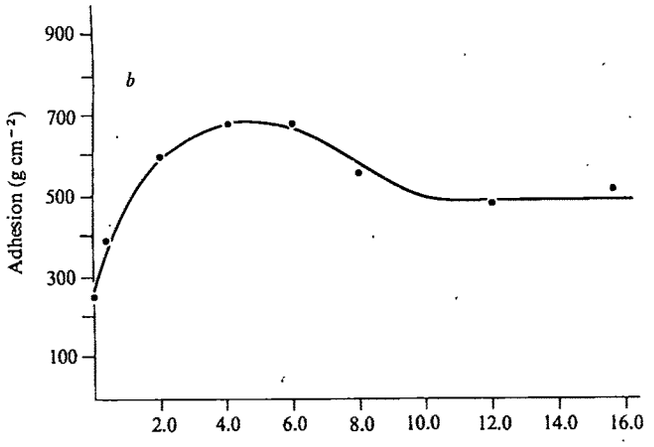
Evidence of hydrophobic interaction in adhesion to tissue

Reaggregation and agglutination of cells *in vitro* have served extensively as model systems in the evaluation of various adhesion phenomena that occur *in vivo*. Such studies have revealed factors that influence cell aggregation[1–3], but the mechanism involved at the molecular level has not been investigated fully. In the agglutination of trypsin-treated and virus-transformed cells by concanavalin A, intercellular cross-linking of receptors by the plant lectin is believed to be necessary after ionic repulsion[4,5]. The adhesion of cells may not, however, depend exclusively on the formation of cross links[6]. Hydrophobic interaction is known to be involved in the aggregation of cell surface components[7], in the solution behaviour of proteins[8] and in adsorption[9], but its contribution to adhesion involving a biological surface has not been considered. This is chiefly because of the difficulty of demonstrating experimentally, by conventional techniques, that hydrophobic interaction can indeed contribute to any significant extent and result in adhesion. I describe here the use of a reactive polyurethane prepolymer to evaluate the conditions required for adhesion to the surface of connective tissue due to hydrophobic interaction.

Castor oil was mixed with the extremely reactive tetrafluoro-1,3-phenylene diisocyanate to produce a syrupy polyurethane prepolymer[10] which contained potentially reactive isocyanate groups[11]. When this preparation was applied to a wound on the skin of a rat (see legend to Fig. 1), further reaction was initiated by the moisture present, and the prepolymer solidified in 0.3–2.0 min. If hydrophobic interaction was adequate, adhesion should have developed after solidification of the prepolymer. Even with various combinations of castor oil and tetrafluoro-1,3-phenylene diisocyanate, however adhesion was insignificant (Fig. 1*a*), and limited to the stratum corneum region of the epidermis. When some tolylene diisocyanate was added to the prepolymer, and the mixture was allowed to polymerise on the tissue *in vivo*, the degree of adhesion changed with the amount added (Fig. 1*b*). Maximum adhesion was found with 4–6 mM tolylene diisocyanate. Adhesion could also be improved considerably by priming the tissue surface with tolylene diisocyanate. This compond could cross link the prepolymer with tissue during polymerisation. If this was the situation, the observed adhesion could not be attributed to hydrophobic interaction. *n*-Hexyl isocyanate, however, which could not form cross links as a monoisocyanate, effected the same degree of adhesion (Fig. 1*b* and *c*) when mixed with the prepolymer. Therefore, the condensation of *n*-hexyl isocyanate with receptors on the tissue surface must have made the tissue surface suitable for adhesion. This conclusion was supported by the observation that the degree of adhesion of the hydrophobic prepolymer changed with the number of carbon atoms in an *n*-alkyl isocyanate (Fig. 1*c*). Maximum adhesion resulted when there were six to eight carbon atoms in the hydrocarbon chain. This was because higher or lower homologues did not have the proper hydrophilic-to-hydrophobic balance in aqueous media[12] for the isocyanate group to react with tissue and effect maximum adhesion.

Under experimental conditions comparable with those on the tissue surface, isocyanates react only with the primary amino and the sulphydryl groups in proteins[13,14], of which there are few in connective tissue. It is remarkable that the conversion of some of the receptor sites is sufficient to alter





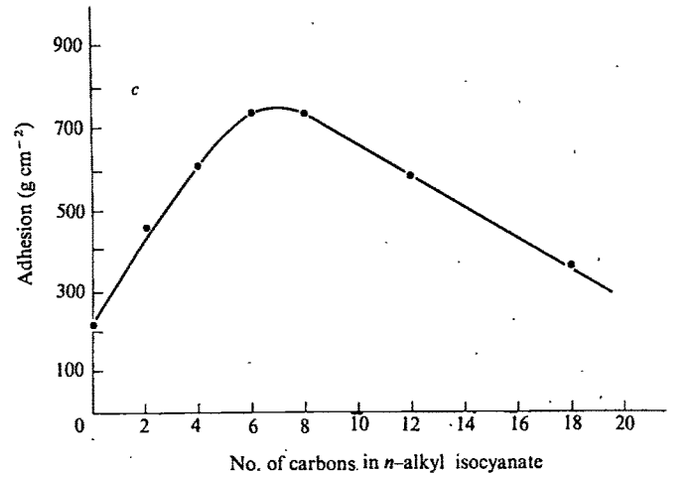


Fig. 1 Adhesion of the hydrophobic prepolymer to tissue. Several samples containing 1 mM castor oil, 4 mM tetrahydrofuran, 1 mM pyridine and a silicone surfactant[10] (0.4%, w/w) were prepared. These samples were converted into prepolymer by mixing with tetrafluoro–1,3–phenylene diisocyanate. The concentrations of tetrafluoro–1,3–phenylene, diisocyanate, tolylene diisocyanate or *n*–alkyl isocyanate required are indicated on the abscissae. Each prepolymer sample of a particular composition was used to close four 1.5 cm stab wounds on the dorsal skin of an anaesthetised Wistar rat. The adhesion of the prepolymer to tissue was assessed on a pneumatic tensiometer[10]. Each point on the graphs was the average of the four determinations on the same animal. *a*, Prepolymer samples contained various concentrations of tetrafluoro–1,3–phenylene diisocyanate only. *b*, Various concentrations of tolylene diisocyanate were added to prepolymer samples made with 4 mM tetrafluoro–1,3–phenylene diisocyanate. The horizontal part of the curve was due to cohesive failure of the solidified prepolymer. *c*, Each sample of the prepolymer was prepared with 4 mM tetrafluoro–1,3–phenylene diisocyanate, and then mixed with 4 mM *n*–alkyl isocyanate which had the required number of carbon atoms as indicated.

the surface hydrophobic character, and effect adhesion in the presence of other unreacted polar groups on tissue components.

These results show that hydrophobic interaction can result in adhesion on a biological surface. The formation of cross links and the nature of tissue surface components do not seem to be critical factors. The adhesion due to this interaction is regulated by the concentration of the converted receptor sites and their hydrophobic characteristics at the interface.

The influence of hydrophobic interaction has been observed to extend to a distance of several microns in the case of the chemotactic attraction of leukocytes by conjugated proteins[15,16]. On the surface of unassociated or dissociated cells, requirements for adhesion due to strong hydrophobic interaction, as I have established here, can be more easily achieved by diffusion[5,17] and configurational changes[15,18] of surface components. Therefore the contribution of hydrophobic interaction should be given full consideration in studies of adhesion phenomena *in vitro*[3,5,6].

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1 Humphreys, T., *Symp. zool. Soc. Lond.*, **25**, 325 (1970).
2 Pessac, B., and Defendi, V., *Nature new Biol.*, **238**, 13 (1972).
3 Curtis, A. S. G., and de Sousa, M., *Nature new Biol.*, **244**, 45 (1973).
4 Nicolson, G. L., *Nature new Biol.*, **239**, 193 (1972).
5 de Petris, S., Raff, M. C., and Mallucci, L., *Nature new Biol.*, **244**, 275 (1973).
6 Wilkins D. J., Ottewill, R. H., and Bangham, A. D., *J. theor. Biol.*, **2**, 176 (1962).
7 Singer, S. J., and Nicolson, G. L., *Science*, **175**, 720 (1972).
8 Dandliker, W. B., and de Saussure, V. A., in *The chemistry of biosurfaces*, (edit. by Hair, M. L.), **1**, 25 (Dekker, New York, 1971).
9 Hansch, D., and Helmer, F., *J. Polym. Sci.*, **6**, 3295 (1968).
10 Llewellyn-Thomas, E., Wang, P. Y., and Vinals, J. N., *Biomat. Med. Dev. Art. Org.*, **1**, 507 (1973).
11 Saunders, J. H., and Frisch, K. C., *Polyurethane: II. technology*, 611 (Interscience, New York, 1964).
12 Becher, P., *Emulsions: theory and practice*, 242 (Reinhold, New York, 1965).
13 Schick, A. F., and Singer, S. J., *J. biol. Chem.*, **236**, 2477 (1961).
14 Gaunt, W. E., and Wormall, A., *Biochem. J.*, **30**, 1915 (1936).
15 Wilkinson, P. C., and McKay, I. C., *Int. Arch. Allergy*, **41**, 237 (1971).
16 Wilkinson, P. C., and McKay, I. C., *Eur. J. Immun.*, **2**, 507 (1972).
17 Frye, L. D., and Edidin, M., *J. Cell Sci.*, **7**, 319 (1970).
18 Yamada, T., and Yamada M., *Nature*, **244**, 297 (1973).

H_2-receptors in peripheral blood vessels

The recent observation that certain histamine responses which were resistant to antihistaminic blockade may now be abolished

by the newly synthesised compounds burimamide (N-methyl-N[1] [4-(4/5)-imidasolyl) butyl] thiourea) and metiamide (N-methyl-N[1] [2/(5 methylimidazol-4-yl) methylthio/ethyl] thiourea) (both compounds, Smith, Kline and French) has culminated in the interpretation of histamine action in terms of both H_1- and H_2-receptors[1]. Until now the attention of workers in this field has centred upon gastric acid secretion. Our own studies, however, have been directed at the receptor control of the microcirculation and we have previously reported failure of conventional antihistaminics to block histamine responses in our experimental model[2]. Here we report results obtained in the canine diarthrodial joint which demonstrate the abolition of histamine responses by H_2 and not by H_1-blocking compounds and thus supporting the conclusion that H_2-receptors are involved in the control of peripheral blood vessels.

The clearance rate of radioactive xenon (^{133}Xe) from a diarthrodial joint following intraarticular injection affords an indirect measure of synovial tissue perfusion[3,4]. Less than 0.2 ml of the gas dissolved in sterile 0.9% sodium chloride was injected into the diarthrodial joints of anaesthetised adult dogs and the clearance followed by means of a thallium-activated sodium iodide scintillation crystal positioned above the joint and connected to a photomultiplier, pulse height analyser and ratemeter recording on a Rikadenki chart recorder. From a semilogarithmic plot of the count rate the half life ($T_{\frac{1}{2}}$) (min) of the clearance rate was calculated. This technique has been used in the past to demonstrate the presence of adrenergic and cholinergic receptors in the synovial microvasculature, and although there is considerable individual variation in clearance rate, the method has provided acceptable reproducibility with repeat studies on the same joint[4].

These studies were carried out on adult dogs weighing between 22 and 32 kg using a standard anaesthetic and experimental technique.

Both agonist and antagonist drugs were injected intra-articularly and various doses of each drug (histamine 1–2.5 μg, metiamide 50–1,000 μg and mepyramine 500 and 1,000 μg were used in these studies.

The relationship between agonist and antagonist was studied in each experiment by injecting first the antagonist followed 10–20 min later by histamine.

Histamine was supplied as histamine acid phosphate (1 mg ml^{-1} (Evans Medical)), mepyramine as mepyramine maleate (50 mg ml^{-1}) (Anthisan, May and Baker) and metiamide was obtained as a powder from Smith, Kline and French Laboratories. All drug dilutions were with isotonic 0.9% sodium chloride or sterile water.

Table 1 ^{133} Xe clearance half life ($T_{\frac{1}{2}}$) values with histamine (H) alone and histamine following H_1 antagonist mepyramine (Mep) given in increasing Mep:H dose ratios

	Histamine			Histamine response following mepyramine with various dose ratios Mep:H					
				100:1		500:1		1,000:1	
	Pre	Post	(μg)	Pre	Post	Pre	Post	Pre	Post
	35	16	(1.0)	60	22	73	25	48	15
	25	16	(1.0)	38	18	39	13	73	24
	68	18	(1.0)	55	31	32	21	36	23
	42	16	(1.0)	150	37	96	51	32	18
	68	30	(1.0)					43	23
	64	33	(2.5)					66	18
	62	35	(2.5)					47	36
	50	13	(2.5)						
	61	14	(2.5)						
	34	16	(2.5)						
Mean	51	21		76	27	60	28	49	22
s.e.m.	5.0	2.7		25	4	15	8	6	3
Mean change		61%			60%		53%		51%

Table 2 ^{133}Xe clearance half life ($T_{\frac{1}{2}}$) values with metiamide (Met) alone and with histamine following metiamide given in increasing Met:H dose ratios

	Metiamide alone			Histamine response following metiamide (Met) in increasing dose ratios (Met:H)							
				< 200:1		200:1		500:1		1000:1	
	Pre	Post	(ug)	Pre	Post	Pre	Post	Pre	Post	Pre	Post
	115	115	(25)	115	29	20	12	31	31*	39	49*
	20	20	(50)	22	11	38	24	47	47*	28	33*
	45	55	(50)	28	13	25	25*	26	26*	150	150*
	49	51	(200)	60	21	32	29*	60	58*		
	50	50	(360)	150	59	50	25	37	33*		
	47	50	(560)	36	15	34	24	35	35*		
	40	34	(500)	50	20	48	48*				
	24	25	(500)								
	35	38	(500)								
	60	58	(1000)								
	150	150	(1000)								
Mean	57	59		66	24	35	28	39	38	72	77
±											
s.e.m.	12	12		18	6	4	4	5	5	39	37
Mean change		1%			59%		19%		0%		

*Indicates instances of complete H_2-receptor blockade.

The results are shown in Tables 1 and 2. Histamine consistently increased the xenon clearance rate in doses of 1 μg and 2.5 μg (Table 1). This response was not abolished by mepyramine maleate in any concentration ratio. Metiamide alone produced no consistent change in xenon clearance rate (Table 2) but completely abolished the action of histamine at dose ratios of 500:1 and 1,000:1 (Met:H). Variable responses were obtained at the ratio of 200:1 and the histamine vasodilator response was clearly apparent at lower concentration ratios.

The abolition of vasodilatation induced by histamine in peripheral blood vessels by metiamide and not by

mepyramine maleate strongly indicates that the effect of histamine in this experimental model is an H_2-mediated response. Other workers have documented involvement of both H_1 and H_2-receptors in larger blood vessels[5,6]. Reed *et al.*[7] have documented H_2-receptor abolition of vasodilatation induced by histamine in gastric mucosa using the amidopyrine technique and our results using a radioactive tracer clearance technique are in accord with theirs. The implications of lipid soluble inert gas clearance rates and their relationship to tissue perfusion have previously been discussed[4]. The evidence supports the contention that large changes in clearance rates, produced pharmacologically, may be interpreted as an alteration in tissue perfusion. The expression of results in terms of absolute flow rate per tissue volume, however, is more contentious.

If, as these results indicate, histamine H_2-receptors are implicated in the control of peripheral blood vessels, a rational explanation is available for the first time for the failure of conventional antihistamine to completely reverse the effect of histamine on systemic blood pressure responses[8]. In addition, the potential interference in vascular responses mediated by histamine offered by metiamide offers an important new approach to the therapy of inflammatory joint disease. It is also conceivable that these methods may be applicable to the study of the relationship between hydrogen ion secretion and peripheral tissue perfusion in diarthrodial joints and in gastric mucosa[7] and we are currently exploring these possibilities.

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1 Black, T. W., Duncan, A. M., Durant C. T., Ganellia, C. R.' and Parsons, E. M., *Nature*, **236**, 385-390 (1972).
2 St Onge, R. A., and Dick, W. C., *Modern Trends in Rheumatology*, 2 (edit. by Hill, A. G. S.) **5**, (Butterworths, London, 1970).
3 Dick, W. C., *et al.*, *Ann. rheum. Dis.* **29**, 131 (1970).
4 Dick, W. C., *Seminars in Arthritis and Rheumatism*, **1**, 301 (1972).
5 Powell J. R., and Brody, M. J., *Int. Symp. on H_2 Receptor Antagonists, London*, (edit. by Wood, C.) 137, (1973).
6 Glover, W. E., Carroll, P. R., and Latt, N., *Int. Symp. H_2-Receptor Antagonists, London*, (edit. by Wood, C.) 169 (1973).
7 Reed, J. D., Smy J. R., Venables, C. W., and Harris, D. W., *Int. Symp. on H_2-Receptor Antagonists. London*, (edit. by Wood, C.) 231 (1973).
8 Douglas, W. W., in *The Pharmacological Basis of Therapeutics* (edit. by Goodman, L. S., and Gilman, A.) 615–627 (McMillan, New York, 1965).

Binding of concanavalin A and ricin to synaptic junctions of rat brain

THE synaptic junction is the intercellular contact where bioelectrical impulses are transferred and transformed by neurotransmitters. The molecular mechanism of impulse transformation is still largely unknown. To understand this process, a detailed knowledge of the structure of the synaptic apparatus is essential. The available information on the morphology and cytochemistry of synapses has recently been reviewed[1]. To arrive at a more precise morphological characterisation of the synaptic junction with respect to carbohydrate moieties[2,3], we investigated the capacity of the plant lectins concanavalin A (con A) and ricin, labelled with ferritin[4], to bind to isolated synaptosomes (that is, pinched-off axon terminals) and synaptic junctions.

A crude mitochondrial pellet obtained from the brains of adult male Sprague–Dawley rats was further fractionated according to the technique of Levitan *et al.*[5] to isolate synaptosomal membranes. This fraction was treated with the detergent Triton X–100 at a concentration of 4 mg ml^{-1} (protein[6] and Triton X–100 in a ratio of 1: 1 by weight), as described by Cotman *et al.*[7]. The resulting suspension was layered on a discontinuous sucrose gradient to isolate the synaptic junctions[7].

Concanavalin A (Calbiochem) was coupled to ferritin (Schwarz/Mann) with glutaraldehyde (Polysciences) at a molar ratio of 1.0 in the presence of 0.2 M α-methyl-glucoside in phosphate buffered saline (PBS) at *p*H 7.3-7.5 (ref. 8). Ferritin-con A and con A were isolated by affinity chromatography on Sepharose G–200 (Pharmacia) and eluted with 0.1 M α-methyl-glucoside. Ferritin-labelled con A was then separated from unreacted con A by density gradient centrifugation[9]. Ricin was similarly coupled to ferritin and purified according to the same procedure with the exception that Sepharose 4B gel (Pharmacia) and galactose were used instead of Sephadex and α-methyl-glucoside[10,11].

The crude mitochondrial and the synaptic junctional fractions were incubated in 0.2–0.5 ml ferritin-con A 1–2 mg ferritin ml^{-1} in PBS molar ratio ferritin: con A about 1: 3) or in ferritin-ricin (0.4–0.8 mg ferritin ml^{-1} in PBS, molar ratio ferritin: ricin about 1: 3) at 20° C for 1 h. As a control for the specificity of the lectin binding, samples were also incubated in the presence of 0.2 M inhibitor (α-methyl-mannoside for con A, galactose for ricin). After the incubation, the suspensions were diluted 20–50-fold with PBS, centrifuged at 10,000*g* for 15 min and washed twice with PBS. The sediments were fixed in 2.5% buffered[12] glutaraldehyde (Polysciences) for 30 min at 20° C, followed by 1% buffered[12] osmium tetroxide for 1.5 h at 4° C, dehydrated through graded acetones and embedded in Araldite. Thin sections were stained with uranyl acetate and lead citrate.

We found that of all the synaptic elements investigated the synaptic cleft had the greatest binding capacity for both ferritin-con A (Fig. 1*a, b, c*) and ferritin-ricin (Fig. 1*d*); the latter, however, bound to a lesser extent than ferritin-con A. In all control experiments, the binding of lectins was completely inhibited by the presence of the appropriate hapten inhibitor. Compared with intact synaptosomes (Fig. 1*a*), the cleft region of isolated synaptic junctions (Fig. 1*b, c*) was considerably more densely coated with labelled con A. Except in the synaptic cleft region, staining of the synaptic junction was negligible. Treatment with Triton, which removes a large part of the presynaptic membrane material from synaptosomes, seems to result in extensive exposure of binding sites located in the cleft region (Fig. 1*b, c, d,*); the penetration of ferritin-labelled con A into the synaptic cleft of intact synaptosomes is obviously restricted (Fig. 1*a*). Furthermore, the extent to which ferritin-con A binds does not depend on the presence of an intact postsynaptic membrane (Fig. 1*b, c*).

From these findings we come to the following conclusions. (1) The synaptic cleft contains a high density of con A receptors (believed to be mannosyl or glucosyl end-groups[13]) and an appreciable number of ricin receptors (believed to be galactose end-groups). (2) The consistency of the cleft material in intact synaptosomes is such as to allow the penetration of the comparatively large ferritin-con A molecules (about 150 Å in diameter[13,14]) (Fig. 1*a*). After fixation

of the synaptosomes with glutaraldehyde, the accessibility of lectin-binding sites in the synaptic cleft is abolished[15]. (3) The lectin-binding carbohydrate groups are firmly bound to the cleft region, since they are insensitive to the detergent treatment; they could be anchored within that part of the postsynaptic web which is in contact with the synaptic cleft. In such an arrangement, the connecting molecular links would run through the postsynaptic membrane. Alternatively, the removal of the postsynaptic membrane by the detergent could simply expose additional binding groups.



Fig. 1 *a*, Synaptosome labelled with ferritin-con A; ferritin-con A is dispersed over the membrane of the axon terminal (recognisable by the synaptic vesicles) and the postsynaptic membrane. Ferritin-con A can penetrate the synaptic cleft (arrow) and bind there specifically (× 27,000); *b*, Synaptic junction stained with ferritin-con A; postsynaptic membrane dissolved by Triton X-100 (× 34,200). *c*, Synaptic junction stained with ferritin-con A; postsynaptic membrane intact (× 34,200). *d*, Synaptic junction stained with ferritin-ricin; postsynaptic membrane dissolved by Triton X-100 (×34,200).

The high density of con A-receptors at the synaptic junctions was subsequently exploited for their purification on con A-Sepharose gel (Pharmacia). In preliminary experiments, a three-fold enrichment (as judged by morphometric analysis of electron micrographs) of synaptic junctions compared with the final material from conventional procedures (density gradient) was obtained.

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1 Pfenninger, K. H., *Progr. Histochem. Cytochem.*, **5**, 1–86 (Gustav Fischer Verlag, Stuttgart, Portland, 1973).
2 Rambourg, A., and Leblond, C. P., *J. Cell Biol.*, **32**, 27–53 (1967).
3 Meyer, W. J., *J. Cell Biol.*, **43**, 92a (1969).
4 Hirano, H., Parkhouse, B., Nicolson, G. L., Lennox, E. S., and Singer, S. J., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2945–2949 (1972).
5 Levitan, J. B., Mushynski, W. E., Ramirez, G., *J. biol. Chem.*, **247**, 5376–5381 (1972).
6 Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265–275 (1951).
7 Cotman, C. W., and Taylor, D., *J. Cell Biol.*, **55**, 696–711 (1972).
8 Stobo, J. D., and Rosenthal, A. S., *Expl Cell Res.*, **70**, 443–447 (1972).
9 de Petris, S., and Raff, M. C., *Eur. J. Immun.*, **2**, 523–535 (1972).
10 Nicolson, G. L., and Blaustein,, J., *Biochim. biophys. Acta*, **266**, 543–547 (1972).
11 Tomita, M., Kurokawa, T., Onozaki, K., Ichiki, N., Osawa, T., and Ukita, T., *Experientia*, **28**, 84–85 (1972).
12 Millonig, G., *Proc. fifth int. Congr. Electron Microscopy, Philadelphia* (edit. by Breese, S. S., jun.), **2**, 8 (Academic Press, New York, 1962).
13 Sharon, L., and Lis, H., *Science*, **177**, 949–959 (1972).
14 Crichton, R. R., *Angew. Chem.*, **85**, 53–62 (1973).
15 Matus, A., de Petris, S., and Raff, M. C., *Nature new Biol.*, **244**, 278–280 (1973).

Toxins of *Vibrio cholerae* and *Escherichia coli* stimulate adenyl cyclase in rat fat cells

RAT epididymal fat cells respond to enterotoxins of *Vibrio cholerae* and *Escherichia coli* with increased membrane adenyl cyclase activity and glycerol release[1–3]. Cholera toxin increases adenyl cyclase activity in many other tissues and cells, including intestine, leukocytes, immunocytes, platelets and liver[4–6]. Here we report studies on the response of fat cells to these bacterial toxins and their effect on membrane adenyl cyclase activation by adrenaline.

E. coli toxin was prepared from a Millipore filtrate of an 18h culture of a strain of *E. coli* previously isolated from a patient with nonvibrio cholera[7,8] grown in medium containing the following concentrations of materials in g l^{-1}: 20 g Bacto-Difco casamino acids, 1.5 g Bacto-Difco yeast extract, 2.5 g NaCl, 8.71 g K_2HPO_4 (0.05 M), and 1.0 ml Trace salts solution (containing 50 g l^{-1} $MgSO_4$, 5.0 g l^{-1} $MnCl_2$, 5.0 g l^{-1} $FeCl_3$ dissolved in 0.001 N H_2SO_4). The mixture was adjusted to *p*H 8.5 with 0.1 n NaOH. Cultures were grown with shaking 500 ml in a 2.5 l low-form flask. After Millipore filtration, the TRY culture media supernatant was concentrated by UM-10 Diaflow filtration to a final concentration of 10 mg ml^{-1}. Highly purified *V. cholerae* toxin was prepared by Finkelstein, according to a method previously described[9]. Both toxins were tested for their ability to produce fluid loss in duodenal loops of mongrel dogs[10]. Fat cells were isolated from the epididymal fat pads of male Sprague-Dawley rats by treatment with collagenase (4 mg ml^{-1}) for 1 h, filtration through silk screen, and subsequent washing three times with buffer. All incubations were carried out in Krebs-Ringer phosphate buffer, *p*H 7.2, with albumin (3.63 g per 120 ml) while shaking at 60 r.p.m. in a 37°C water bath. Toxins were added at time zero at concentrations of 1 mg ml^{-1} (*E. coli*) and 1 μg ml^{-1} (*V. cholerae*) to fat cells 0.5 g 1.0 ml. At the end of 10 min, all samples were diluted with buffer to final volume of 5 ml (except the 5 min point which was diluted at 5 min). Incubation was terminated by centrifugation of cells and media for 1 min at 1,500 r.p.m. and removal of infranatant buffer for glycerol determination[11]. Fat cells were lysed by exposure to chilled (4°C) distilled water and mixing twice on a vortex shaker. Membranes were isolated in a pellet by centrifugation at 12,000*g* for 10 min at 4° C. Pellets were resuspended in cold Tris–$MgCl_2$ buffer (75 mM Tris, 25 mM $MgCl_2$, *p*H

8.0) adjusted to make a protein concentration of 25–100 μg in 55 μl of total reaction mixture. Adenyl cyclase activity was subsequently measured by conversion of ^{32}P–ATP to ^{32}P–cyclic AMP according to the method of Krishna[12]. The responsiveness of membrane adenyl cyclase to adrenaline was evaluated by adding 5.5 μM adrenaline to the adenyl cyclase assay of membranes from control cells and exposed cells exposed to toxin.

The stimulation of membrane adenyl cyclase by *E. coli* toxin was at its maximum (642 ± 269 pmol cyclic AMP per mg protein per 10 min increase over control) by 30 min, falling to control levels within 60 min (Fig. 1*a*). The response to cholera toxin, however, was not measurable until after 60 min and continued to rise to a plateau of 1244±121 pmol cyclic AMP mg^{-1} protein per 10 min increase over control adenyl cyclase activity at 3–4 h. The action of a single dose of cholera toxin on gut cells is known to continue for at least 12 h but measurement of duration is limited in our system by viability of isolated fat cells[4]. Statements about the effects after 3 h are not therefore possible.

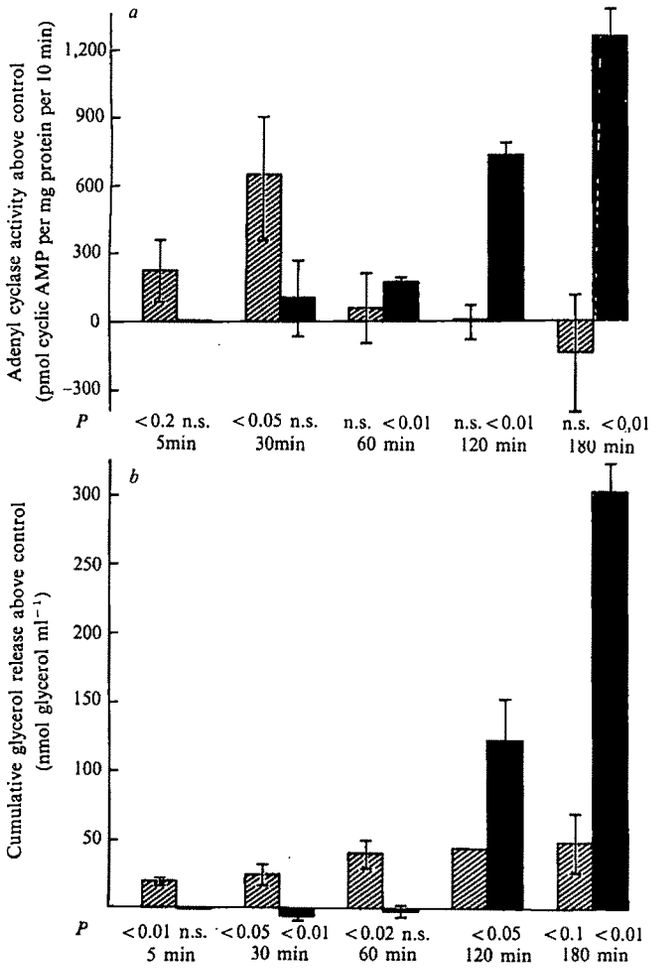


Fig. 1 Time course of the response of isolated rat epididymal fat cells to *E. coli* ▨ or *V. cholerae* (■) enterotoxins (9195 and 1071, respectively) measured by adenyl cyclase activity in pmole cyclic AMP per mg protein per 10 min above control values (*a*), or cumulative glycerol release in nmol ml^{-1} above controls (*b*). Values expressed as mean ± s.e.m.

Glycerol release in response to cholera toxin began after 60 min and continued beyond 3 h. Neither the adenyl cyclase activation nor the glycerol release induced by cholera toxin was significantly altered by the five-fold dilution of cells and toxin after the initial 10 min incubation, indicating rapid and strong binding of toxin to cell. A significant lipolytic response to *E. coli* toxin was seen at 5 min. This activity was heat labile (boiling for 10 min). By 3 h a total 48 nmol ml^{-1} of glycerol had been released. Cells which were not diluted as described previously and were exposed continuously to 1 mg ml^{-1} of *E. coli* toxin released 138 nmol ml^{-1} of glycerol by 3 h. This difference in response to the same initial concentration and total dose of toxin indicates a more transient or less avid interaction with cells. A similar action of *E. coli* toxin on canine intestine has been reported[10]. Preliminary experiments with fat cells indicate that washing cells previously exposed to toxin has no effect on response to cholera toxin, but washing cells exposed to *E. coli* toxin at 5 min can prevent a further rise in adenyl cyclase activity and glycerol release.

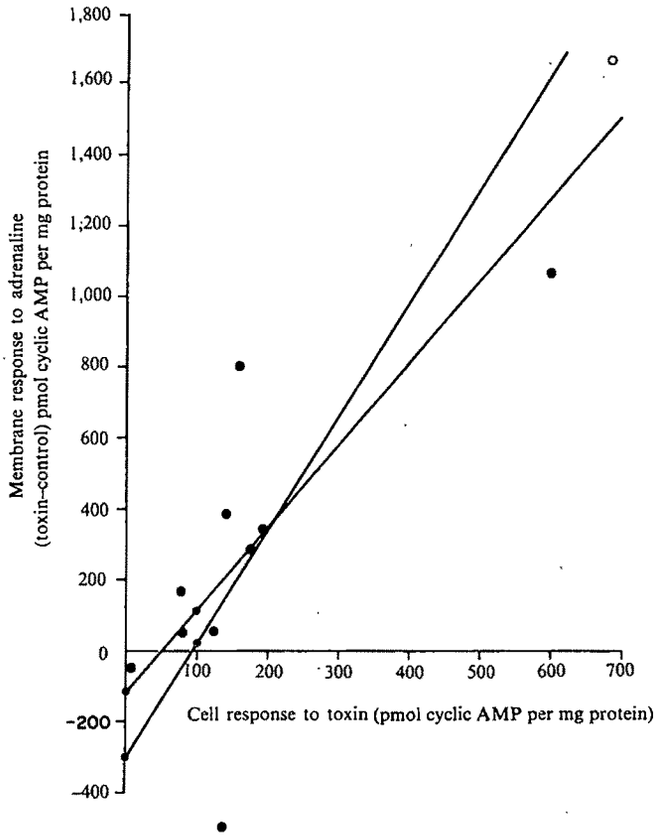


Fig. 2 Responsiveness to adrenaline of membranes of cells previously exposed to *E. coli* ($n=14$) or *V. cholerae* ($n=1$) toxins. ●, Cells exposed to E. coli toxin; ○, cells exposed to cholera toxin. $R=0.86$ $P < 0.01$. The regression lines indicate the slopes at 95% confidence limits.

In spite of the marked difference in the time course of adenyl cyclase stimulation by these two bacterial toxins, both have a significant effect on the responsiveness of membrane adenyl cyclase to further stimulation by adrenaline. Exposure of membranes to 5.5 μM adrenaline at the time of adenyl cyclase assay resulted in a significantly greater increase in adenyl cyclase activity in membranes previously exposed to either toxin than in control membranes (Fig. 2). This heightened response was greatest in membranes showing the greatest response to either toxin.

Enterotoxins of *E. coli* and *V. cholerae* produce gut fluid loss by similar actions on ion movement across gut mucosa. They each produce an altered ion flux by stimulating adenyl cyclase and thereby increasing cyclic AMP levels[10,13]. Each affects tissues other than intestinal mucosa, producing effects compatible with increased levels of cyclic AMP. There is also a close antigenic relationship between the heat-labile components of *E. coli* and enterotoxins demonstrated by immunological cross reactivity with specific antisera[14].

Although the relationship to toxin receptor and regulator to the hormonal receptor and regulator is not known, other studies have indicated that toxin action does not interfere with normal channels of hormone action[1,16]. Here, we have demonstrated not only a lack of interference, but also a marked facilitation by toxin of cellular response to a hormonal stimulus. The enhanced responsiveness of cell membrane adenyl cyclase to adrenaline is related directly to the response of cells to toxin. Thus, stimulation of whole cells by toxin sensitises their membranes to subsequent catecholamine challenge. The mechanism of this enhanced hormonal responsiveness is not clear.

V. cholerae infections are restricted to the gut lumen and the action of its enterotoxin is only on intestinal epithelium. It will be of interest to determine whether the sensitisation of intestinal epithelium to hormonal stimuli plays a role in cholera in addition to the known direct action of cholera toxin. The *E. coli* enterotoxin(s), however, may not be restricted in their action to the intestine as *E. coli* frequently causes tissue infections and septicaemia. Should enterotoxin production occur in the blood stream or other tissues, a variety of biological effects might be expected based both on the direct stimulation of adenyl cyclase in the involved tissues and on enhanced responsiveness to normal hormonal stimuli. Such effects are seen when cholera toxin is given parenterally to animals[17]. Some effects such as hypersensitivity to catecholamines have already been observed and attributed, perhaps erroneously, to endotoxin[18].

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1 Vaughan, M., Pierce, N. F., and Greenough, W. B. III., *Nature*, **226**, 658 (1970).
2 Hewlett, E. L., and Greenough, W. B. III., *Clin. Res.*, **19**, 459 (1971).
3 Evans D. J. Jr., Chen, L. C., Curlin, G. T., and Evans, D. G., *Nature new biol.*, **236**, 137 (1972).
4 Sharp, G. W. G., and Hynie, S. *Nature*, **229**, 266 (1971).
5 Zieve, P. D., Pierce, N. F., and Greenough, W. B. III., *Johns Hopkins med. J.*, **129**, 299 (1971).
6 Lichtenstein, L. M., Henney, C. S., Bourne, H. R., and Greenough, W. B. III., *J. clin. Invest.*, **52**, 691 (1973),
7 Evans, D. G., Evans, D. J., jun., and Pierce, N. F., *Bact. Proc.*, (in the press).
8 Lindenbaum, J. L., Greenough, W. B. III, Benenson, A. S., Oseasohn, R., Risvi, S., and Saad, A., *Lancet*, **i**, 1081 (1965).
9 Finkelstein, R. A., and LoSpalluto, J. J., *J. infect Dis.*, **121**, S63 (1970).
10 Guerrant, R. L., Ganguly, U., Casper, G. T., Moore, E. J., Pierce, N. F., and Carpenter, C. C. J., *J. clin. Invest.*, **52**, 1707 (1973).
11 Mosinger, B., and Vaughan, M., *Biochim. biophys. Acta*, **144**, 569 (1967).
12 Krishna, G., Weiss, B., and Brodie, B. B., *J. Pharm. exp. Ther.*, **163**, 379 (1968).
13 Al-Awqati, Q., Wallace, C. K., and Greenough, W. B. III., *J. infect Dis.*, **125**, 00 (972).
14 Smith, N. W., and Sack, R. B., *J. infect Dis.*, **127**, 164 (1973).
15 Serebro H. A., Iber, F. L., Yardley, J. H., and Hendrix, T. R., *Gastroenterology*, **56**, 506 (1969).
16 Katz, M. S., and Greenough, W. B. III., *Clin. Res.* **20**, 531 (1972).
17 Pierce, N. F., Graybill, J. R., Kaplan, M. M., and Bouwman, D. L., *J. lab. clin. Med.*, **79**, 145 (1972).
18 Zweifach, B. W., Nagler, A. L., and Thomas, L., *J. exp., Med.*, **104**, 881 (1956).

Subterminal motor nerve abnormalities in psychotic patients

THERE is considerable evidence of neuromuscular dysfunction in most patients with all types of the functional psychoses. Examples of such dysfunction are (1) increased activity of skeletal-muscle-type creatine phosphokinase (CPK) in serum in the acute stage of psychosis[1–4]; (2) abnormally large increases in serum CPK activity with a standardised exercise test performed by patients in symptomatic remission[5]; (3) morphological abnormalities in skeletal muscle fibres at biopsy, particularly scattered atrophic fibres and extensive areas of Z-band streaming[6–8]; and (4) abnormalities of eye movements in tracking a pendulum[9]. The skeletal muscle fibre abnormalities found in psychotic patients are likely to be neurogenic in origin[10].

We now report neurological abnormalities of subterminal motor axons in 21 out of 29 psychotic patients. Our subjects were 16 acute and three chronic schizophrenics, one patient with a paranoid psychosis, four manic-depressives—manic or hypomanic phase—five psychotic depressives and two nonpsychotic hospitalised depressed patients. Diagnoses were made by consensus of two psychiatrists and one social worker without knowledge of the



Fig. 1 The subterminal nerve ends in five branches. The two branches on the right end on the same skeletal muscle fibre. The stain was methylene blue, ×360.

biological data. All patients with the diagnosis of schizophrenia satisfied the criteria of the New Haven Schizophrenia Index[11]. The patient groups consisted of 15 males and 14 females; their median age was 24 (range, 17–45). The control group consisted of three male and three female volunteers who were accepted only after a thorough history revealed no personal or familial medical illness that might affect neuromuscular pathology. Their median age was 23 (range, 22–35).

The peroneus brevis was biopsied under local anaesthesia. The subterminal motor nerves were stained supravitally with methylene blue[12] and the absolute and functional terminal innervation ratios (ATIR and FTIR respectively)[13] were determined by carefully following at least 100 sub-

terminal motor nerves selected randomly from all of the slides prepared from each patient. All histological work was performed on a blind basis by coding the slides. To test the reliability of the method, investigators made independent assessments of coded slides. The inter-rater correlation coefficient of six innervation ratios was $r=0.932$ ($P<0.01$). We have also computed the percentage of axons with more than two branches, since such extensive branching has been considered to be diagnostic of denervation and reinnervation. Two other small samples from the same muscle were obtained for histochemical studies and phase microscopy by methods described previously[6,7].

TIRs for patients and controls are summarised in Table 1.

Table 1 Absolute and functional terminal innervation ratios

Group	N	Absolute TIR Mean s.d.	Range	Functional TIR Mean s.d.	Range
Chicago controls	6	1.08±0.049	1.05–1.16	1.07±0.052	1.05–1.16
Controls of Cöers[8,12]	56	—	—	1.11±0.05	1.01–1.20
All psychotics	29	1.49±0.46‡	1.00–2.90	1.39±0.34†§	1.00–2.35
All schizophrenics	19	1.46±0.50†	1.00–2.90	1.36±0.36†§	1.00–2.35
Affective psychoses	9	1.57±0.34†	1.04–1.93	1.48±0.31†§	1.03–1.87
Neurotic depression	2	1.11±0.01*	1.10–1.12	1.11±0.01*	1.09–1.12

The ATIR is defined as the number of end plates innervated by a given number of axons divided by that number of axons. Similarly, the FTIR is defined as the number of muscle fibres innervated by a given number of axons divided by that number of axons. The mean ATIR and FTIR of the experimental groups were compared with those of the Chicago controls by means of a Mann-Whitney U test, two-tailed: * $P > 0.5$; † $P > 0.01$; ‡ $P > 0.005$.

The mean FTIR of the experimental subjects were compared with those of the controls of Cöers and colleagues[14] using a two-tailed t-test: § $P < 0.001$; ¶ $P > 0.5$.

The TIRs of the six controls were in excellent agreement with those reported by Cöers *et al.*[13–15]. The 95% upper limit of normal for the ATIR was 1.16; for the FTIR it was 1.15. The TIRs of the two nonpsychotic patients were normal. Of the 29 psychotic patients, 21 had an ATIR greater than 1.16 and 19 had an FTIR greater than 1.15. The mean TIRs for all psychotic patients, and for the subgroups consisting of all schizophrenics (acute and chronic combined) and the affective psychoses (manic-depression, manic phase and psychotic depression), were significantly greater than those of our six controls and the 56 controls of Cöers *et al.*[15]. Although groups were small, comparisons of group means gave no evidence that acute compared with chronic status or paranoid compared with nonparanoid status had any relationship to the degree of abnormality of the TIRs. There also were no significant relationships between the TIRs and age, sex or race.

The mean TIRs of the 12 psychotic patients who had received the largest total of phenothiazines (8–270 g) were not significantly greater than those of the 12 patients who had received the smallest doses (0–5.8 g). Increased ratios were found in four of six psychotic patients who had received a total of less than 1 g of chlorpromazine.

The type of branching of the subterminal motor axons in the muscle of the psychotic patients is illustrated in Figs 1 and 2. The 95% confidence limit for the percentage of fibres with three or more branches was 6.2% in the six controls. Cöers *et al.* found a mean of 0.65% of subterminal axons with three or more branches in the palmaris longus and tibialis anterior muscles of 13 controls[16]. Fifteen of the twenty-nine psychotic patients had more than 6.2% of axons with three or more branches (range, 6.6–25.4%) with four patients having 20% or more of their axons with three or more branches.

The relationship between the TIRs and the results of the histochemical and phase microscopy studies of muscle fibres in the psychotic patients will be described in detail elsewhere[17]. Here we wish to indicate some important relationships between muscle fibre abnormalities and subterminal motor nerve changes. Three of the psychotic patients with an excessive percentage of fibres with three or more branches had several groups of 50–100 fibres of the same fibre type rather than the usual checkerboard pattern, or atrophic fasicles (Fig. 3). Previous studies have demonstrated a relationship between elevated FTIRs and type grouping[18]. The correlation between the FTIR and the number of small angular fibres in the sample taken for histochemistry was highly significant ($r=0.719$, $N=29$, $P<0.01$). On the other hand, there was no correlation between either TIR and the percentage of fibres with extensive areas of Z-band streaming. The TIRs of the 22 psychotic patients with abnormalities in muscle fibre morphology were, however, significantly greater than those of the seven psychotic patients without such abnormalities ($P<0.05$, two-tailed). Since scattered small angular fibres and Z-band streaming have both been described in neuropathic motor disorders[19–21], it seems likely that neuronal dysfunction is the basis for both the muscle pathology and the increased subterminal axonal branching in psychotic patients. Additional support for this hypothesis comes from the observation that the ATIR and FTIR were not significantly different in the psychotic patients. This pattern strongly suggests a neurogenic motor disorder, because in primary muscle disease the FTIR remains near unity, while the ATIR increases[13].

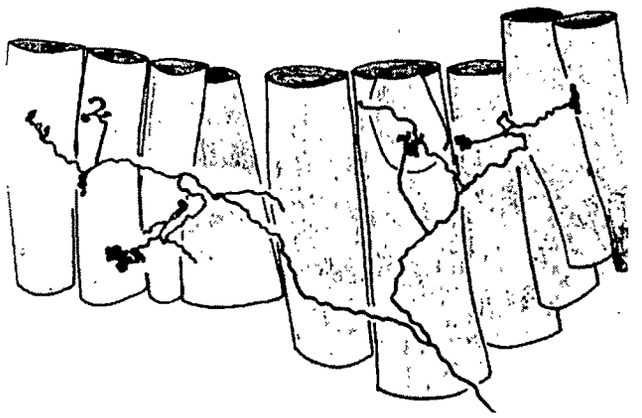


Fig. 2 Camera lucida drawing. The motor nerve can be followed to at least eight recognisable end plates. Several sprouts (branches without end plates) are evident.

In addition to the branches with terminal arborisations, excessive formation of sprouts, or immature branches, was found in 11 of 29 psychotic patients (Fig. 2). Cöers and Woolf have found that sprouting is characteristic of anterior horn cell disease and vitamin B_{12} deficiency whereas in peripheral neuritis there is little sprouting because the healthy motor neurone somata support the formation of healthy branches rather than sprouts[13]. The presence of sprouting along with fibre type grouping and excessive numbers of small angular fibres, indicates the α-motor neurone as a probable locus of neuropathology in these patients. It sems likely that the effectiveness of branching is one reason why relatively few scattered atrophic fibres are present in the psychotic patient.

These studies therefore show that there are neuropathological changes in patients with the so-called functional psychoses. The significance, if any, of these abnormalities of the motor nerves for motor performance in psychotic patients is an intriguing question. Evidence for impaired psychomotor performance in schizophrenics has been re-

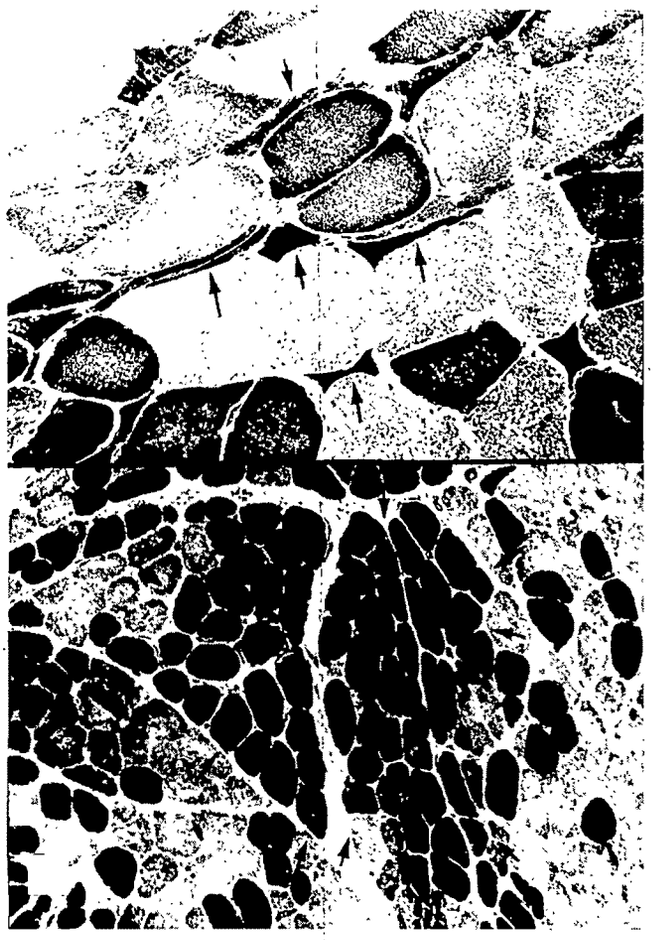


Fig. 3 Top, four small, angular atrophic type I muscle fibres are indicated by arrows. (Nicotinamide adenine dinucleotide-tetrazolium reductase, ×167.40.) Bottom, two groups of type I fibres are indicated by arrows. (Nicotinamide adenine dinucleotide-terazolium reductase, ×86.80.)

viewed recently[22]. It is conceivable that the abnormalities of motor nerves reported here might affect a number of critical fine motor processes such as eye movements, larynx and finger dexterity such that they contributed to the schizophrenics' difficulties in adaptation.

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1 Meltzer, H., *Science*, **159**, 1368–1370 (1968).
2 Meltzer, H. Y., and Moline, R., *Arch. gen. Psychiat.*, **23**, 481–491 (1970).
3 Schweid, D. E., Steinberg, J. S., and Sudak, H., *Arch. gen. Psychiat.*, **26**, 263–265 (1972).
4 Gosling, R., Kerry, R. J., and Owen, G., *Br. med. J.*, **3**, 327–329 (1972).
5 Meltzer, H., and Moline, R., *Arch. gen. Psychiat.*, **22**, 390–397 (1970).
6 Meltzer, H. Y., and Engel, W. K., *Arch. gen. Psychiat.*, **23**, 492–502 (1970).
7 Fischman, D. A., Meltzer, H. Y., and Poppei, R. W., *Arch. gen. Psychiat.*, **23**, 503–515 (1970).
8 Meltzer, H. Y., McBride, E., and Poppei, R. W., *Neurology Minneapolis*, **23**, 769–780 (1973).
9 Holzman, P. S., Proctor, L. R., and Hughes, D. W., *Science*, **181**, 179–181 (1973).
10 Meltzer, H. Y., *Arch. gen. Psychiat.*, **27**, 125–132 (1972).
11 Astrachan, B. M., Harrow, M., Adler, D., Brauer, L., Schwartz, A., Schwartz, C., and Tucker, G., *Br. J. Psychiat.*, **121**, 529–539 (1972).
12 Evans, R. H., Haynes, J., Morris, C. J., and Woolf, A. L., *J. neurol. neurosurg. Psychiat.*, **33**, 783–785 (1970).
13 Cöers, C., and Woolf, A. L., *The inervation of muscle* (Blackwell, Oxford, 1965).
14 Reske-Nielsen, E., Coërs, C., and Harmsen, A., *J. neurol. Sci.*, **10**, 369–384 (1970).
15 Coërs, C., Telerman-Toppet, N., and Gérard, J-M., *Arch. Neurol.*, **29**, 210–214 (1973).
16 Coërs, C., Reske-Nielsen, E., and Hermsen, A., *J. neurol. Sci.*, **19**, 351–356 (1973).
17 Meltzer, H. Y., and Crayton, J. W., *Biol. Psychiat.* (in the press).
18 Morris, C. J., *J. neurol. neurosurg. Psychiat.*, **32**, 440–444 (1969); *J. neurol. Sci.*, **11**, 129–136 (1970).
19 Engel, W. K., in *Proc. fifth Int. Cong. Neuropath.*, Zurich, 1965, 613–624 (Excerpta Medica Foundation, Amsterdam, 1966).
20 Shafiq, S. A., Milhorat, A. T., and Gorycki, M. A., *Neurology Minneapolis*, **17**, 934–948 (1967).
21 Mendell, J. R., and Engel, W. K., *Neurology Minneapolis*, **21**, 358–365 (1971).
22 Holzman, P. S., *Psychopharmacologia Berlin*, **24**, 29–41 (1972).

Effects of bicuculline on functions of inhibition in visual cortex

Further to a recent report[1] on the effects of intravenous bicuculline on neurones in the cat visual cortex, we feel that we should present some preliminary results from our own experiments.

In the mammalian cerebral cortex, bicuculline acts as a reversible inhibitor of the action of γ-aminobutyric acid (GABA) (ref. 2), the principal putative inhibitory neurotransmitter[3]. In the visual cortex, intracortical inhibitory interneurones may act to increase the precision with which a neurone is selectively responsive to a particular visual stimulus, for example, one specific orientation of a moving edge or bar[4,5]. The direct excitatory input to the cell from the lateral geniculate nucleus, (LGN), may only broadly 'tune' the cell in its responses to orientation, while an inhibitory input (even more broadly tuned for orientation), through intracortical interneurones, could suppress the responses to stimuli away from the optimal orientation[6–9] (Fig. 1*a*). This would give improved specificity for one particular orientation at the expense of general decreased responsiveness. According to this theory, then, blockage of intracortical inhibitory neurotransmission, such as with bicuculline, should induce an increase in the breadth of orientation tuning; a proportionately greater increase in responsiveness (Fig. 1*a*); and, perhaps, a rise in spontaneous discharge if there is tonic activity of inhibitory interneurones[10]. Also, bicuculline might reduce the specificity for stimulus length (hypercomplex cells)[11], direction and velocity of movement[8,10], and binocular suppressive interactions[12], all of which could depend on intracortical inhibitory interactions.

Pettigrew and Daniels have reported[1] that bicuculline administered intravenously increased the breadth of orientation tuning, responsiveness, and spontaneous activity of complex cells, modified the receptive fields, and sometimes abolished direction selectivity. Hypercomplex cells lost their preference for short bars. Simple cells, however, became less responsive and sometimes more narrowly tuned. Thus, their reported results partially support and partially conflict with the theory described here.

In our experiments, adult cats were paralysed with Flaxedil and artificially ventilated with N_2O/O_2 mixture[13]. Extracellular recordings were made in area 17 (verified by later histology) using glass-insulated tungsten microelectrodes and conventional amplification and display techniques. Quantitative estimations of the characteristics of each cell were made by

averaging the number of spikes given in response to several sweeps of a bright bar of optimum dimensions across the face of an oscilloscope, at many different orientations. Linear regression lines were fitted to these data to form tuning curves[14] (Fig. 1*b* and *c*). Changes in these curves after drug application were assessed, using empirical criteria for their reliability: changes in breadth of tuning of >15% and in peak response (at the optimal orientation) of >20% were considered significant[14], while *t* tests were used to assess shifts in spontaneous firing level (Table 1). Several hours were allowed between experiments on cells in the same cat to ensure complete recovery from the drug. The mechanical stability of the preparation for topical application is erratic in some experiments and has meant that few cells have been fully analysed so far.

First, the effects of intravenous bicuculline (0.2 mg kg^{-1}; K & K Laboratories) were studied on two simple and three complex cells, with essentially negative results. For perhaps a minute after injection, the EEG showed very large slow waves or epileptiform spikes (effects were inconsistent). Data were collected within 13–29 min after injection, and from these an orientation tuning curve was constructed and compared with that obtained before drug application.

No systematic changes were seen (Table 1). Intravenous application of bicuculline has unknown potential actions on neural systems (such as the retina[15], LGN (ref. 16) and reticular system[17]), and can cause tachycardia and hypertension[18]. Some of the findings of Pettigrew and Daniels[1], especially the consistently decreased responses of simple cells, cannot be easily explained in terms of neural effects within the primary visual cortex alone.

For topical application experiments, the electrode was introduced through a closed perspex chamber cemented over a 3–5 mm diameter craniotomy above the visual cortex. The chamber was filled with warmed saline through long thin tubing to damp down pulsations and the pressure was maintained at 10–20 mm H_2O. The saline could be replaced with 10^{-4}M or 10^{-5}M bicuculline as hydrochloride in saline.

Topical bicuculline broadened the tuning of all three simple cells tested (though one by only 12%) and the other predictions of the theory described above were verified completely in two of these cells (Table 1 and Fig. 1*b*). In two simple cells the receptive fields were plotted again and they had increased three-fold and five-fold in area. The possible correlation of this with alterations in responsiveness remains a matter for future investigation. One simple cell, which was clearly direction selective before application, showed reduced direction selectivity afterwards.

Of the complex cells tested, the first three gave straightforward results (Table 1). Two showed increases in breadth of tuning. One of these two fulfilled all predictions of the theory under test and also showed a quantitative reduction of direction selectivity; bars moving in the nonpreferred direction caused a 25% inhibition of spontaneous activity before drug application; but with bicuculline they caused a clear response, 24% of that in the preferred direction ($P < 0.001$). This cell was still present 3–3.5 h after drug removal, when its responses had returned to the condition before drug administration. The third complex cell showed no consistent effect of the drug. The fourth complex cell was unusual in having clear inhibitory 'side-flanks' in its orientation tuning curve before application of the drug. Topical bicuculline abolished these side flanks as well as increasing the breadth of tuning and the peak response (Fig. 1*c*).

The next day, a fifth complex cell was found in the same location in the cortex, with very similar receptive fields and tuning curve to the fourth complex cell. Immediately after the application of bicuculline there was actually a decrease in both peak response and breadth of tuning (Table 1), but after 45 min exposure, both parameters had increased above the level before the drug was given. But after more than 2 h under the influence of bicuculline (and even with an additional intravenous application; 0.4 mg kg^{-1}) statistically significant inhibitory side flanks to the tuning curve clearly persisted, with strong inhibition even for a stimulus perpendicular to the best orientation. This indicates the presence of some non-orientation-specific inhibition[19], possibly not GABA mediated[20], which may operate in addition to the orientation-specific inhibition[8,9] in some or all cells.

One cell was classified as pure direction selective, responding to movement of any stimulus in the optimal direction[21]. Its breadth of tuning for orientation increased by about 50%, and so did its peak response. This was, in fact, the only cell found showing an almost exact 'iceberg' effect, with constant slopes to the tuning curve before and after the drug, perhaps because of the removal of nonorientation-specific inhibition.

One hypercomplex cell has also been tested (in cortical layer VI : 10^{-4}M bicuculline). Before the application of bicuculline, a long bar (16°) caused slight inhibition of

Table 1 Actions of bicuculline on orientation tuning curves

Method of application	Cell type	Cortical layer	Change in breadth of tuning (%)	Change in peak response (%)	Ratio of changes (peak: breadth)	Change in spontaneous activity	Notes
Intravenous 0.2 mg kg^{-1}	Simple		(−15)	(+10)		(+)	
			(− 5)	(−17)		+	
	Complex	IV	(+ 3)	−31		−	
		VI	+22	−27		(−)	
		VI	(− 1)	+40		+	
Topical 10^{-4} or 10^{-5}M	Simple	V	(+12)	+31	2.6	(+)	*
		III	+31	+151	4.9	+	† (Fig. 1*b*)
		II	+36	(−18)		(+)	†‡
	Complex	VI	+75	+23	0.3	(+)	*
		V	+20	+88	4.4	(−)	‡
		III	(− 3)	−36		+	
		VI	+26	+100	3.8	(−)	(Fig. 1*c*)
		VI	−17	−40		−	
	Pure direction selective	V	+49	+51	1.0	(+)	†

Data were collected starting 1–5 min and finishing 13–29 min after intravenous injection, and 5–20 min to 35–60 min after topical application. All cells studied topically were within 1.6 mm of the surface. (One complex cell was studied which was 4 mm deep, beyond the probable range of drug diffusion; no effect of bicuculline was seen, and the cell has been excluded from these results.) From tuning curves like those in Fig. 1*b* and *c* the breadth of tuning (average half width[14]) and the peak response (evoked impulses at the optimal orientation) were measured before and during the application. Changes in these parameters are expressed as % increases or decreases. Values in parentheses failed to meet the criteria of significance (> 15% for breadth of tuning, >20% for peak response) established from control measurements of the inherent variability of these parameters without the application of the drug[14]. To test the prediction that the peak response should show a greater % increase than the breadth of tuning, the ratio of the two is shown for cells showing a positive change in both: ratios greater than 1.0 support the hypothesis. Changes in spontaneous activity are shown simply as an increase or a decrease (+ or −), those in parentheses being nonsignificant ($P > 0.01$). Not all cells were tested for changes in field size. Both cells examined for reduced direction selectivity (one simple, one complex) showed the effect.

* 10^{-5}M bicuculline (all others 10^{-4}M).
† Receptive field size increased.
‡ Direction selectivity reduced.

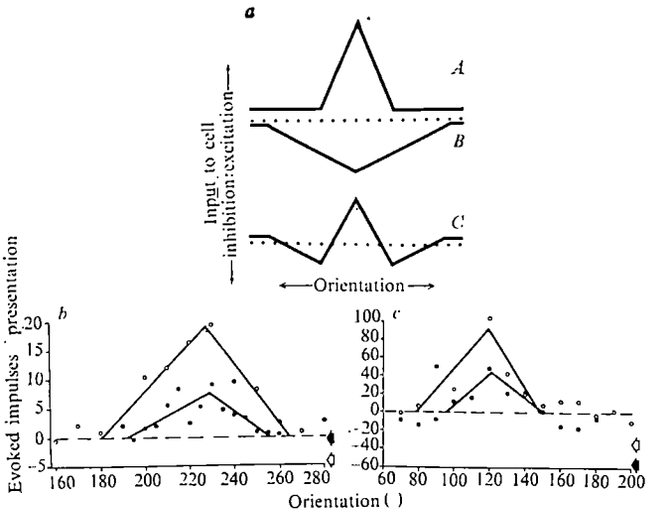


Fig. 1 *a* Diagrammatic illustration of the theory described in the text, whereby a broadly tuned excitatory input, *A*, and a more broadly tuned inhibitory input, *B*, summate (perhaps linearly, as shown here) to give the final orientational tuning curve *C*. Bicuculline should convert the cell's tuning curve from type *C* to type *A*. Normally, in cortical cells, spontaneous discharge is too low for inhibitory 'side flanks' to be seen clearly (the dotted lines represent zero firing level for the cell). Alternatively, inhibitory effects might be nonlinear, perhaps presynaptic, affecting the gain of the excitatory mechanisms[7]. *b*, Effects of bicuculline on a simple cell, D20R4 (10^{-4}M bicuculline, 15–64 min after application). *c*, Effects of bicuculline on a complex cell, D18L6 (10^{-4}M, 5–50 min after application). ●, Responses before application; ○, responses after application. The spontaneous activity was counted over the same gating period used to measure the responses. For clarity, the responses are displayed on the ordinate as the extra impulses generated by the stimulus relative to these spontaneous levels. Inhibition appears as negative values, that is reductions of the spontaneous discharge. Zero firing levels before (◀) and after (◁) the administration of the drug are shown.

spontaneous activity, but with bicuculline, a long bar produced a clear response, 24% ($P < 0.05$) of that elicited by a short bar (1.5°). Two and a half to three hours after removal of the drug, long bars again inhibited the cell slightly.

There are numerous difficulties in interpreting these results quantitatively: one is the general problem of assessing the significance of experimental changes in the characteristics of neurons that exhibit inherent variability in responsiveness[10,14,22] and a second is the uncertain nature of diffusional barriers, including distance, when a drug is applied topically. Finally, bicuculline itself occasionally has only weak or even anomalous actions as a GABA antagonist[23,24]. In spite of these problems, however, all but one of the neurones in this sample showed some if not all of the predicted effects of bicuculline. These results, together with Sillito's very recent report of the actions of iontophoretically-applied bicuculline[24], warrant further investigations into the functions of GABA in the visual cortex.

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1 Pettigrew, J. D , and Daniels, J. D., *Science*, **182**, 81–83 (1973).
2 Curtis, D. R., Duggan, A. W., Felix, D., and Johnston, G. A. R., *Nature*, **226**, 1222–1224 (1970).
3 Krnjević, K., *Nature*, **228**, 119–124 (1970).
4 Hubel, D. H., and Wiesel, T. N., *J. Physiol. Lond.*, **160**, 106–154 (1962).
5 Hubel, D. H., and Wiesel, T. N., *J. Physiol. Lond.*, **195**, 215–243 (1968).
6 Andrews, D. P., *Vision Res.*, **7**, 975–997 (1967).
7 Carpenter, R. H. S., and Blakemore, C., *Expl Brain Res.*, **18**, 287–303 (1973).
8 Benevento, L. A., Creutzfeldt, O. D., and Kuhnt, U., *Nature new Biol.*, **238**, 124-126 (1972).
9 Blakemore, C., and Tobin, E. A., *Expl Brain Res.*, **15**, 439-440 (1972).
10 Bishop, P. O., Coombs, J. S., and Henry, G. H., *J. Physiol. Lond.*, **219**, 659–687 (1971).
11 Hubel, D. H., and Wiesel, T. N., *J. Neurophysiol.*, **28**, 229–289 (1965).
12 Bishop, P. O., in *Handbook of Sensory Physiology* (edit. by Jung, R.) VII3A, 225–305 (Springer, Berlin, 1973).
13 Blakemore, C., Donaghy, M. J., Maffei, L., Movshon, J. A., Rose, D., and Van Sluyters, R. C., *J. Physiol. Lond.* **237**, 39–41 (1974).
14 Rose, D., and Blakemore, C., *Expl Brain Res.* **20**, 1–17 (1974).
15 Straschill, M., and Perwein, J., *Pflügers Arch. ges. Physiol.*, **312**, 45–54 (1969).
16 Phillis, J. W., *Int. Rev. Neurobiol.*, **14**, 1–48 (1971).
17 Tebécis, A. K., Hösli, L., and Haas, H. L., *Experientia*, **27**, 548 (1971).
18 Precht W., Baker, R., and Okada, Y., *Expl Brain Res.*, **18**, 415–428 (1973).
19 Bishop, P. O., Coombs, J. S., and Henry G. H., *J. Physiol. Lond.*, **231**, 31–60 (1973).
20 Phillis, J. W., and York, D. H., *Brain Res.*, **5**, 517–520 (1967).
21 Blakemore, C., and Van Sluyters, R. C., *J. Physiol. Lond.*, **237**, 195–216 (1974).
22 Henry, G. H., Bishop, P. O., Tupper, R. M., and Dreher, B., *Vision Res.*, **13**, 1771–1779 (1973).
23 Straughan, D. W., Neal, M. J., Simmonds, M. A., Collins, G. G. S., and Hill, R. G., *Nature*, **233**, 352–354 (1971).
24 Sillito, A. M., *J. Physiol. Lond.* (in the press).

Constitutivity of the HCG-receptor protein in the testis of rat and man

IN a series of embryological experiments on rabbits and rats, Jost *et al.*[1] have shown that the manifestation of the male or female phenotype is strictly dependent on the presence or absence, respectively, of testosterone. The Müllerian inhibitor formed by the seminiferous tubules[2] only seems to be responsible for the retrogression of the Müllerian ducts. Thus, testosterone synthesised in foetal testes[3] is the chief organiser of masculine differentiation; a female phenotype results from the absence of testosterone. Accordingly, Ohno[4] defined maleness as the induced and femaleness as the noninuced state of genital differentation. The androgenic effects on the development of the genital structures occur during successive critical phases. For instance, the stabilisation of the Wolffian ducts in the rat occurs between days 17 and 18 (ref. 1) while the prostate is imposed on day 21 of foetal life. The imprinting of the rat hypothalamus into the male direction, responsible for the masculine secretory pattern of pituitary gonadotrophins and behaviour pattern, is established during the first two days of postnatal life[5]. Castration, hypophysectomy or injections of cyproterone acetate (a highly potent antiandrogen) before these critical stages prevent the differentiation processes but by later treatment the development is no longer affected. Similar developmental patterns may be applicable to man except for hypothalamus differentiation which is fixed as early as in the fifth month of intrauterine life[6]. Full maturity of the genital structures and particularly the onset of sperm production, are both dependent on testicular androgens, so occur rather late during the postnatal life of all mammalian species.

Testicular androgens are synthesised by stimulus of the gonadotrophic hormones, luteinising hormone (LH) or human chorionic gonadotrophin (HCG). Recent studies have shown that the gonadotrophic effect is mediated by a

Table 1 Uptake of ^{125}I-HCG by rat testicular tissue taken from pre and postnatal rats. The spleens of the prenatal animals were used as controls.

	c.p.m. bound without unlabelled HCG	c.p.m. bound plus unlabelled HCG (30 μg)	Specific uptake of ^{125}I-HCG (g × 10^{-10}) per 30 min per mg protein
Rat testis prenatal	3,478 ± 319*	731 ± 183	1.16
Rat spleen prenatal	710 ± 41	725 ± 120	0
Rat testis day 5	1,966 ± 9.9	627 ± 68	0.35
Rat testis day 10			0.85†
Rat testis day 20			1.76†
Rat testis adult			3.18†

Binding studies, purification and iodination of HCG were performed as described earlier[9]. All assays were performed in triplicate. The amount of specifically bound HCG was determined as the difference between the c.p.m. bound in samples with ^{125}I-HCG alone and in samples containing an excess of unlabelled HCG.
* Standard error of the mean.
† See ref. 9.

specific HCG-receptor in the testicular Leydig cells; the stimulation of testosterone synthesis by HCG in Leydig cells is preceded by the binding of the polypeptide hormone to its specific receptor[7]. The presence of the specific HCG receptor in the developing Leydig cell should therefore be a prerequisite for testosterone synthesis and also male organogenesis in the foetus. As testosterone synthesis is required during both pre and postnatal development, it might be assumed that the HCG receptor is present throughout these periods, appearing with the first formation of Leydig cells in the primordial testis. These cells are first seen in the rat on day 14 (ref. 1) and in man around the 60th day of foetal life[8]. Here we report experiments to test this hypothesis by measuring specific binding of ^{125}I–HCG by foetal and newborn rats and human testicular tissue.

The procedure used was similar to that described earlier[9]. For the study of prenatal animals at a definite developmental stage, mating was determined by examining the presence of copulation plugs in the female rats. On the 20th day of pregnancy the animals were killed and the foetal testes removed. The testes of between 60 and 70 animals were taken together and homogenised in cold Tris-HCl buffer (0.1 M, *p*H 7.4) containing $MgCl_2$ (0.005 M) and sucrose (0.1 M); when 5-d-old rats were used, the testes of 15 animals were pooled in each assay. In the 10, 46 and 75-yr-old humans individual tests were removed 3–5 h after accidental death; the neonate died from pneumonia. Portions (1 ml) of the homogenate were incubated with 7 ng ^{125}I-HCG alone (about 5×10^4 c.p.m.), or with the addition of 30μg unlabelled HCG respectively at 32° C for 30 min (Table 1). After incubation unbound radioactivity was separated by centrifugation.

The results clearly demonstrate the presence of a specific HCG receptor in the pre and postnatal testis of the rat and man (Tables 1 and 2). In contrast to the rat, specific HCG-binding by testicular tissue in man does not vary much with age; only the 10-yr-old boy showed a relative high binding capacity. It is not yet known whether there is any correlation between high binding capacity and the onset of puberty. In rats the fluctuation in binding might be due to the varying amount of Leydig cells present during the respective developmental stages[10]. There is evidence[11] that the pattern of change in Leydig cell numbers may be similar in rat and human testicular development. Further studies are in progress to discover any correlations between hormone binding and human testicular developmental stages.

The important point is, however, that the binding capacity for HCG of the Leydig cell at each developmental stage in rat and man is sufficient to evoke a maximum response; Catt and Dufau[7] have shown that less than 1% of the HCG-receptor sites must be occupied for maximum testosterone synthesis. We conclude from our results that one of the first events occurring in the differentiating Leydig cell is the 'switching on' of the receptor synthesis so that the Leydig cells can start testosterone synthesis. The receptors become functional to guarantee that androgenic hormone synthesis is sufficient for the stepwise maturation of the androgen-dependent genital structures. Defects in the gonadotrophin receptor may result not only in infertility of the adult male but also in defects in the differentiation of male primary and secondary sexual organs. Such defects may only be apparent in the homozygous state, heterozygosity may not reveal deviations, as only a very small quantity of HCG receptors must be occupied to evoke biological response of the Leydig cells.

Table 2 Uptake of ^{125}I-HCG by human testicular tissue at different developmental stages.

	26th foetal week	Neonate (day 4)	10 yr	46 yr	75 yr
Specific uptake of ^{125}I-HCG (g × 10^{-10}) per 30 min per mg protein.	0.451 ± 0.05	0.208 ± 0.017	1.800 ± 0.12	0.293 ± 0.03	0.217 ± 0.019

For experimental procedure see text and legend of Table 1.

Constitutivity has already been supposed for the androgen receptor in the rat ventral prostate[12] and for the oestrogen receptor in the rat uterus[13]. To our knowledge, the results presented here represent the first example of constitutivity of a hormone receptor protein in mammals.

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1 Jost, A., Vigier, B., Prépin, J., and Perchellet, J. P., *Rec. Progr. Horm. Res.*, **29**, 1 (1973).
2 Josso, N., *Endocrinology*, **93**, 829 (1973).
3 Noumoura, T., Weisz, J., and Lloyd, C. W., *Endocrinology*, **78**, 245 (1966).
4 Ohno, S., *Hereditas*, **69**, 107 (1971).
5 Harris, G. W., *Endocrinology*, **75**, 627 (1964).
6 Dörner, G., *Sexualhormonabhängige Gehirndifferenzierung und Sexualität* (Springer-Verlag, Wien-New York, 1972).
7 Catt, K. J., Dufau, M. L., *Nature new Biol.*, **244**, 219 (1973).
8 Reyes, F. I., Winter, J. S. D., and Faiman, C., *J. clin. Endocr. Metab.*, **37**, 74 (1973).

[9] Frowein, J., Engel, W., Weise, H. Ch., *Nature new Biol.*, **246**, 148 (1973).
[10] Lording, D. W., and De Kretser, D. M., *J. Reprod. Fert.*, **29**, 261 (1972).
[11] Mancini, R. E., Vilar, O., Lavieri, J. C., Andrada, J. A., and Heinrich, J. J., *Am. J. Anat.*, **122**, 203 (1963).
[12] Sullivan, J. V., and Strott, C. A., *J. biol. Chem.*, **248**, 3202 (1973).
[13] Luck, D. N., Gschwendt, M., Hamilton, T. H., *Nature new Biol.*, **245**, 24 (1973).

Fibril structure of collagen in egg capsule of dogfish

In spite of a wealth of information relating to the composition and structure of mesenchymal collagens, relatively little is known about the so-called 'secreted' collagens of epithelial origin which are much more limited in distribution. The materials of the selachian egg capsule has been classified as such a collagen though this claim is based on a poorly oriented high angle X-ray diffraction pattern obtained from skate egg capsules[1] and on the colorimetric detection of hydroxyproline in hydrolysates of the same material[2]. Partial amino acid analysis of this material[2] indicated a composition markedly different from pure collagens. To date there has been no report of fibrous periodicity in selachian egg capsules.

Here, we provide evidence that the inner layers of the egg capsule wall of the selachian, *Scyliorhinus caniculus* are built up from fibrils which contain a collagen-like protein, probably in association with other protein(s). The unique and highly ordered structure of these fibrils is described.

Fresh egg capsules were obtained from the Scottish Marine Biological Association Laboratories. Electron microscopical examination of ultrathin sections of the egg capsule wall fixed in glutaraldehyde and osmium tetroxide and embedded in Epon showed that the inner layers of the capsule wall consist of laminae (approximately 0.4 μm thick)

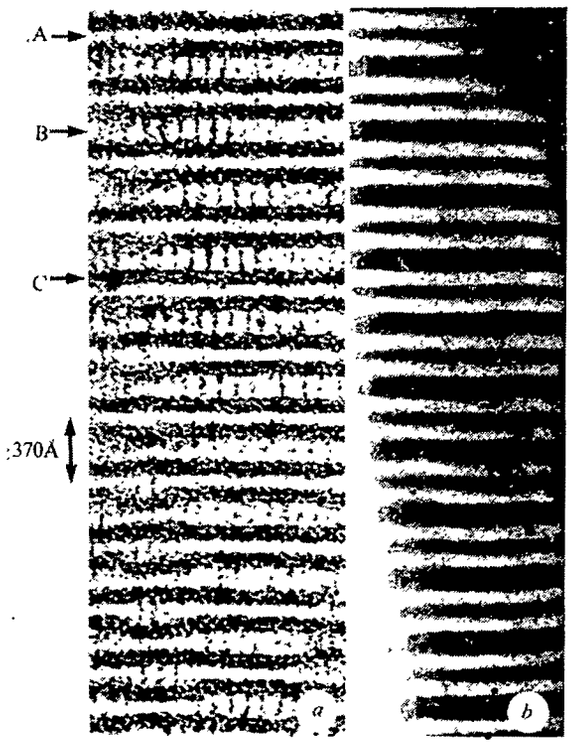


Fig. 1*a*, Axial banding pattern. Positively stained ultrathin sections. *b*, Negatively stained dispersed fibrils.

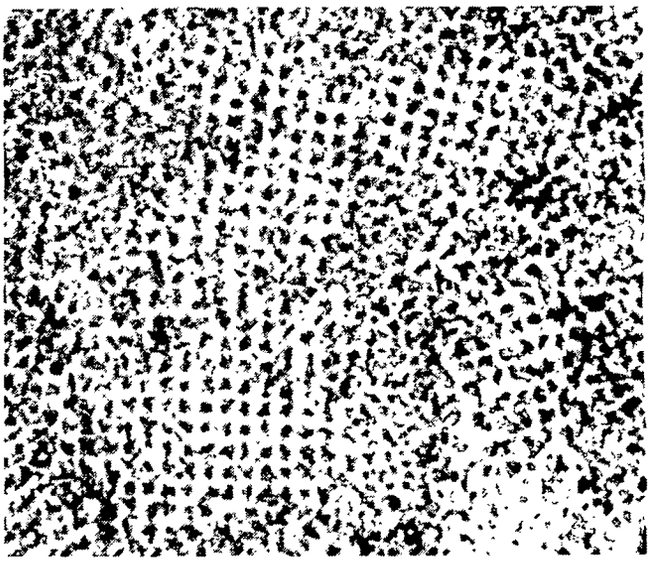


Fig. 2 Transverse section showing the intrafibrillar lattice (stained with uranyl acetate and lead citrate (X 335,000)).

which seem to be built up entirely from closely packed fibrils. In a single lamina, the long axes of the fibrils are parallel. Laminae appear stacked upon each other to give a cross ply construction such that there is an angle of approximately 45° between the long axes of the fibrils in adjacent laminae. The outermost layer of the capsule shows a different construction and is less transparent and more pigmented than the inner layers. In ultrathin sections it seems to consist of closely-packed elliptical granules (0.2–0.5 μm in diameter). Small quantities of fibrils similar to those of the inner layers are found in the interstices between the granules. Amino acid analyses of the outermost and the inner layers (Table 1) were obtained using a Locarte analyser. The outermost layer differed from the inner layers and showed a high tyrosine content (18 residues/100 residues).

The inner layers showed an amino acid composition clearly different from pure mammalian collagen (Table 1). Hot trichloracetic acid[3], however, extracted protein with a composition showing a resemblance to pure mammalian collagen and an even closer resemblance to the 'secreted' collagen of the byssus threads of *Mytilis edulis* (Table 1).

A moderately well oriented high angle X-ray diffraction pattern was obtained after slowly stretching the marginal band of the capsule to an extension of 50–70%. This pattern had the following features in common with collagen[5]: a strong meridional arc at 2.9 Å, and a weaker one at 4 Å. A broad diffuse equatorial arc at 4.5 Å, a faint diffuse equatorial spot at 6 Å and a strong one at 12 Å which on wetting became a streak with a maximum intensity at about 15 Å. Strips of the inner layers showed a sharp thermal transition (mean half shortening temperature 78° C), shrinking by about 30–40% of their resting length.

Taken together, this evidence indicates that the fibrils which comprise the inner layer of the capsule contain a protein with the configuration of a collagen together with other protein(s) not in this configuration. In this respect the fibrils appear to resemble those of elastoidin[6].

Fibrils of the inner layers of the capsule seen in ultrathin sections stained with uranyl acetate and lead citrate have a mean diameter of 1,300 Å and show an axial, centrosymmetrical banding pattern with a mean repeat period of 370 Å (Fig. 1*a*). This banding pattern is built up from alternating narrow bands (A; 70 Å wide) and broad bands (B; 120 Å wide), both of low density, separated by bands (C; 90 Å wide) of high density. The C bands can be resolved into two dense sub-bands. Fibrils dispersed from the inner layers by homogenisation and sonication show, on negative staining

Table 1 Amino acid composition in residues per 100 amino acid residues. Corrected for hydrolytic losses.

	Inner layer	Outer layer	TCA extract of inner layer trace	TCA extract of byssus threads*	Bovine skin collagen†
cysteic acid	1.2	1.2		—	—
OH Pro	0.9	0.4	4.4	7.1	9.2
Asp	11.0	13.3	6.6	6.7	4.8
Thr	5.6	3.6	4.6	3.4	1.7
Ser	6.2	7.0	4.8	4.2	3.8
Glu	6.7	7.0	6.5	8.9	7.2
Pro	7.9	7.7	9.9	8.3	12.9
Gly	16.0	16.5	30.0	33.7	33.4
Ala	6.5	3.6	8.0	8.4	10.5
Val	5.2	2.1	3.2	3.3	1.9
Met	trace	0.6	2.7	0.2	0.7
Ileu	3.3	1.5	1.8	1.7	1.1
Leu	3.5	1.5	2.1	2.2	2.5
Tyr	8.0	18.1	2.4	0.8	0.5
Phe	3.2	2.5	1.3	1.0	1.3
His	3.1	5.5	1.5	1.1	0.5
OH Lys	—	—	0.1	0.2	0.7
Lys	7.1	4.7	3.5	7.1	2.5
Arg	4.5	2.9	6.6	4.5	4.8

* 'Secreted' collagen of the byssus threads of *Mytilus edulis* (D. P. K., and S. O. Anderson, unpublished).
† Extracted as gelatin[4].

with PTA or ammonium molybdate at *p*H 6.4, a banding pattern closely similar to that seen in positively stained ultrathin sections but in reversed contrast (Fig. 1*b*).

Two observations indicate that the transverse arrangement of molecules within the B bands is highly ordered. First, where the fibrils are sectioned transversely, a highly ordered array of dense dots is seen (Fig. 2). These lie in a nearly square, rhombic lattice (lattice angle 93.5°; mean length of equilaterals 80 Å). Second, in longitudinal sections where a fibril is appropriately orientated about its long axis, a pattern of evenly spaced longitudinal filamentous densities is seen traversing the B band (Fig. 1*a*). These show spacings of either approximately 80 Å or approximately 60 Å and

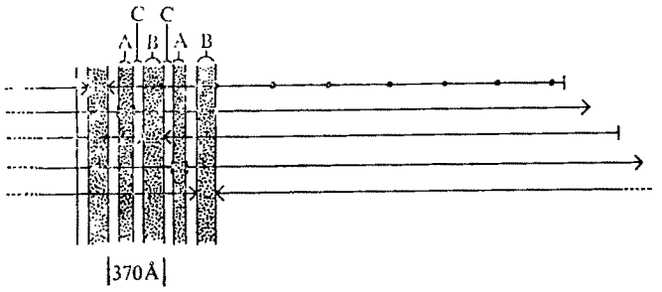


Fig. 3 Diagram illustrating the proposed longitudinal arrangement of molecules in the fibrils. Negative stain is thought to penetrate the gaps between the head ends and the tail ends. Arrowheads and bars indicate only the opposite ends of the molecule and do not imply which is the amino end.

may correspond respectively to images of the rhombic lattice viewed in the plane of the equilaterals or in the plane of the diagonals.

Although we have been unable to devise a model to account for all the features in our micrographs, the centrosymmetrical nature of the axial banding pattern and the alternation of narrow and broad bands into which negative stain can penetrate indicate that fibrous molecules are arranged longitudinally in a head-to-head, tail-to-tail fashion with gaps between the head ends of different size to the gaps between the tail ends (Fig. 3).

Although we have, so far, been unable to determine the molecular length of the collagen in this system, if it were close to that of a mammalian collagen (2,990 Å, ref. 7), the molecules would need to be staggered by about $\frac{1}{8}$ of the molecular length to produce the repeat of 370 Å (Fig. 3). Eight collagen molecules staggered in this way and packed together transversely in some form of nearly square array could account for the axial periodicity and could provide the building unit for the nearly square lattice seen in transverse sections of the fibrils.

The collagen-containing fibrils of the inner layers of the dogfish egg capsule show a unique fine structure indicating a highly ordered longitudinal and transverse arrangement of molecules. Our demonstration by electron microscopy of a nearly square unit cell (sides 80 Å) in transverse sections of these fibrils is particularly interesting in the light of the evidence from low angle X-ray diffraction for a similar lattice (sides 77 Å) (ref. 8) in the collagen fibrils of rat tail tendon.

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1 Bear, R. S., *Adv. Protein Chem.*, **7**, 69 (1952).
2 Gross, J., Dumsha, B., and Glazer, N., *Biochim. biophys. Acta*, **30**, 293 (1958).
3 Fitch, S. M., Harkness, M. C. R., and Harkness, R. D., *Nature*, **176**, 163 (1955).
4 Bailey, A. J., *Comp. Biochem.*, **26B**, 297 (1968).
5 Ramachandran, G. N., Sasisekharan, V., and Thathachari, Y. T., in *Collagen* (edit. by Ramanathan, N.) (J. Wiley, New York, 1960).
6 Kimura, S., and Kubota, M., *Bull. Jap. soc. scient. Fish.*, **34**, 535 (1968).
7 Piez., K. A., Miller, E. J., Lane, J. M., and Butler, W. T., in *Chemistry and Molecular Biology of the Intercellular Matrix*, **1** (edit. by Balazs, E. A.) (Academic Press, New York and London, 1970).
8 Miller, A., and Parry, D. A. D., *J. molec. Biol.*, **75**, 441 (1973).

Release of cyclic AMP from rat superior cervical ganglia after stimulation of synthesis *in vitro*

THE formation of adenosine 3′,5′-monophosphate (cyclic AMP) from adenosine 5′-triphosphate (ATP) in central nervous tissues seems to be controlled by catecholaminergic and histaminergic mechanisms[1]. Also in a peripheral autonomic ganglion, the intact superior cervical ganglion (SCG), accumulation of cyclic AMP was observed in response to electric stimulation[2] and to β-adrenergic agents[3], and histamine[4,5].

Cyclic AMP is excreted in large amounts in the urine[6], and

Table 1 Accumulation of cyclic AMP in SCG and release of cyclic AMP to the medium during incubation in the presence of adrenaline; acetycholine; adrenaline after preincubation with practolol; or adrenaline and acetycholine together

	N	Control	*N*	Adrenaline 10^{-4}M for 15 min	*N*	Acetylcholine 10^{-3}M for 15 min	*N*	Adrenaline plus practolol	*N*	Adrenaline plus acetylcholine
Cyclic AMP in ganglia (pmol ± SD per ganglion)	3	4.2 ± 0.2	3	24.9 ± 0.05*	3	4.8 ± 0.02	3	5.2 ± 0.8	3	24.9 ± 1.2*
Cyclic AMP in medium (pmol ± SD per ganglion)	3	0.6 ± 0.1	3	1.2 ± 0.2†	3	0.5 ± 0.06	3	0.6 ± 0.1	3	1.2 ± 0.1†

*P < 0.01 Student's *t*-test. †P < 0.05 Student's *t*-test.

marked changes in the excretion rate correlate with mood changes in manic depressed patients and with response to central active drugs[7-9]. It has therefore been suspected and is supported by indirect evidence[8] that the central nervous system may be one important source of cyclic AMP in body fluids.

We have reported experimental data showing a relationship between the rate of accumulation of cyclic AMP in the cerebrospinal fluid and the amount of catecholamine synthesis in the brain[10]. These findings prompted us to study more directly the release of cyclic AMP from nervous tissues when the synthesis of the nucleotide was stimulated by biogenic amines *in vitro*.

Male Sprague Dawley rats, weighing 200 to 300 g, were killed by cervical dislocation. The superior cervical ganglia (SCG) were rapidly removed, desheathed, and kept on ice until used for incubations. The ganglia were incubated in groups of two to four in 1 ml of Krebs-Henseleit solution *p*H 7.4 containing 200 mg ml^{-1} of ascorbic acid at 37° C under an atmosphere of 95% O_2 and 5% CO_2 with or without the drugs added. Blocking agents were tested by a preincubation period of 20 min. At the end of the experiments the ganglia were homogenised and chromatographed on anion exchange columns as described[3]. Total cyclic AMP was measured in column eluates of ganglia and in incubation media according to Gilman[11]. The formation of ^{14}C-cyclic AMP was measured after preincubation of the ganglia with ^{14}C-adenine for 1 h as described for brain slices[12]. Labelled cyclic AMP was determined by thin layer chromatography on silica-gel sheets[13]. Cyclic AMP phosphodiesterase activity was determined at micromolar substrate concentration according to Thompson and Appleman[14], lactate dehydrogenase activity by the method of Wroblewski and LaDue[15], and protein by the method of Lowry *et al.*[16].

The levels of cyclic AMP in freshly dissected SCG of rats ranged from 1.9 to 4.2 pmol per ganglion, corresponding to 1.6 to 2.3 pmol per mg fresh tissue. If SCG were incubated for 5–10 min in the absence of drugs, only small amounts of cyclic AMP were detectable in the medium (Fig. 1). Adrenaline (10^{-4} M), noradrenaline (10^{-3} M), isoprenaline (10^{-5} M), and histamine (10^{-3} M) rapidly increased the levels of cylic AMP in SCG tissue in a dose-related manner. The time course of the accumulation of cyclic AMP on incubation with adrenaline is shown in Fig. 1.

Elevated cyclic AMP concentrations were observed in the medium after 3 min incubation of the ganglia with adrenaline (10^{-4} M) or histamine (10^{-3} M). The rise of the cyclic AMP concentration seemed to continue after the peak accumulation was reached in the ganglia but the medium levels decreased

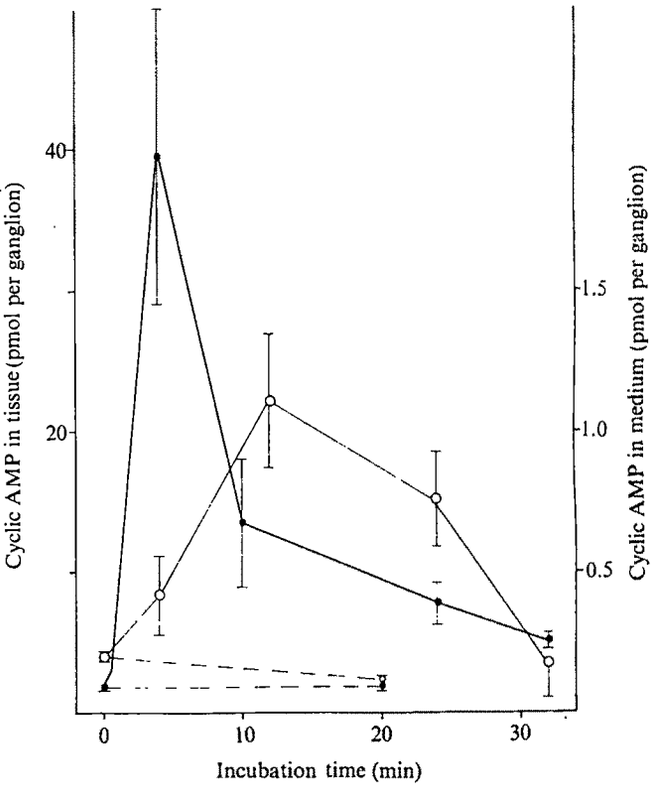


Fig. 1 Time course of the accumulation of cyclic AMP in superior cervical ganglia and in the incubation medium during incubation with L-adrenaline (10^{-4} M). Cyclic AMP was determined according to Gilman[11]. ●, Cyclic AMP content of ganglia; O, cyclic AMP appearing in the medium; - - - , time course in control incubations without the amine. Each point represents the mean of four experiments. Vertical bars show double standard errors of the mean.

again after 12 to 15 min of stimulation (Fig. 1). Acetylcholine (10^{-3} M) and carbamylcholine (10^{-9}–10^{-3} M) did not alter the cyclic AMP concentration in the SCG and in the incubation medium. Moreover, acetylcholine did not influence the accumulation and release of cyclic AMP induced by adrenaline. Preincubation with propanolol or practolol, two β-adrenergic blocking agents, completely blocked the effects of adrenaline on cyclic AMP in SCG and the medium (Table 1).

The assay for cyclic nucleotide phosphodiesterase activity in the incubation medium revealed the appearance of protein

Table 2 Medium content of total protein, cyclic nucleotide phosphodiesterase activity and lactate dehydrogenase activity after incubation of superior cervical ganglia for various times

Treatment	Protein (μg per ganglion)	Cyclic nucleotide phosphodiesterase (pmol cyclic AMP hydrolysed per mg protein per min)	Lactate dehydrogenase (mU per ml per mg protein per min)
Control (10 min)	11.5	13.5	7.4
Histamine 10^{-3} M (10 min)		6.7	5.3
Control (40 min)	9.6	12.0	24.3
Histamine 10^{-3} M (40 min)		4.8	18.7

and enzyme activity in the absence of stimulating drugs (Table 2). While the activity of lactate dehydrogenase which serves as a cytoplasmic marker enzyme increased in the medium during incubation with three- to four-fold increases from 10–60 min, bulk release of protein as well of phosphodiesterase activity occurred mainly during the initial period of incubation with in general no further increases in concentration and specific activity after 10 min of incubation. When ganglia were incubated with adrenaline or histamine the phosphodiesterase activity was not increased in the medium but slightly decreased while the total release of proteins from the ganglia into the medium was unchanged. This behaviour may indicate that cyclic nucleotide phosphodiesterase in an activated state is less prone to loss from intracellular compartments than in an inactive form.

After preincubation of SCG with ^{14}C-adenine for 60 min, less than 1% of the total radioactivity was found to correspond to ^{14}C-cyclic AMP. Of the total ^{14}C-cyclic AMP formed 35–45% appeared in the medium if measured after 15 min incubation in the absence of drugs. The addition of adrenaline or histamine strongly stimulated the rate of conversion to ^{14}C-cyclic AMP. Of the total radioactivity 6 and 5% was associated with cyclic AMP after incubation with adrenaline (10^{-4} M) and histamine (10^{-3} M) respectively for 15 min. The ratio of labelled cyclic AMP in the medium to ^{14}C-cyclic AMP in the ganglia remained essentially unchanged after stimulation as compared to the resting condition. This behaviour indicates that the preincubation with ^{14}C-adenine rather selectively labels a separate ATP precursor pool for cyclic AMP, as was observed earlier[12,17] and, in addition, suggests a membrane associated localisation of the precursor pool leading to preferential release of labelled product after the stimulation of adenylate cyclase by biogenic amines.

Our experiments have shown that from a peripheral sympathetic ganglion of the rat cyclic AMP is released following the stimulation of its intracellular formation by biogenic amines such as adrenaline and histamine *in vitro*. Acetylcholine neither affects the rate of synthesis nor the release of cyclic AMP. Whether under physiological conditions the release or overflow of cyclic AMP after stimulation by neurohormones serves to remove, and thus inactivate, excessive cyclic AMP unbound to protein kinases, or whether released cyclic AMP has some function of its own, is at present unclear. Evidence in favour of a release of cyclic AMP from central nervous tissues *in vivo* has also been obtained in humans[18].

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[1] Kakiuchi, S., and Rall, T. W., *Molec. Pharmac.*, **4**, 379 (1968).
[2] McAfee, D. A., Schorderet, M., and Greengard, P., *Science*, **171**, 1156 (1971).
[3] Cramer, H., Johnson, D. G., Hanbauer, I., Silberstein, S. D., and Kopin, I. J., *Brain Res.*, **53**, 97 (1973).
[4] Lindl, T., and Cramer, H., *IRCS* (73–8) 3–5–12.
[5] Lindl, T., and Cramer, H., *Biochim. biophys. Acta*, **343**, 182 (1974).
[6] Chase, L. R., Aurbach, G. D., *Proc. natn. Acad. Sci. U.S.A.*, **58**, 518 (1967).
[7] Paul, M. I., Ditzion, B. R., Janowsky, D. S., *Lancet*, **i**, 88 (1970).
[8] Abdulla, Y. H., and Hamadah, K., *Lancet*, **i**, 378 (1970).
[9] Paul, M. I., Cramer, H., and Goodwin, F. K., *Arch. gen. Psychiat.*, **24**, 327 (1971).
[10] Cramer, H., and Lindl, T., *Psychopharmacologia*, **26**, supplement, 49, (1972).
[11] Gilman, A., *Proc. natn. Acad. Sci., U.S.A.*, **67**, 305 (1970).
[12] Shimizu, H., Daly, J. W., and Creveling, C. R., *J. Neurochem.*, **16**, 1609 (1969).
[13] Dighe, P. K., Pahuja, D. N., and Sha, D. H., *J. Chromatogr.*, **40**, 449 (1969).
[14] Thompson, W. J., and Appleman, M. M., *Biochemistry*, **10**, 311 (1971).
[15] Wroblewski, F., and La Due, J. S., *Proc. Soc. exp. Biol. Med.*, **90**, 210 (1955).
[16] Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265 (1951).
[17] Schultz, J., and Daly, J. W., *J. biol. Chem.*, **248**, 843 (1973).
[18] Cramer, H., Ng, N.K.Y., and Chase, T. N., *J. Neurochem.*, **19**, 1601 (1972).

Origins of metal ions in biology

A FERREDOXIN may have been one of the earliest proteins formed. The evidence for this assumption was recently presented by Hall *et al.*[1] based on the primary structures of several ferredoxins. Here, further evidence is reported for the early evolution of ferredoxins, and the origins of ferredoxins and other metalloproteins are discussed from an inorganic point of view based on thermodynamic data.

The atmosphere predominating during the time of chemical evolution and early life is generally assumed to have been a reducing one, characterised by a moderate pressure of methane (0.01–0.001 atm) and a very low oxygen pressure[2]. At such a reducing condition, thermodynamic data for the system, air–sea–sediment[3], indicate that at equilibrium the redox potentials in the seas were as low as −325 mV ($p^E = -5.5$): inorganic sulphur would be in the form of the iron sulphide, FeS_2(s) (pyrite), and perhaps partly as $Fe_{0.86}S$(s) (pyrrhotite); the major excess of iron would be in the form of Fe_3O_4(s) and a minor amount dissolved in the form of Fe^{2+} (≤ 0.2 mM).

Crystals of FeS_2(s) and $Fe_{0.86}S$(s) are well-known semiconductors having the capacity to transfer electrons. This leads to the following hypothesis: inorganic electron carriers in the form of FeS_2(s) and $Fe_{0.86}S$(s) were present prebiotically in the area where the first proteins evolved. When polypeptides containing cysteine residues formed, they reacted spontaneously with these iron sulphides forming bonds similar to those present in ferredoxins (compare refs 4–6). Also, iron in the form of Fe^{2+} would react forming iron proteins similar to rubredoxin[5]. Thus, very early, at least two different kinds of iron-sulphur proteins most likely formed, and these proteins probably developed independently into two different classes of electron carriers. It should be noted that during these reducing conditions low-valent molybden could enter solid solutions of iron sulphide (molybdenite, MoS_2(s), would be the prevailing molybden compound), and the sulphur-rich Mo-Fe proteins[7] might have evolved from such solid solutions (compare ref. 8).

The great abundance of iron in sea sediment supports the idea that the formation of iron-sulphur proteins was spontaneous and very common in early life processes. Also, the relative high concentration of dissolved iron (0.2 mM) would facilitate reactions with porphyrins, and thus the formation of haem proteins. Likewise, the Mn(II) concentration was much higher in the primordial seas[3], perhaps as high as 50 mM. This may explain why manganese seems to be one of the most important elements in early redox processes; for instance, in the photosynthesis of the primitive blue-green algae[9], and in the reactions of an early superoxy dismutase, apparently a manganese enzyme rather than a copper enzyme[10].

During the early reducing conditions, copper ions were most likely trapped into extremely insoluble Cu(I) sulphides; they could not be released into aqueous and biological phases until the air–sea–sediment system became more oxidised by evolving oxygen. Then copper proteins developed, such as plastocyanin and cytochrome oxidase, which were required for further biological evolution. It can be estimated, using Sillén's data[3,11], that Cu(I) complexes of thiols and amines could form at redox potentials of about +90 and +230 mV, and free Cu^{2+} at about +250 mV. Thus, thermodynamic data strongly support an evolution of metalloproteins, beginning with iron and manganese proteins and continuing with copper proteins.

When oxygen successively oxidised the air–sea–sediment quite insoluble phases of iron and manganese formed[3], FeOOH(s) and MnO_2(s). And at present, the concentrations of dissolved iron and manganese in sea water[12] are less than 10^{-7} M. Therefore, although copper was released by oxidation, iron and manganese became immobilised.

Also of particular evolutionary interest is the successive biological importance of weakly interacting metal ions; Mg^{2+} and Ca^{2+}, for instance. Magnesium ions are important in reactions simulating prebiotic condensations[13] and in early and fundamental cell reactions, such as protein biosynthesis, anaerobic energy production, and photosynthesis; calcium ions are of specific importance in processes characterising later and higher forms of life, such as nerve transmission, hormone secretion, and muscle contraction. One explanation for the early role of Mg^{2+} may be ascribed to its abundance; its high concentration in the oceans (54 mM) may have been even higher during the early reducing conditions, since on the primitive Earth NH^{+}_{4}, Fe^{2+}, and Mn^{2+} would partly replace Mg^{2+} in the silicate phases[3].

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1 Hall, D. O., Cammack, R., and Rao, K. K., *Nature*, **233**, 136-138 (1971).
2 Ponnamperuma, C., *Q. Rev. Biophys.*, **4**, 77-106 (1971).
3 Sillén, L. G., *Arkiv Kemi*, **25**, 159-176 (1966).
4 Malkin, R., and Rabinowitz, J. C., *Biochem. biophys. Res. Commun.*, **23**, 822-827 (1966).
5 McCarthy, K., and Lovenberg, W., *J. biol. Chem.*, **243**, 6436-6441 (1968).
6 Sugiura, Y., and Tanaka, H., *Biochem. biophys. Res. Commun.*, **46**, 335-340 (1972).
7 Hardy, R. W. F., Burns, R. C., and Parshall, G. W., *Bioinorganic Chemistry*, (edit. by Gould, R. F.) 219-247 (American Chemical Society, Washington, DC, 1971).
8 Schrauzer, G. N., and Schlesinger, G., *J. Am. chem. Soc.*, **92**, 1808-1809 (1970).
9 Olson, J. M., *Science*, **168**, 438-446 (1970).
10 Keele, B. B., Jr., McCord, J. M., and Fridowich, I., *J. biol. Chem.*, **245**, 6176-6181 (1970).
11 Sillén, L. G., and Martell, A. E., *Stability Constants*, (Second ed.), (Chem. Soc. Spec. Publ. No. 17, 1964; Supplement No. 1, Chem. Soc. Spec. Publ. No. 25, 1971).
12 Sillén, L. G., *Oceanography*, **0**, 549-581 (1959).
13 Lohrmann, R., and Orgel, L. E., *Nature*, **244**, 418-420 (1973).

Artificial induction of the sexual cycle of *Neurospora crassa*

CHEMICAL regulation of sexual development in fungi was suggested in the 1880s by Sachs and de Bary (see ref. 1). Burgeff[2] later demonstrated that the so-called + and − strains of *Mucor mucedo* if separated by a colloidion membrane, produced zygophores that grew toward each other. The presence of diffusible substances which function in the sexual cycle has since been demonstrated in, for example, *Achlya*[1,3–8], *Allomyces*[9,10], *Ascobolus*[11–13], *Mucor*[14–20], *Saccharomyces*[21–23], and *Neurospora*[24–27]. Varied functions have been attributed to these extracts. In some well studied systems, such as *Achlya* and *Mucor*, chemical substances have been reported at almost all stages of sexual development, each serving to initiate the next stage[4,17].

But in spite of all this work relatively little is known about the chemical basis of the incompatibility of heterothallic fungi. Burgeff's work with *Mucor*[2], was confirmed in both *M. mucedo* and *Blakeslea trispora*[28]. In *N. crassa*, protoperithecia have been induced to develop into fruiting bodies, albeit sterile, by filtrates from cultures of the opposite mating type or from mated cultures[24]. Similar results have been obtained with lipid extracts from single and mated cultures of *N. crassa*[27], and from the resulting fruiting bodies a few ascospores were isolated which segregated in an aberrant manner with respect to the mating-type alleles. Unfortunately no tests for other genetic markers were reported.

Table 1 Effect of mycelial extracts from mated cultures on plates of pan 1 and leu 3 assessed by the presence of mature perithecia with spores.

Strain tested	Duration of mating before lysate extraction (h) 0	1	6	12	24	48	96	168	240	336
panlA	−	−	−	+	+	+	+	+	−	−
panla	−	−	−	+	+	+	+	+	−	−
leu3A	−	−	−	+	+	+	+	+	−	−
leu3a	−	−	−	+	+	+	+	+	−	−

−, No perithecia developed.
+, Perithecia developed.

These and similar reports gave rise to the suggestion that physical contact between compatible mating partners is not essential. Some diffusible substance, termed progamone by Plempel[29], induces the sexual cycle in a single strain. If the incompatibility block could thus be totally relieved, then artificial induction of a single strain should be followed by the orderly progression of sexual development. The conclusive demonstration of this induction would be the isolation of resulting ascospores which carry genetic markers from the single parent. This has never been reported, perhaps because the true incompatibility substances have not yet been isolated, or because artificial induction by these substances is followed by aberrant events in the ascogonium leading to strange segregation ratios, as Islam and Weijer[27] suggested.

It is significant that previous efforts to overcome incompatibility have relied largely on lipid extracts. Their incomplete success in inducing sexual development might indicate that incompatibility substances, if they exist, are not lipid in nature. Recent suggestions have been that some sex substances are proteinaceous[23,30,31]. We report here the effect of crude protein extracts on single mating cultures of *N. crassa*. They provide the first conclusive evidence of artificial indication of sexual development in a fungus.

Table 2 Average number of mature perithecia in leu3 plates treated with mycelial extracts.

	Duration of mating before lysate extraction (h) 0	1	6	12	24	48	96	168	240	336
No. of perithecia per plate	0	0	0	1,810	3,760	5,840	3,010	2,080	0	0

Figures represent the composites of five plates each.

Emerson strains, obtained from the Fungal Genetics Stock Center, Humbolt, California, provided the extracts. Single cultures were grown for 7 d in large flasks of standard crossing medium[32], fertilised with conidia of the opposite mating type and extracted at intervals ranging from 1 to 336 h later (Table 1). Extraction procedures were conducted at 4° C or less. Mycelial mats were homogenised in a Virtis blender with 0.15 M phosphate buffer followed by filtration through cheese cloth, to remove mycelial fragments, and subsequent centrifugation of the filtrate for 30 min at 25,0000*g*. The supernatant was dialysed against 0.05 M phosphate buffer, concentrated by pervaporation and filter sterilised. All extracts were tested to ensure the absence of viable material. Extracts were assayed by adding them to plates of single strain cultures of leu3 (allele *R*—156) and pan1 (allele 5531) and subsequent 14 d incubation at 25° C. Plates were then inspected for the presence of mature perithecia with spores.

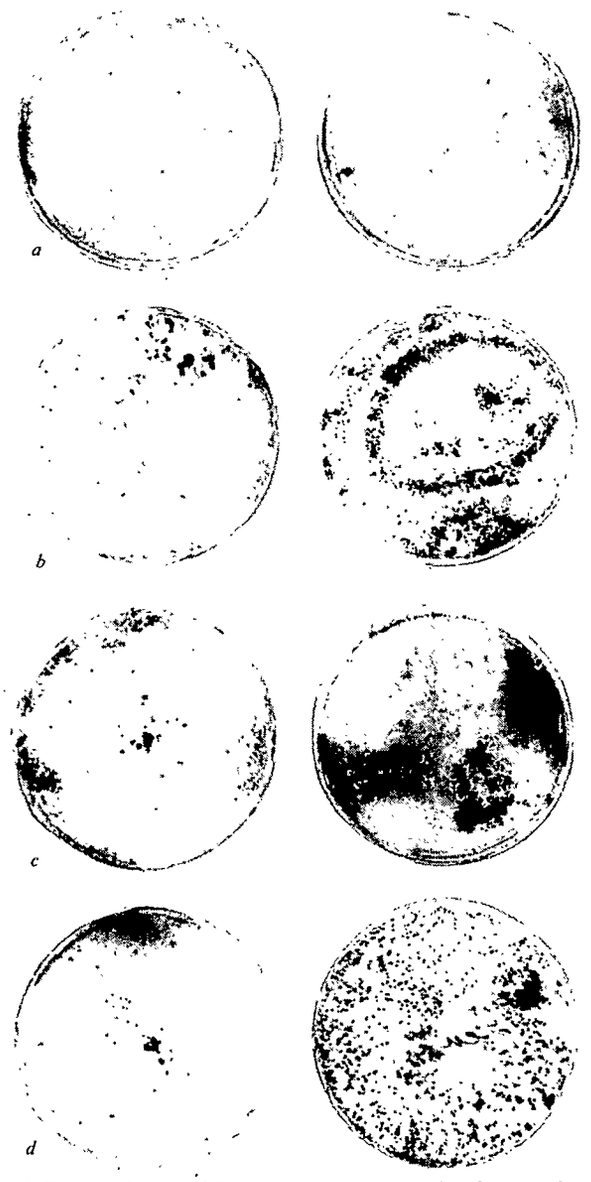


Fig. 1 Effect of mycelial extracts on unmated cultures of pan1 (left hand side) and leu3a (right hand side). *a*, Controls (buffer only added); *b*, with extract from 12 h culture of Em A × Em a; *c*, with extract from 96 h culture of Em A × Em a; *d*, with extract from 168 h culture of Em A × Em a.

The effect of the various mycelial extracts on single cultures of each of the mating types of leu3 and pan1 are shown in Tables 1 and 2 and illustrated in Fig. 1. Tetrads were isolated from mature perithecia obtained from eleven different plates (Tables 3 and 4).

The data in Table 1 indicate that the crude extract contains a sex factor capable of inducing the sexual cycle in a single mating culture of the heterothallic fungus. This substance is present in the mycelial extract of a mated culture; it appears first 12 h after mating and is no longer recoverable 240 h after mating. Preliminary investigations based on lack of activity in medium extracts, suggested that it is not a diffusible substance.

We have demonstrated further that after artificial induction sexual development proceeds normally, culminating in the production of mature ascospores which carry genetic markers from the single parent only (Tables 3 and 4). This is conclusive evidence of artificial induction. The isolation and purification of the active ingredient should ultimately provide the necessary clue to the chemical mechanism of incompatibility. The perithecia produced appear to be morphologically normal but some contain relatively few asci. The incidence of aborted spores (asci with four or eight aborted spores) does not seem to be significantly greater than in normal crosses. Some perithecia contained asci which showed a 4:4 segregation of genetic markers (Table 3). These may have been due to accidental fertilisation by contaminant wild type spores.

Table 3 Tetrad analysis of asci isolated from perithecia present in unmated cultures of leu3a treated with mycelial extracts

Plate No.	No. of asci with eight germinated spores	No. of asci with four germinated spores	Segregation patterns of mating-type marker*(a-A) 8:0	4:4	4:0	Segregation patterns of leu marker* (leuΔ-leuΔ) 8:0	4:4	4:0
201	6	0	6	0	0	6	0	0
202	4	0	4	0	0	4	0	0
401	3	0	3	0	0	3	0	0
402	3	0	3	0	0	3	0	0
406	4	0	4	0	0	4	0	0
203	0	11	0	0	11	0	0	11
401	10	0	0	10	0	0	10	0

*No. of asci in each class.

Table 4 Tetrad analysis of asci isolated from perithecia present in unmated cultures of pan1A treated with mycelial extracts

Plate No.	No. of asci with eight germinated spores	No. of asci with four germinated spores	Segregation patterns of mating-type marker* (A:a) 8:0	4:4	4:0	Segregation patterns of leu marker* (pan^-:pan^+) 8:0	4:4	4:0
416	4	0	4	0	0	4	0	0
417	3	0	3	0	0	3	0	0
418	6	0	6	0	0	6	0	0
419	4	0	4	0	0	4	0	0
427	2	0	2	0	0	2	0	0

*No. of asci in each class.

The sex inducer, termed erogen B in line with previous terminological suggestions[33], has not yet been characterised chemically. Preliminary tests based on its inactivation by heat and protease but not by nucleases indicate that it is a protein. It is interesting that the extracts from single mating cultures (unfertilised) are inactive in terms of their ability to induce the sexual cycle in strains of the opposite mating type. Thus, erogen B seems to be produced only when both mating types are present in the same culture. Similar results are reported in *B. trispora* and *M. mucedo* where trisporic acid synthesis is initiated only when living mycelia of both mating types are placed in the same culture and stops immediately if one of the partners is removed[34]. One might predict, from the simplest interpretation of a complementary stimulant model of the chemical mechanism of incompatibility, that the active ingredient, erogen B, from one mating type should successfully induce the opposite mating type. We suggest that the incompatibility mechanism is more complex and that another sex factor, erogen A, is involved. Further details of this work are bing published elsewhere.

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1 Raper, J. R., *Am. J. Bot.*, **47**, 794 (1960).
2 Burgeff, H., *Bot. Abh. Heft.*, **4**, 5 (1924).
3 Raper, J. R., *Am. Sci.*, **31**, 110 (1951).
4 Raper, J. R., *Symp. Soc. exp. Biol.*, **11**, 143 (1957).

5 Barksdale, A. W., *Am. J. Bot.*, **47**, 14 (1960).
6 Barksdale, A. W., *Mycologia*, **55**, 627 (1963).
7 Barksdale, A. W., *Ann. N.Y. Acad. Sci.*, **144**, 313 (1967).
8 Barksdale, A. W., *Science*, **166**, 831 (1969).
9 Machlis, L., *Physiol. Pl.*, **11**, 181 (1958).
10 Machlis, L., *Physiol. Pl.*, **11**, 845 (1958).
11 Bistis, G., *Am. J. Bot.*, **43**, 389 (1956).
12 Bistis, G., *Am. J. Bot.*, **44**, 436 (1957).
13 Bistis, G., and Raper, J. R., *Am. J. Bot.*, **50**, 880 (1963).
14 Banbury, G. H., *Nature*, **173**, 499 (1954).
15 Banbury, G. H., *Expl Bot.*, **6**, 235 (1955).
16 Plempel, M., *Arch. Microbiol.*, **25**, 154 (1957).
17 Plempel, M., *Naturwissenchaften*, **47**, 472 (1960).
18 Plempel, M., and Braunitzer, G., *Z. Naturb.*, **13b**, 302 (1958).
19 Gooday, G. W., *Phytochemistry*, **7**, 2103, (1968).
20 Gooday, G. W., *New Phytol.* **67**, 815, (1968).
21 Levi, J. D., *Nature*, **177**, 753 (1956).
22 Yanagashima, N., *Planta*, **87**, 110 (1969).
23 Yanagashima, N., *Physiol. Pl.*, **24**, 260 (1971).
24 Ito, T., *Bot. Mag. Tokyo*, **69**, 369 (1956).
25 Howe, jr., H. B., and Prakash, V., *Can. J. genet. Cytol.*, **11**, 689 (1969).
26 Vigfusson, N. V., Walker, D. G., Islam, M. S., and Weijer, J., *Folia Microbiol.* **16**, 166 (1971).
27 Islam, M. S., and Weijer, J., *Folia Microbiol.* **17**, 316 (1972).
28 Ende, H. van den, *J. Bact.*, **96**, 1298 (1968).
29 Plempel, M., *Planta*, **59**, 492 (1963).
30 Duntze, W., Mackay, V., and Manney, T. R., *Science*, **168**, 1472 (1970).
31 Ende, H. van den, and Stegwee, D., *Bot. Revs.*, **37**, 22 (1971).
32 Westergard, M., and Mitchell, H. K., *Am. J. Bot.*, **34**, 573 (1947).
33 Machlis, L., *Mycologia*, **64**, 235 (1972).
34 Ende, H. van den, Weichmann, A. H. C. A., Reyngoud, D. J., and Hendricks, T., *J. Bact.*, **101**, 423 (1970).

Bacteria can swim without rotating flagellar filaments

THERE are two simple ways in which the observed screw-like propulsive motion of bacterial flagella can be understood. Either, first, the flagellum is like a rigid corkscrew rotating in a sort of journal bearing in the cell wall (hypothesis *A*) or, second, the flagellum is firmly attached to the cell wall and moves by the propagation of helical waves (hypothesis *B*).

Berg and Anderson[1] argue in favour of hypothesis *A*. Their most positive argument involves the idea that in multiflagellated bacteria a bivalent antibody firmly attaching itself to two neighbouring flagella would prevent motion according to hypothesis *A* but not on hypothesis *B*. Thus, they interpret the observations of Di Pierro and Doetsch[2] on the effect of univalent and bivalent flagellar antibodies on both peritrichous and uniflagellar bacteria as support for the 'rotating corkscrew' picture of flagellar motion.

Unfortunately, they have left out of their analysis a simple geometrical factor, which altogether removes from their argument the power to discriminate between the two hypotheses.

Figure 1 (based on Fig. 1 Berg and Anderson[1]) shows two cross-sectional views (Fig. 1*a* and *b*) along the helical axes of two neighbouring filaments at two successive instants of time according to hypotheses *A* and *B* respectively. In Fig. 1*a* the cross sections rotate, whereas in Fig. 1*b* they do not. The difference in pattern of relative motion of the spots marked on the surfaces of adjacent filaments is plain.

But now consider Fig. 1*a′* and *b′*, which show projected 'elevations' of the situations depicted in Fig. 1*a* and *b*. Again the spots are shown but on short segments of the neighbouring filaments in this view. Notice the relative motion of the spots in Fig. 1*b′* along the local filament axis, which is not apparent in the view in Fig. 1*b* alone. A bivalent antibody attached to the two spots in Fig. 1*b* and *b′* could therefore inhibit motion just as strongly as it could in Fig.

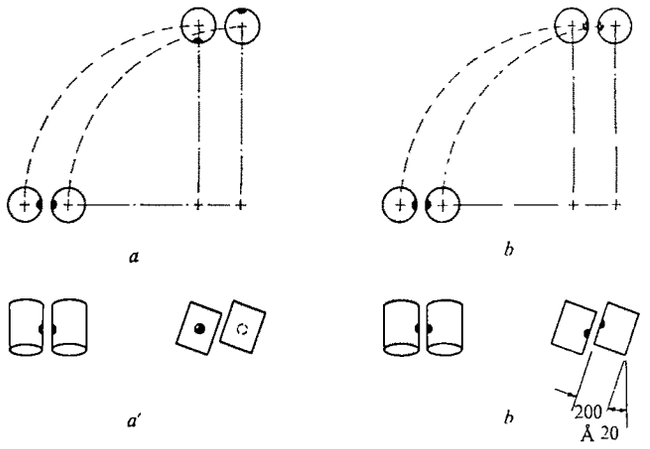


Fig. 1 A schematic view of two adjacent filaments rotating individually (*a* and *a′*) or propagating helical waves (*b* and *b′*) at two successive intervals of time, *a* and *b* Are sectional views in a plane normal to the helical axes (+). *a′* and *b′* Are elevations of segments of the filaments, projected from *a* and *b* in the plane of the helical axes.

1*a* and *a′*. The relative motion of the spots on hypothesis *B* is an oscillatory lengthwise rubbing, and we can estimate its amplitude easily by taking the helix angle in Fig. 1*b′* as about 20° and the diameter of the filaments as about 200Å, after Asakura[3]. Then the extreme displacement of one spot to another is ±70 Å, that is a total throw of 140 Å or about three times the diameter of a flagellin subunit.

Hence, the fact that bivalent flagellar antibodies 'lock' adjacent filaments can only be taken to rule out hypothesis *B* if the antibodies are flexible, and indeed much more flexible than seems likely from what is known about their size and construction.

Now antibodies have a Y-shaped form, with two arms which attach to the host at the tips[4]. From an X-ray crystallographic investigation of the Fab′ fragment of an antibody Padlan *et al.*[5] have determined its overall length to be 80 Å. Thus the length of an arm can be assumed to be about 70 Å. It seems likely that the tips are attached to the host with strong restrictions on the angle of attachment, and so it is difficult to see how such a molecule could stay attached to two points on adjacent flagella executing the large relative motion I have described without having extremely flexible arms.

The arguments of Berg and Anderson therefore do not prove that hypothesis *B* is wrong. Indeed, I believe hypothesis *B* to be correct because the propagation of helical waves in a filament requires only small relative movements between adjacent flagellin subunits, whereas a rigid-body motion of the entire filament would require a very specially constructed bearing. Moreover, as Klug[6] has pointed out, the problem of propagating a helical wave in a filament is largely subsumed in the problem of assembling a helical filament from identical flagellin subunits, which would still be an outstanding problem on hypothesis *A*.

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1 Berg, H. C., and Anderson, R. A., *Nature*, **245**, 380–382 (1973).
2 Di Pierro, J. M., and Doetsch, R. N., *Can. J. Microbiol.*, **14**, 487–489 (1968).
3 Asakura, S., *Adv. Biophys.*, **1**, 99–155 (1970).
4 Green, N. M., *Adv. Immun.*, **11**, 1–30 (1969).
5 Padlan, E. A., Segal, D. M., Spande, T. F., Davies, D. R., Rudikoff, S., and Potter, M., *Nature new Biol.*, **245**, 165–167 (1973).
6 Klug, A., *Symp. int. Soc. Cell Biol.*, **6**, 1–18 (1967).

Toxicity of piperonyl butoxide to *Boophilus microplus*

SYNERGISM studies with the cattle tick *Boophilus microplus* have been effected by enclosing larvae in packets impregnated with olive oil solutions of the test chemicals and recording mortality after 48 h[1,2]. In these conditions larvae exposed continuously to 0.4% piperonyl butoxide were apparently unaffected. Subsequently, in studies to determine the sensitivities of the mixed function oxidase systems in organophosphorus (and carbamate) resistant and susceptible strains of *B. microplus*, larvae were immersed in aqueous colloids[3] of piperonyl butoxide. Concentrations greater than 0.02% proved to be toxic and an LC_{50} of 0.044% at 24 h after treatment was determined. This unexpected result aroused interest in the acaricidal potential of piperonyl butoxide. Although it is generally regarded as a nontoxic synergist[4], some workers have claimed it is toxic to houseflies at fairly high dosage[5] but others found it to be nontoxic[6]; toxicity to the mite *Acarus siro* (formerly *Tyroglyphus farinae*) has been recorded[7,8].

We decided that testing of the material alone and mixed with carbaryl, against parasitic stages of the tick in the field, was warranted. The mixture was tested because of the well known synergism of carbaryl and other carbamate insecticides by piperonyl butoxide against resistant houseflies. This synergistic action is believed to be due to piperonyl butoxide reducing the abnormally rapid rates of oxidative degradation of the carbamates by resistant flies[9]. The ticks used in our tests, however, were of the Biarra strain[10], and are resistant to carbaryl because of low sensitivity of their acetylcholinesterase to inhibition by carbaryl, while their detoxication systems are normal[11].

Cattle naturally infested with all stages of Biarra ticks were sprayed with aqueous emulsions of piperonyl butoxide formulated with one third its volume of Triton X-100. (*B. microplus* is a one-host tick with a cycle of approximately 21 d, and infested cattle normally carry mixed populations of larvae, nymphs and adults.) Cattle were also sprayed with a commercial wettable powder formulation of carbaryl at 0.3% active ingredient, and with mixtures of carbaryl and the piperonyl butoxide formulation. Counts of semiengorged females 4.5 mm to 8 mm in length were made at intervals up to 21 d after treatment and a survival index was calculated from counts of ticks on untreated control animals[12]. The results in Table 1 show that piperonyl butoxide is lethal to all stages of the tick when used at relatively high concentrations. The acaricide carbaryl allowed 28% survival of resistant Biarra ticks compared with 1% survival expected with susceptible Yeerongpilly ticks (W. J. R., unpublished data). With the addition of piperonyl butoxide at concentrations $\geq 0.3\%$, however, less than 1% of the resistant strain survived.

As much lower concentrations of piperonyl butoxide have been shown to inhibit the degradation of carbaryl in laboratory tests on larvae (C. A. S., unpublished data), this type of protective action doubtless contributed to the effectiveness of the spray mixtures. The lethal effect of piperonyl butoxide itself may also have contributed to the enhanced toxicity of the mixtures and it is not certain, therefore, whether piperonyl butoxide acts with carbaryl predominantly as a synergist or mainly as a toxicant participating in some type of joint action.

It seems likely that the toxic action of piperonyl butoxide resulted from inhibition of a mixed function or closely related oxidase, the recognised type of target of piperonyl butoxide[13]. This indicates that the cattle tick is vitally dependent on some part of this system. In support of this we have preliminary evidence that another methylenedioxyphenyl compound sulfoxide, which inhibits cattle tick mixed function oxidase, is also toxic.

The immediate significance of these findings is that an acaricide which had failed against a resistant strain has been restored to full effectiveness in the field by the addition of a compound which has no significant mammalian toxicity[14]. A more important aspect in the long term could be that from this class of compound a practical acaricide could emerge.

Table 1 Field control of the Biarra strain of cattle tick with sprays containing piperonyl butoxide or carbaryl or mixtures of the two chemicals

Spray Composition (%) Carbaryl	Piperonyl butoxide	Cattle per treatment	Survival index* on days† after treatment 1	2	5	7	9	12	14	16	19	21	Mean 1–21 days
–	0.03	4	99	64	92	132	52	105	86	42	36	38	75
–	0.3	4	53	28	3	16	33	40	33	14	4	1	23
–	3.0	4	95	27	0.8	0.7	1	5	4	1	0.4	0.4	14
0.3	–	2	39	11	3	39	92	16	19	11	9	40	28‡
0.3	0.03	2	35	22	3	4	33	61	41	24	5	1	23
0.3	0.3	2	1	0	0	0.7	5	0.7	0.4	0.8	0.8	1	1
0.3	3.0	2	3	0	0	0.7	4	0.3	0	0	0	0	1

*Calculated as follows: (Tick count on treated animals/Tick count on control animals) × (Pretreatment count on control animals/Pretreatment count on treated animals) × 100.
On control cattle daily totals of more than 200 semiengorged females were recorded before and after treatment.
†Stages of tick at treatment were: adult females, day 1–8; nymphs, day 9–15; larvae, day 16–21.
‡In similar conditions less than 1% of the acaricide-susceptible Yeerongpilly ticks survived (W. J. R. unpublished data).

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1 Knowles, C. O., and Roulston, W. J., J. *Aust. ent. Soc.*, **11**, 349 (1972).
2 Knowles, C. O., and Schuntner, C. A., J. *Aust. ent. Soc.*, (in the press).
3 Roulston, W. J., Schuntner, C. A., and Schnitzerling, H. J., *Aust. J. biol. Sci.*, **19**, 619 (1966).
4 Negherbon, W. O., *Handbook of Toxicology*, **3**, 619 (W. B. Saunders Philadelphia, 1959).
5 Dove, W. E., *Am. J. trop. Med.*, **27**, 339 (1947).
6 Wachs, H., *Science*, **105**, 530 (1947).
7 Parkin, E. A., *Pest infest. Res.*, **20** (1950).
8 Hope, J. A., *Pest infest. Res.*, **34** (1960).
9 Metcalf, R. L., in *The Enzymatic Oxidation of Toxicants* (edit. by Hodgson, E.), 162 (Raleigh, North Carolina State University, 1968).
10 Roulston, W. J., and Wharton, R. H., *Aust. Vet. J.*, **43**, 129 (1967).
11 Roulston, W. J., Schnitzerling, H. J., and Schuntner, C. A., *Aust. J. biol. Sci.*, **21**, 759 (1968).
12 Wharton, R. H., Roulston, W. J., Utech, K. B. W., and Kerr, J. D., *Aust. J. agric. Res.*, **21**, 985 (1970).
13 Wilkinson, C. F., in *The Enzymatic Oxidation of Toxicants* (edit. by Hodgson, E.), 131 (Raleigh, North Carolina State University, 1968).
14 Negherbon, W. O., *Handbook of Toxicology*, **3**, 620 (W. B. Saunders, Philadelphia, 1959).

Treehopper (Membracidae) alarm pheromones

ALARM pheromones are characteristic of many species of social Hymenoptera and Isoptera. The suggestion that alarm pheromones occur in gregarious, nonsocial arthropods[1] was confirmed by the report that the heteropteran *Dysdercus intermedius* Distant produces hex-2-en-1-al from its scent glands to repel members of its own species[2]. Aphids, other heteropterans which form aggregates on the plants on which they feed, also produce alarm pheromones. From specialised structures (cornicles) they secrete droplets[3] containing volatile pheromones that cause aggregates to disperse when attacked by predators[4] or parasites[5]. We now report alarm pheromones in three species of myrmecophilous treehoppers (Membracidae), two of which brood the egg mass (Fig. 1*a*) and all of which form feeding aggregates on their host plants (Fig. 1*b*). The release of these pheromones is unique in that specialised secretory structures are not involved.

Aggregations of *Vanduzeea arquata* (Say) on *Robinia pseudoacacia* L., and *Entylia bactriana* (Germar), and *Publilia concava* (Say) on *Ambrosia trifida* were studied in the field and laboratory. Whole treehoppers were crushed on 8-mm diameter filter paper disks which were held by fine forceps 1 cm from the centre of treehopper aggregations. A positive response was recorded when treehoppers moved at least 1 cm from their feeding sites on leaves within 1 min of presentation of the disk. In intra- and interspecific tests, at least five individuals were included in each trial and each combination was tested a minimum of five times. As a control, all test insects were exposed to untreated paper disks before testing with extracts. In field tests, responding individuals first raised their abdomens and expelled a droplet of honeydew from the anus before walking from their feeding sites, often moving to the opposite side of the leaf. Both nymphs and adults of all three species possessed repellent substances and each of the three species responded to compounds produced by the other two species (Table 1). Nymphs were more responsive to their conspecific pheromone than adults. Nearly all responding treehoppers reacted to treated disks within 20 s of initial exposure. No treehoppers responded to the untreated paper disks.

In further tests, filter paper disks treated with three to five drops of honeydew or haemolymph (collected by tibial excision) elicited no response from 63 treehoppers in eight aggregations. Only disks treated with a greenish milky fluid obtained by puncture of the body wall elicited a positive response from 42 of 63 test insects. We found no evidence of an external gland involved in the release of repellent substances. Treehoppers do not respond to cornicle droplets produced by several species of Aphidinae[6] or to synthetic aphid alarm pheromone, *trans*–*β*–farnesene, nor do aphids respond to the repellent substances from treehoppers.

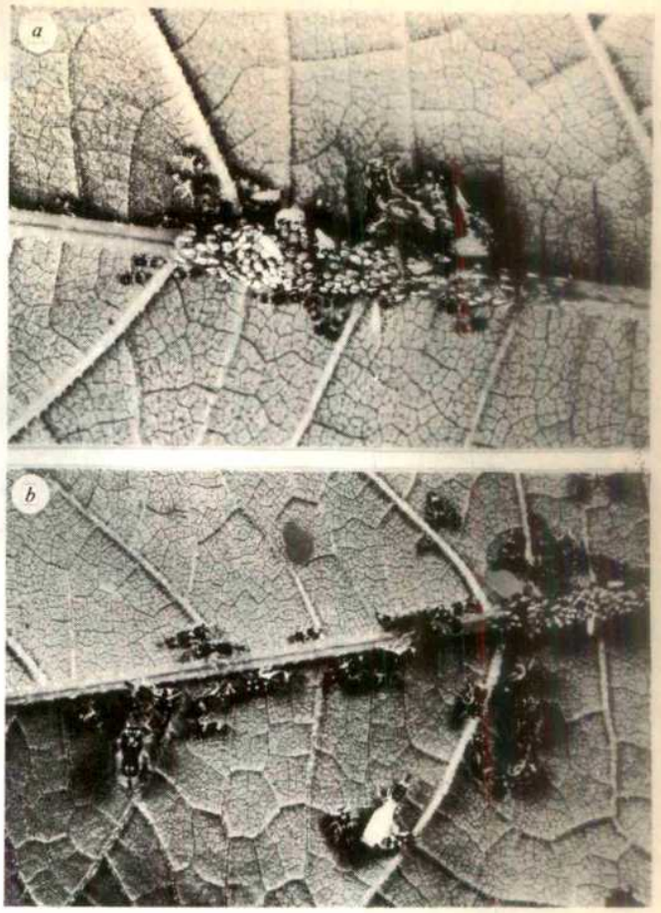


Fig. 1 Adult female *Entylia bacteriana* with her egg mass and recently hatched nymphs, (*a*). Aggregation of middle instar *Publilia concava* near egg mass (*b*).

Tests designed to determine if coccinellid predators would elicit the alarm response in attacked treehopper aggregations proved frustrating. Both larval and adult beetles usually avoided treehoppers, even when predators were starved 24 h before testing. In only two instances did larval coccinellids attack early instar treehoppers. After puncture of the body wall by the predator's mouth parts, alarm reactions were noted among other treehoppers which then relocated on the opposite side of the leaf.

Our results demonstrate that treehoppers produce alarm pheromone(s) which repel other members of the species. Treehoppers apparently cannot release the pheromone unless the body wall is punctured, such as by an attacking predator. Release of alarm substances in this manner has been described before for sea urchins[7] and certain species of schooling fishes[8,9]. It is not certain whether these compounds are specially elaborated pheromones or chemicals which also

Table 1 Interspecific and intraspecific response of treehoppers to alarm pheromones

Species tested	Source of pheromone* *Vanduzee* Adult	*Vanduzee* Nymph	*Entylia* Adult	*Entylia* Nymph	*Publilia* Adult	*Publilia* Nymph
Vanduzeea arquata						
Adult	62.6	53.0	N	N	N	N
Nymph	66.6	65.6	50.0	72.3	51.4	64.1
Entylia bactriana						
Adult	N	N	39.0	24.3	N	N
Nymph	42.8	31.4	45.5	72.9†	27.8	51.5
Publilia concava						
Adult	N	N	N	N	19.4	12.7
Nymph	18.7	60.4	14.6	22.0	16.6	45.9†

* Percentage of individuals responding to pheromone from single treehoppers. From 35–110 individuals in five or more replicates were tested for each combination.

† Significantly more nymphs than adults ($P < 0.05$) responded to pheromone from the species.

N, Not tested.

serve other functions in these organisms. This is the first report of an alarm pheromone in an insect which requires the rupture of the body wall for its release.

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1 Blum, M. S., *A. Rev. Entomol.*, **14**, 57 (1969).
2 Calam, D. H., and Youdeowei, A., *Insect Physiol.*, **14**, 1147 (1968).
3 Kislow, C. J., and Edwards, L. J., *Nature*, **235**, 108 (1972).
4 Nault, L. R., Edwards, L. J., and Styer, W. E., *Environ. Ent.*, **2**, 101 (1973).
5 Goff, A. M., and Nault, L. R., *Environ. Ent.* (in the press).
6 Bowers, W. S., Nault, L. R., Webb, R. E., and Dutky, S. R. *Science*, **177** 1121 (1972).
7 Snyder, N., and Snyder, H., *Science*, **168**, 276 (1970).
8 von Frisch, K., *Z. Physiol.*, **29**, 46 (1941).
9 Thines, G., and Vandenbussche, E., *Anim. Behav.*, **14**, 296 (1966).

New germination inhibitor from *Aegilops ovata* L.

IT has been previously reported[1] that the dispersal units of *Aegilops ovata* L. contain a germination inhibitor. Leachates of the hulls inhibited the germination of *Lactuca* achenes. This inhibition was considerably stronger in light than in darkness, an unusual feature, since all germination inhibitors already described inhibit the germination of *Lactuca* achenes in darkness more than in light. We therefore felt it essential to isolate and characterise this specific inhibitor present in *Aegilops ovata*.

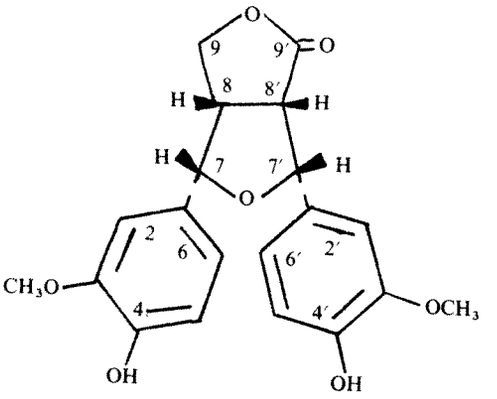
Preliminary investigations yielded a number of compounds namely vanillin, *p*-hydroxybenzaldehyde, ferulic, vanillic, *p*-hydroxybenzoic, *p*-coumaric and syringic acids. The inhibitory activity of these compounds did not, however, account for the initial inhibition observed with the crude extract. Repeated careful chromatography ultimately produced a pure fraction which showed the expected physiological activity. The compound in this fraction, obtained in pure form, was assigned structure (I) on the basis of its NMR, infrared and mass spectra. Structure (I) does not belong to any of the known groups of lignans[2], and is a monoepoxylignanolide (MEL), since the molecule has an epoxide as well as the five-membered ring lactone.

Table 1 Effect of mono-epoxylignanolide (MEL) on the germination of *Lactuca sativa* cv. "Great Lakes" achenes in light and in darkness at 25° C

Amount of MEL per filter paper (mg)	Light condition	Percentage germination* 24 h	48 h	72 h
1	L	0.5±0.5	10±3.7	20±4.2
	D	91.5±1.7	–	–
0	L	95 ±2.4	95.5±2.1	–
	D	95 ±0.6	95 ±0.6	–

L, In light; D, in darkness; *± standard error (4× 50 seeds)

The NMR spectrum of compound I shows the following signal pattern: 2,2′,5,5′,6, and 6′ H at δ6.80 (m, 6×H), 7 and 7′ H at 5.24 (d, 2×H, J=3.0 Hz, 8′ H at 3.40 (dd, J=9.0 and 3.0 Hz), 8 H at 3.18 (m), 9 H at δ4.1 (dq J_{ab}=10.0 Hz, J_{ax}=6.0 Hz and J_{bx}=4.0 Hz), C(3) and C(3′) —OCH_3 groups at δ3.78 (s, 6×H). The two phenolic –OH protons



Compound I

at C(4) and C(4′) are at δ5.80 disappearing on exchange with D_2O. The fragmentation pattern in the mass spectrum contributed to the elucidation of the structure. The loss of fragments m/e 84 and 85 from the molecular ion peak (M^+ 372) correspond to the five-membered ring lactone. In addition the fragments m/e 151 and 137 (base peaks) arise from the loss of $\dot{O} \equiv C.C_6H_4(OCH_3)$ (OH) and $H_2\dot{C}.C_6H_3(OCH_3)$ (OH) respectively. The presence of a five-membered ring lactone in compound I was further substantiated by its infrared spectrum, ν_{max} 1,770 cm^{-1} characteristic of such a system[3].

For the biological assay Whatman No. 1. (diameter 52 mm) filter papers were impregnated with 1 mg quantities of the pure substance. After careful evaporation of the solvent each filter paper was placed in a Petri dish covered with an untreated filter paper and distilled water (2 ml) added. After equilibration over 3 h, 50 achenes of *Lactuca sativa* cv. "Great Lakes" were introduced and the Petri dish kept in a germinator at 25° C either in continuous white light or in darkness. Each test (four replicates) was monitored by a blank (four replicates) in which the lower filter paper was untreated.

The results (Table 1) clearly indicate that the MEL has a unique activity in that it inhibits germination in white light (fluorescent and incandescent) while it is inactive in darkness. This is the first instance to be reported where a compound has such a property. The inhibitory activity of MEL in white light, however, diminishes with time.

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1 Datta, S. C., Evenari, M., and Gutterman, Y., *Israel J. Bot.*, **19** 463–483 (1970).
2 Freudenberg, K., and Weinges, K., *Tetrahedron*, **15**, 115 (1961)
3 Nakanishi, K., in *Infrared Absorption Spectroscopy*, (Holden-Day, New York, 1964).

Dry deposition of sulphur dioxide on wheat

ABOUT 6 million tonnes of sulphur dioxide (SO_2) are emitted annually into the atmosphere over Britain. Atmospheric concentrations and the quantity of SO_2 exported are determined by the rates of emission, dispersal and removal. Removal as sulphate by rainfall over Britain accounts for about 15% only of annual emission[1]. As long ago as 1950 Meetham[2] suggested that turbulent transfer was an important process for removing SO_2 molecules at the Earth's surface, a process usually referred to as 'dry deposition' even when the surface is wet. Measurements of the dry deposition rates on short grass and water have not been made until recently[3,4]. From limited evidence for short grass Garland *et al.*[1] suggested that dry deposition over Britain may remove 40% of the SO_2 emitted annually. The surface factors which may control dry deposition rates have not yet been established, however, nor have dry deposition rates on field crops been measured, although it has been suggested that the sulphur supplied in this way may avoid deficiences on some British soils[5]. Here we report the results of an extensive series of measurements of dry deposition rates on a field of winter wheat and point to factors which may limit the deposition rate.

Table 1 Characteristic measurements of the resistances to deposition of sulphur dioxide on wheat. The SO_2 concentration χ and deposition velocity v_g are referred to $z = 1$ m.

Date	Time (GMT)	v_g mm s^{-1}	χ μg m^{-3}	$r_a + r_c$ s mm^{-1}	r_c s mm^{-1}	Wet (W) or dry (D)
10/6/1973	10.40	1.5	81	0.65	0.61	D
24/6/1973	11.00	5.4	21	0.18	0.15	D
5/7/1973	18.00	2.0	11	0.49	0.30	D
18/7/1973	19.06	6.4	22	0.16	0	W
24/7/1973	21.00	4.5	26	0.30	0	W
25/7/1973	00.50	5.8	35	0.17	0.06	W
29/7/1973	10.05	1.1	77	0.90	0.85	D
29/7/1973	17.10	3.7	72	0.27	0.23	D
30/7/1973	02.00	7.6	13	0.13	0.04	W
30/7/1973	09.00	4.1	83	0.24	0.20	D
11/8/1973	19.00	4.1	22	0.25	0.10	W
12/8/1973	04.00	4.1	35	0.24	0.05	W

Because the surface is a sink for SO_2 the volume concentration χ decreases towards the surface and the flux F of SO_2 may be determined from measurements of the variation of concentration with height using the standard micrometeorological equation

$$F = K(z) \frac{\partial \chi}{\partial z}$$

where $K(z)$ is the eddy diffusivity for SO_2 at height z, assumed equal to that for momentum. At Sutton Bonington, 10 miles south of Nottingham, values of χ were measured at five heights up to 2 m over a uniform 8 ha field of winter wheat (var Maris Ranger) using the techniques described by Garland *et al.*[1]. Eddy diffusivities corrected where necessary for the effects of atmospheric stability[6] were determined from analysis of wind speed and temperature profiles in the lowest 2 m of the atmosphere.

Deposition rates are often characterised by the velocity of deposition v_g defined by $v_g(z) = \frac{F}{\chi(z)}$ as this quantity is independent of concentration, but to investigate the controlling factors in the deposition process it is convenient to consider the resistance to deposition, $r = \frac{1}{v_g}$ as the sum of an aerodynamic resistance r_a and a surface resistance r_c. The surface resistance is a measure of the affinity of the surface for SO_2 uptake, that is $r_c = 0$ for a perfect sink. Values of r_a were calculated from wind and temperature profiles making allowance for the different processes responsible for mass and momentum transfer at the surface of foliage[7]. The accuracy of the values of v_g depends mainly on the gradient of SO_2 concentration. The chemical analysis was accurate to about ± 0.5 μg^{-3} SO_2 and the uncertainty in v_g ranged from about $\pm 5\%$ to $\pm 50\%$.

Measurements were made over 1 or 2 h intervals during the period from June 8 until August 12, 1973, which may be arbitrarily divided. Before the last week in July most of the leaves were green and the crop was growing. By the first week in August, the crop was senescent and it was harvested on August 13.

Table 1 shows characteristic results of measurements before and after senescence. The large proportion of the total deposition resistance $r_a + r_c$ contributed by the surface resistance r_c clearly indicates that surface processes limit dry deposition rates in most conditions. When the crop was fully senescent, two regimes were distinguished. When the leaves were dry the SO_2 concentration gradient was generally below the limit of detection, that is $\delta\chi/\delta z < 0.5$ μg^{-3} m^{-1}. This implies that v_g was very small (< 0.8 mm s^{-1}) because the surface resistance was large (> 1.2 s mm^{-1}). There were eight occasions when this occurred but none before August 3. In the late evening the leaves were frequently wetted by dew and the values of r_c decreased substantially. The effect of dew was also observed before senescence, indicating that for wet crops, physiological control of deposition was unimportant. On several occasions when measurements continued sequentially over 24 h the variations in r_c and v_g reveal some interesting features.

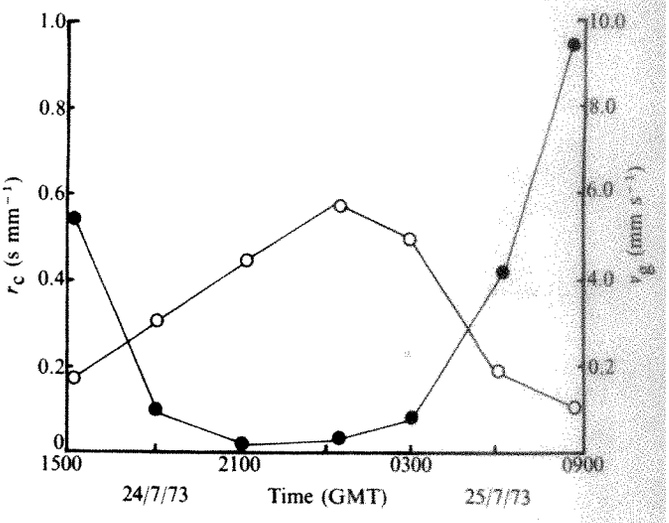


Fig. 1 Variation with time of the deposition velocity v_g (○) and surface resistance r_c (●) for sulphur dioxide on wheat.

Figure 1 shows the diurnal variation of r_c and v_g on July 24. At 1600 h GMT, values of r_c were representative for a dry crop. By 1800 h, dew could be detected on the flag leaves of the crop and the value of r_c was decreasing. As dewfall continued r_c decreased until midnight but then began to increase although dew was still present. The changes in r_c may be explained by a decrease in *p*H of the dew which decreases the solubility of SO_2 (ref. 8). A similar variation of r_c with time in the presence of dew has been observed in other 24 h experiments. On a night with strong winds and cloud, and consequently no dew, however, r_c remained fairly constant with time.

The mean v_g from 12 occasions when the crop was wet was 4.0 ± 1.4 mm s^{-1} and the corresponding mean for r_c was 0.14 ± 0.12 s mm^{-1}. For 16 occasions when the crop was dry but physiologically active the mean values of v_g and r_c were 3.3 ± 2.0 mm s^{-1} and 0.40 ± 0.26 s mm^{-1} respectively. The values of v_g and r_c do not differ significantly, but the former is considerably below the value of 8.5 mm s^{-1} for the mean deposition velocity to short grass[1]. By extrapolating a mean value of v_g, Garland *et al.*[1] calculated that the annual dry deposition of SO_2 over Britain was 110 kg ha^{-1}, assuming a mean concentration of 40 μg^{-3}. If our mean value for v_g (3.6 mm s^{-1}) is more representative of arable crops then with the same assumptions it implies an annual SO_2 deposition of about 47 kg ha^{-1}.

Our results indicate that surface wetness has a major influence on dry deposition rates and that the surface resistance is the controlling step in deposition to vegetated surfaces. A further implication is that in winter, when surfaces are wet for long periods, dry deposition rates of SO_2 may be considerably enhanced.

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1 Garland, J. A., Clough, W. S., and Fowler, D., *Nature*, **242**, 256 (1973).
2 Meetham, A. R., *Quart. J. R. met. Soc.*, **76**, 359 (1950).
3 Shepherd, J. G., *Atmos. Environ.*, **8**, 69–74 (1974).
4 Garland, J. A., Atkins, D. H. F., Readings, C. J., and Caughey, C. J., *Atmos. Environ.*, **8**, 75–9 (1974).
5 Cowling, D. W., and Jones, L. H. P., *Soil Sci.*, **110**, 346 (1970).
6 Pruitt, W. O., Morgan, D. L., and Lourence, F. J., *Quart. J. R. met. Soc.*, **99**, 370 (1973).
7 Thom, A. S., *Quart. J R. met. Soc.*, **98**, 124 (1972).
8 Junge, C., and Ryan, T. G., *Quart. J. R. met. Soc.*, **84**, 46 (1958).

Two spot ladybirds as indicators of intense local air pollution

Most individuals of the two spot ladybird, *Adalia bipunctata*, may be designated as red with black spots, or as black with red spots. Although intermediate forms are found, they usually constitute less than 1% of the population in Britain. The most commonly occurring black forms are under the control of a single gene and are genetically dominant over the red forms[1]. In parts of continental Europe[2] and in Britain[3,4], the frequency of black is highest in industrial and other areas with much atmospheric pollution; up to 97% melanics are found in Liverpool and Glasgow. Multiple regression analysis suggests that there is an association of high melanic frequency with smoke, but not with sulphur dioxide[5], this conclusion is supported by results from Birmingham, where melanic frequencies fell soon after the introduction of extensive smoke control areas, which had only a relatively small effect on sulphur dioxide levels[6].

Samples previously reported from South Wales[3] indicate a consistently low melanic frequency, the maximum being 6% in a sample of 35 individuals from Pontypridd. This is in accord with the occurrence of generally low levels of smoke pollution, in spite of considerable industrialisation[7].

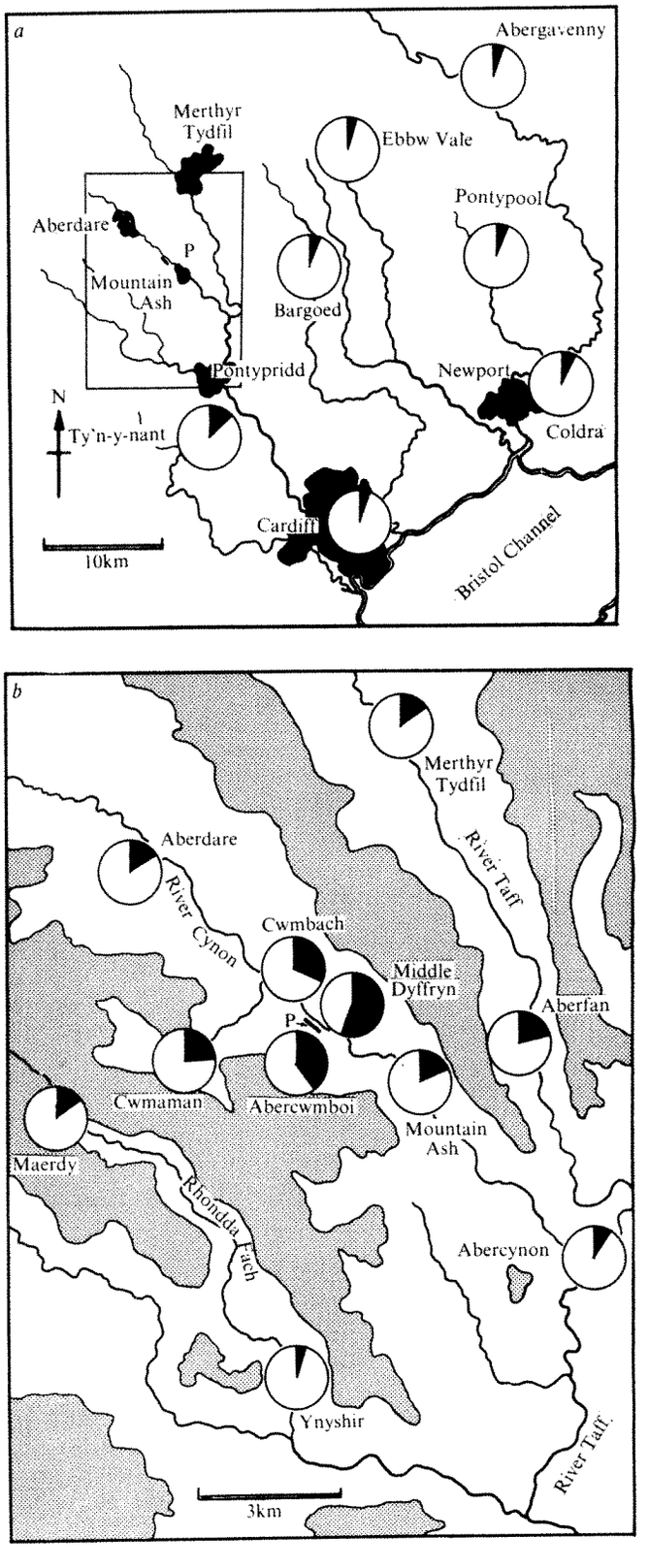


Fig. 1*a* Map of the eastern valleys of South Wales showing the position of the Phurnacite plant (P), the melanic frequencies at the more distant sampling sites and the area shown in detail in *b*. The relative size of the black segments is proportional to melanic frequency. *b*, Map of the Cynon Valley showing melanic frequencies. For clarity, the Aberewmboi and Middle Dyffryn sites are shown at greater than their true distance (0.3 km) from P ⊙ Ground over 1,000 feet above sea level is stippled.

Table 1 Details of samples of the two spot ladybird, *Adalia bi-punctata*, collected in south east Wales in June 1973

	Distance from phurnacite plant (km)	Numbers			%
		Red	Black	Total	Black
Cynon Valley					
Aberdare	4.9	42	7	49	14.3
Cwmaman	2.8	188	60	248	24.2
Cwmbach	1.2	42	20	62	32.3
Abercwmboi	0.3	110	78	188	41.5
Middle Dyffryn	0.3	44	51	95	53.7
Mountain Ash	2.6	22	5	27	18.5
Abercynon	7.6	100	9	109	8.3
Rhondda Fach					
Maerdy	5.5	72	12	84	14.3
Ynyshir	7.3	130	6	136	4.4
Taff Valley					
Merthyr Tydfil	6.4	62	11	73	15.1
Aberfan	4.4	93	26	119	21.8
Cardiff	25.9	29	1	30	3.3
Elsewhere					
Ty'n-y-nant	15.1	100	12	112	10.7
Bargoed	12.4	60	3	63	4.8
Ebbw Vale	17.0	69	2	71	2.8
Abergavenny	30.5	49	2	51	3.9
Pontypool	27.4	75	4	79	5.1
Coldra (Newport)	35.2	61	4	65	6.2

A sample of 95 *Adalia bipunctata* was obtained from the Cynon valley in June 1973, however, of which 53.7% were black. The ladybirds were found on sallow (*Salix* spp.) and stinging nettle (*Urtica dioica*) over an area of about 75 m × 20 m. The site is in the ruins of the Middle Dyffryn colliery and is 300 m to the east of the ovens of the National Coal Products Phurnacite plant at Abercwmboi, which produces smokeless fuel from coal; it is on the edge of the wood in which the effects of air pollution on the leaves of oak trees were investigated[8].

To discover how extensive was the area of relatively high melanic frequency, samples were collected from other sites in this part of South Wales (Fig. 1); the results are summarised in Table 1. The sample from the village of Abercwmboi, with 41.5% melanics, was obtained roughly the same distance from the ovens as the first sample, but to the west of them. The difference in frequency between the two samples does not quite reach formal significance; $\chi_1^2 = 3.78$, $P = 0.052$. The melanic frequency falls steadily with increasing distance from the Phurnacite plant (Fig. 2), and there is no indication that other areas with locally high frequencies occur in South Wales. The site at Ebbw Vale (2.8% black) is 200 m from large steel and tinplate works, and that at Ty'n-y-nant (10.7% black) is 500 m from coke ovens and chemical works which are, however, much smaller than the Phurnacite plant, but may be responsible for the apparent small increase in melanic frequency.

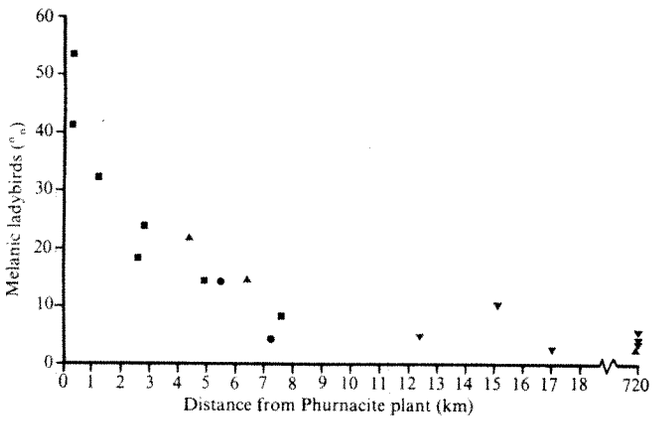


Fig. 2 The relationship between melanic frequency in samples of *Adalia bipunctata* and the distance of the collecting site from the Phurnacite plant at Abercwmboi. ■, Sites in the Cynon valley; ●, sites in the Rhondda Fach; ▲, sites in the Taff valley; ▼, other sites in south east Wales.

There is extensive damage to the trees near to the Phurnacite plant, but this is largely confined to those on the east side, the prevailing winds being westerly. Williams *et al.*[8] concluded however that, although sulphur dioxide was having a marked effect on the trees, high sulphur levels in the leaves resulted from increased epidermal permeability caused by fine particles in the pores of the stomata, rather than from locally high levels of atmospheric sulphur dioxide. Both sulphur dioxide and smoke have lower mean annual levels at a recording point 1.3 km to the ESE of the Phurnacite plant (44 $\mu g\ m^{-3}$ and 30 $\mu g\ m^{-3}$ respectively) than in the urban areas of Mountain Ash (49 $\mu g\ m^{-3}$ and 55 $\mu g\ m^{-3}$) 2.1 km to the south-east, and Aberdare (60 $\mu g\ m^{-3}$ and 41 $\mu g\ m^{-3}$) 4 km to the north-west; but the fallout of particulate matter or grit is very much higher immediately to the east of the Phurnacite plant than elsewhere. In 1967 (the most recent year for which almost complete records are available) total solids at the Middle Dyffryn site were 2.16 $g\ m^{-2}\ d^{-1}$, compared with 0.21 $g\ m^{-2}\ d^{-1}$ at Abercwmboi (from data supplied by Dr J. Ll. Williams). This high level of particulate matter renders it impossible to use the biological method of smoke and sulphur dioxide assessment[9] to provide a more detailed comparison of melanic frequencies and air pollution, for I had taken care to avoid proximity to sources of intense pollution. Although I found the reflectance of tree trunks to be the most important single indicator of sulphur dioxide levels over southern Britain, reflectance near the Phurnacite plant is largely influenced by the amount of particulate matter deposited on the surface. The other biological parameters used suggest that both mean smoke and sulphur dioxide levels are lower at the Middle Dyffryn site than in Mountain Ash.

If air pollution from the Purnacite plant has a direct selective effect on the melanic frequencies, then it seems to be an effect of gases, other than sulphur dioxide, or of the particulate matter comprising grit and smoke. At Aberfan, a melanic frequency of over 20% is found 4.3 km from Middle Dyffryn, and separated from it by high ground on which the insect does not occur; furthermore, the decline in melanic frequency with increasing distance from the Phurnacite plant seems to be the same in samples from the Rhondda Fach and Taff valleys as it is in the Cynon valley (Fig. 2). This suggests that the frequencies are influenced not only by outward gene flow, but also by the Phurnacite plant possibly having an appreciable effect over such distances.

I thank Richard Steward and Dr D. R. Lees for their help with various aspects of this work, and Dr J. Ll. Williams, Medical Officer of Health for Mountain Ash and Aberdare, for the data on pollution levels. The work was supported by a grant from the Nuffield Foundation.

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1 Lus, J. J. *Izv. Byuro Genet.*, **6**, 89–163 (1928).
2 Lusis, J. J., *Latv. Ent.*, **4**, 3–29 (1961).
3 Creed, E. R., *Heredity*, **21**, 57–72 (1966).
4 Creed, E. R., in *Ecological Genetics and Evolution* (edit. by Creed, E. R.) 134–151 (Blackwell, Oxford and Edinburgh, 1971).
5 Lees, D. R., Creed, E. R., and Duckett, J. G., *Heredity*, **30**, 227–232 (1973).

[6] Creed, E. R., *Evolution, Lancaster, Pa.*, **25**, 290–293 (1971).
[7] Warren Spring Laboratory, *National Survey of Air Pollution* 1961–71, **2** (HMSO, London, 1972).
[8] Williams, R. J. H., Lloyd, M. M., and Ricks, G. R., *Environ. Pollut.*, **2**, 57–68 (1971).
[9] Creed, E. R., Lees, D. R., and Duckett, J. G., *Nature*, **244**, 278–280 (1973).

Deltatheroides–like mammals from the Upper Cretaceous of North America

THE family Deltatheridiidae[1] has been redifined to include primitive therian mammals of tribosphenic grade referrable to only two genera *Deltatheridium*[1] and *Deltatheroides*[2], from the Djadokhta and Barun Goyot Formations, of uncertain age within the Upper Cretaceous of Mongolia[2]. The Deltatheridiidae, long considered Eutheria, although of disputed taxonomic dimensions and phyletic position within the infraclass[1–5], seem now best classified as therians of metatherian–eutherian grade, but referrable to neither Metatheria nor Eutheria[2]. A combination of apparent marsupial-like and placental-like features in the known parts of the dentition and skull of members of the Deltatheridiidae insures that the family will be of continuing interest.

I here record the first recognition of the Deltatheridiidae *sensu* Butler and Kielan-Jaworowska[2] from territories outside of Asia, an occurrence documented by fossils collected at Upper Cretaceous horizons in the Western Interior of North America. The new deltatheridiids are known only from isolated teeth and tooth fragments, which have been found but rarely in the large samples of North American late Cretaceous mammals in collections. Nevertheless, the detailed resemblances of these fossils to molars of the Asiatic Deltatheridiidae, especially to those of *Deltatheroides cretacicus*[1], make reliable the identifications recorded here. The fossils are: (1) University of Alberta (UA) no. 4085b (see Fig. 1*a*), a single upper molariform tooth from the late Maestrichtian (Lancian)[6,7] Upper Edmonton Formation, Alberta, and first described[8] as a possible deciduous P^4 of the palaeoryctid *Cimolestes magnus*[9]; (2) University of California (Berkeley) Museum of Paleontology no. 46359, a lower molar (see Fig. 1*b*), and American Museum of Natural History no. 59482, a lower molar trigonid, both from a previously unidentified species occurring in the late Maestrichtian Lance Formation, Wyoming[10]; and (3) UA 4248, a previously unidentified lower molar talonid, from the late Campanian (Judithian)[6,7] Oldman Formation, Alberta. The Lance and Oldman specimens are virtually identical in coronal architecture, other than slightly larger size, to M_2 or M_3 of *Deltatheroides cretacicus* (this species is being redescribed elsewhere by Z. Kielan-Jaworowska). Occlusal relations and comparison with upper molars of the Asiatic deltatheridiids suggest that the tooth from the Upper Edmonton Formation is probably an M^1 from the same or closely related species as are the lower molar specimens.

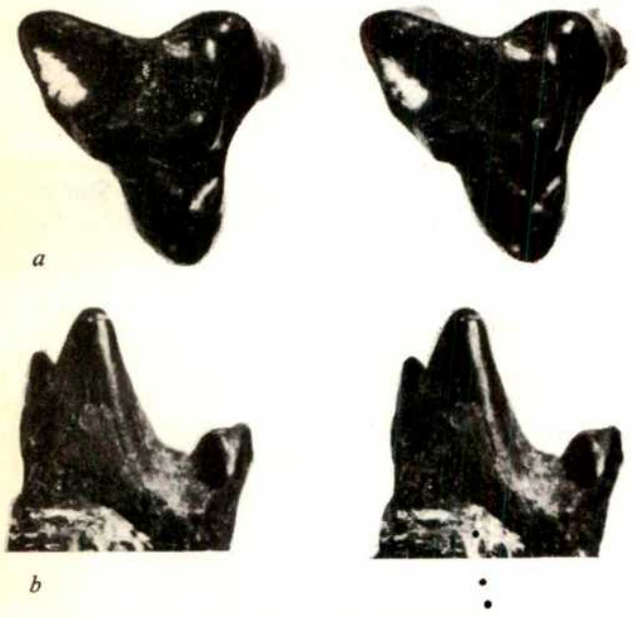


Fig. 1 Stereophoto pairs of isolated molars of cf. *Deltatheroides sp.*, Upper Cretaceous, North America. *a*, UA 4085b, M^1 (?), Upper Edmonton Formation, Alberta, Canada; *b*, UCMP 46359, lower molar, Lance Formation, Wyoming (×about 7.6).

The close morphological resemblances between the North American and the Asian fossils generate inferences that: (1) the known Asian and North American deltatheridiids diverged relatively recently before their oldest discovered occurrences in the Djadokhta and Oldman Formations; and, as a corollary, (2) migration of the descendant deltatheridiid species from Asia to North America or *vice versa* (and the known evidence does not now permit a choice) took place soon after this divergence. Although these inferences concerning the near contemporaneity of earliest appearances of Asiatic and North American deltatheridiids must remain tentative—at least until more extensive sampling of the local mammalian faunas of North America and East Asia can be completed—they are nevertheless in accord with other evidence that had earlier and independently indicated the improbability of a relatively great Cretaceous age of the Djadokhta mammals[11]: relict triconodonts and symmetrodonts survived in North America to at least as late as the time of deposition of the early Campanian (Aquilan)[6,7] Upper Milk River Formation (still before the time of first appearance of North American deltatheridiids), but fossils of neither triconodonts nor symmetrodonts have been found in the deltatheridiid-bearing Djadokhta Formation or in younger Upper Cretaceous rocks in East Asia.

W. Clemens, A. Crompton, Z. Kielan-Jaworowska, M. McKenna and B. Slaughter permitted study of specimens in their care; A. Lindoe photographed the specimens; and D. Krause critically read the manuscript. This work was supported by the National Research Council of Canada and the University of Alberta.

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[1] Gregory, W., and Simpson, G., *Am. Mus. Novit.*, **225**, 1–20 (1926).
[2] Butler, P., and Kielan-Jaworowska, Z., *Nature*, **245**, 105–106 (1973).
[3] Patterson, B., *Fieldiana, Geol.*, **13**, 1–105 (1956).
[4] Van Valen, L., *Bull. Am. Mus. nat. Hist.*, **132**, 1–126 (1966).
[5] McKenna, M., Mellett, J., and Szalay, F., *J. Paleont.*, **45**, 441–442 (1971).
[6] Russell, L., *Contr. Life Sci. Div. R. Ont. Mus.*, **61**, 1–24 (1964).
[7] Clemens, W., Fox, R., Sloan, R., in *Abst. 1973 Ann. Mtg geol. Soc. Amer.*, **5**, 577 (1972).
[8] Lillegraven, J., *Paleont. Contr. Univ. Kansas*, **50**, 1–122 (1969).
[9] Clemens, W., and Russell, L., in *Vertebrate Paleontology in Alberta*, 32–40 (Univ. Alberta, Edmonton, 1965).
[10] Clemens, W., *Univ. Calif. Publs geol. Sci.*, **94**, 1–102 (1973).
[11] Fox R., *Nature*, **239**, 170–171 (1972).

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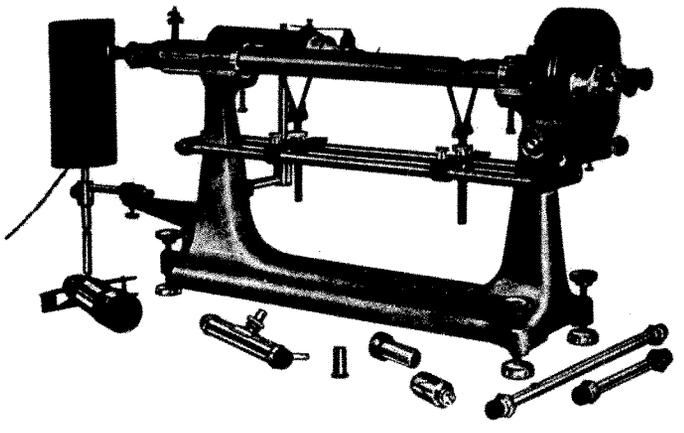
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book reviews

Prehistoric snails

Land Snails in Archaeology, with Special Reference to the British Isles. By John G. Evans. Ppxii+436. (Seminar: New York and London, January 1973.) £7.90.

ARCHAEOLOGISTS are becoming increasingly interested in the reconstruction of the environment and ecology of the ancient sites they excavate, and one of the sources of evidence available to them is the careful study of the shells of ancient land snails. These are particularly important in the limestone and chalk regions of Britain where pollen grains do not survive to give direct evidence of the local flora.

This book sets out to give an account of the growth of interest in the study of land snails by archaeologists during the past century, the methods of identification and the results that can be achieved by careful sampling and interpretation.

The possibility of using snail shells as environmental indicators was first suggested by General Pitt-Rivers when he found their remains in the ditch of the iron age hill fort at Cissbury, Sussex, in 1869. Most of the early work on snails in ancient contexts in this country was carried out by A. S. Kennard and B. B. Woodward. Recent developments largely brought about by the author of this book, and recorded in detail in it, include the much more accurate sampling procedures, the interpretation of the environment from the standpoint of both moisture and temperature. "The techniques of snail shell analysis is now refined to the extent that we are able to recognise and differentiate between the effects of climatic, local environmental and anthropogenic influences on ancient snail populations."

Now it is essential that further study be made of the geographical ranges of present day snail populations in this country. Also urgently needed are quantitative data on modern snail populations from habitats whose physical, chemical and biological aspects are fully described. The reconstruction of the past environment depends on establishing the snail fauna of the locality: snail faunas in which 90% of the snails are of one species reflect a severe habitat—salt marsh, ploughed field or sand dune. A wide range of species may be found in well established woodland—upwards of fifty species is not uncommon, with none of them accounting for more than 15% of the total fauna.

The study of snail faunas ultimately rests on the identification of the remains of the snail shells surviving from the past. The author provides a number of pages of outline drawings of the 200 species involved, including illustrations of immature forms, which should be a splendid guide for those undertaking this identification work. It might have also been helpful to supplement this with photographs both of complete shells, and the most common ways in which they survive as fragments. The species are described in detail, with the environment, distribution and archaeological finds all listed for each species.

Chapter six deals with the ecological groups into which snail faunas may be classified, and may be one of the most interesting sections of the book for those concerned with reconstructing past ecological conditions. They are divided up into shade-loving species, (including the Zonitidae, *Carychium tridentum* and *Discus rotundus* and other woodland species); intermediate or catholic species; open country species (including *Vertigo pygmaea, Pupilla muscorum, Vallonia costata, V. excentrica* and *Helicella itala*); marsh and freshwater slum species. Besides these, four other groups which are not entirely ecological can be distinguished: alien species (introduced into Britain by man at various times since the Bronze Age), burrowing species (*Pomatias elegans* and *Cecilioides acicula* in particular), anthropohobic species, confined to wild habitats in remote woodland (including *Lauria angelica, Ena montana, Helicodonta obvoluta* and *Limax tenellus*); and synanthropic species which live in man made habitats such as gardens, hedgerows and refuse heaps (for example, *Hygromia striolata, Helix aspersa* and *Limax flavus*).

Some snail species are now extinct, which were relatively common in the Boreal period, and thus may be useful for giving some clue for dating the deposits in which they occur, in particular *Vertigo genesii* and *Discus ruderatus*.

The conclusions from work so far are that there was an all-pervading forest cover in Mesolithic and Neolithic times, and that forest clearance at a number of Neolithic sites was followed by a period, immediately before the burial of the soil profile, of short turfed grassland. So far snails have not indicated cultivation, except at South Street, Avebury where the ground was ploughed in Beaker times, possibly to create better grazing land. Regeneration of the forests seems to have been prevented for many centuries, probably by grazing, and was never very successful. In the latter half of the first millenium BC the Iron Age farmers began to bring the chalk and limestone soils into more intensive cultivation.

This book marks the beginning of a new phase in the study of ancient land snails, where carefully controlled sampling and analysis can provide the data for the reconstruction of snail faunas, and thus of ancient environments. There are some problems to be overcome, notably the lack of adequate information about some of the modern snail populations, and the fact that some snail species seem to undergo changes in habitat preference (for example *Vallonia costata* and *Hellicella itala* which occurred abundantly in prehistoric arable habitats, but are absent from cultivated soils today). It is also possible that some habitats known in the past are now quite extinct.

Land Snails in Archaeology should be on the shelf of every practical archaeologist, and of all those interested in reconstructing the past environments of ancient sites.

JANE M. RENFREW

Mermaid morphology

Morphology of the Sirenia: A Macroscopic and X-ray Atlas of the Osteology of Recent Species. By Hans E. Kaiser. Pp. viii+76. (Karger: Basel, London and New York, 1974.) Sfr.94; £13.65; $29.15.

THE true function of this slim but expensive book of photographs is somewhat difficult to discern, though much effort has gone into its production. Here is an ordered series of original photographs, together with a number of positive X-ray photographs, designed to illustrate the macroscopic osteology of the three genera of modern Sirenia. Each photograph is accompanied by a detailed textual description of what is to be seen in that particular picture. But unfortunately a number of the photographs are lacking

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Ecological Stability
Edited by M. B. USHER and M. H. WILLIAMSON
May 1974: 208 pages: illustrated: hardback: £3.60
The unifying theme of this book is in the analytic investigations of populations, the quantification of population processes and the application of mathematical models to simulate and predict these phenomena. The chapters are based on, but are not identical to, twelve papers given at a 'workshop' meeting at York University in the summer of 1973. The papers fall into four inter-related groups: relations between predator and prey; relations between host and parasite; relations of species within a trophic level; and the effects of special heterogeneity on the stability of species.

CHEMISTRY TEXTBOOK SERIES
Pericyclic Reactions
G. B. GILL and M. R. WILLIS
April 1974: 248 pages: limp cover: £2.95
This book provides a comprehensive introduction to the main theories that have been advanced to explain the stereospecificity of pericyclic reactions. It has been written for use as a text for both undergraduate and postgraduate courses; there are worked examples, formal problems with solutions, and references to the review literature.

The Chemistry of the Non-Metals
P. POWELL and P. L. TIMMS
April 1974: 274 pages: illustrated: limp cover: £2.85
This undergraduate level textbook is concerned with the non-metallic elements and the compounds they form with one another. An attempt is made to correlate the chemistry of these compounds as a whole and emphasis is given to discussing common features of structure and bonding within the different classes of compound.

Further information on these titles and a list of stockists is available from the publishers on request.

in clarity, and others are marred by the excessive prominence of the wire ties holding the specimen together. Additionally there are a few line drawings of ossicles, vestigial pelvic bones and so on. But nowhere are scales or measurements indicated except in the initial photograph of the mounted skeletons of *Hydrodamalis*, *Dugong* and *Trichechus* where the overall lengths are stated.

Description is entirely dominant over discussion and comment. The author evidently has little interest in the extraordinary and changing tooth patterns in the dugong and manatee in the course of the life of the individual: pointers on certain pictures simply say "Dentes molares". The non-erupting tusks of the female dugong are nowhere referred to, nor the presence, form and function of the horny buccal pads which are so important in the Sirenia. The work is based on several specimens and not upon series, of skulls, for example.

G. C. L. BERTRAM

International eel

Der Aal: Biologie und Fischerei. By Friedrich-Wilhelm Tesch. Pp. 306. (Parey: Hamburg and Berlin, 1973.) DM 78.

THE physiology and migrations of eels raise fundamental and controversial research questions. This book will give a further impetus to work in this field, for it surveys the entire literature (about 900 references) dealing with the Atlantic as well as the Indopacific species of Anguillidae.

Two-thirds of the book are devoted to zoological aspects, with an especially thorough discussion of anatomy, variation, taxonomy, life history, feeding and migrations. The separation of two Atlantic species, the American, *Anguilla rostrata* and the European, *A. anguilla* is defended against the background of a recent controversy. Dr Tesch attributes their geographical separation to the time required for the transition from larva to elver. The European species requires for this three years, so that larvae brought prematurely near American shores will not be ready to respond by movement to freshwater. American larvae become elvers in one year and any transported eastwards will perish as they will not be near the coast at the appropriate ontogenetic stage.

The chapters on environmental influences on productivity of inland waters, growth, behaviour and even sex determination and sex ratios are particularly detailed. Certain current biochemical studies, however, are not discussed in sufficient detail (and Sick's haemoglobin studies were electrophoretic not chromatographic, page 41).

In addition to the main body of information, minor but fascinating issues are not forgotten: the methods and difficulties of determining age and sex of specimens, the poison in the uncooked eel blood, the survival of a specimen with very little haemoglobin, the swimming ability of the larva, and so on.

The last third of the book deals expertly with methods of fishing and other related issues. The increasing economic importance of the eel is clearly demonstrated. Characteristically, for our age, the collection of elvers for food and the impediments to elver immigration presented by technical works, are leading in Japan to scarcity of eels. This is met by fish farming, and there are over 1,200 eel farms in that country. The methods used in these are described.

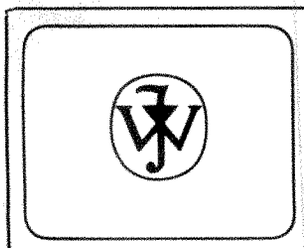
This is an excellent book, impeccably produced and with very helpful illustrations and tables. I hope that it will be translated into English.

E. M. PANTELOURIS

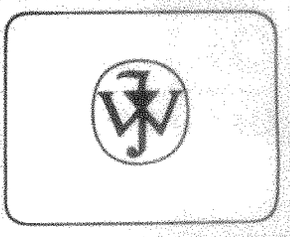
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Erratum

The price of *Computational Physics* by David Potter (reviewed in *Nature* **248**, 295; 1974) is £6.95 and not as stated, and there is no paperback edition.



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CLINICAL ONCOLOGY: A Quantitative Approach

By **N. M. Emanuel** and **L. S. Evseenko,** *Meditsina Publishing House, Moscow.*

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April 1974 280 pages £11.25

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A new approach to the chemistry and structure of micro-organisms. Describes micro-organisms in terms of their constitutional and dynamic molecular components and views them as dynamic assemblies of specific macromolecules that constantly interact among themselves and with the environment. Divided into two major parts: a general treatment of properties and function of microbial molecules; and a systematic description of molecular constitutions and interactions of the different microbial assemblies (bacteria, viruses, rickettsiae, fungi, etc.).

April 1974 494 pages £14.06

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By **Avram Goldstein, Lewis Aronow** and **Sumner M. Kalman,** *all of Stanford University School of Medicine.*

Explains principles that govern drug action, absorption, metabolism, distribution and toxicity. Discusses clinical use of drugs and evaluation of drug effects and safety. Uses appropriate drugs to illustrate principles. New in this edition: receptor isolation, the revolutionary advances in analytical chemistry applied to the detection and quantitation of drugs and drug metabolites, environmental toxicity, the immunochemical basis of drug allergies and drug-drug interactions in man.

May 1974 870 pages £11.40

INTENSIVE CARE

By **Ake Wahlin, Lars Westermark** and **Ansje van der Vliet,** *Central Anaesthesiological Dept, Karolinska Sjukhuset, Stockholm.*

Written with the non-specialist in mind, this book explains the routines in intensive care which have been developed in Scandinavia. Its object is to introduce and guide students in all aspects of medical care to the proper planning and running of an intensive care unit. Its practicability is assured since it is based largely on experience gained by the authors in the intensive care unit of the Central Anaesthesiological Department of the Karolinska Hospital, Stockholm, Sweden. This English edition is based on the third Swedish edition of the book.

April 1974 324 pages £6.75

THE MAMMALIAN MYOCARDIUM

Edited by **Glenn A. Langer,** and **Allan J. Brady,** *University of California, Los Angeles.*

A comprehensive analysis of mammalian cardiac function with particular focus on cellular and subcellular function. Synthesizes and summarizes information from a variety of related disciplines.

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The series "The Chemistry of the Functional Groups" is planned to cover in each volume all aspects of the chemistry of one of the important functional groups in organic chemistry, in this case the compounds containing quinonoid group. The emphasis is laid on the functional group treated and on the effects which it exerts on the chemical and physical properties, primarily in the immediate vicinity of the group in question, and secondly on the behaviour of the whole molecule. The authors concentrate on important developments and on material which has not been well covered in other literature. (Bound in two parts).

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By **N. S. Isaacs,** *University of Reading.*

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April 1974 564 pages £12.00

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by **Raymond C. Bartlett, M.D.,** *Hartford Hospital, Connecticut.*

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announcements

International meetings

June 2–6, **Radiation Protection: Philosophy and Implementation** (Symposium Organiser, Room 1324, CEGB, Sudbury House, 15, Newgate Street, London EC1A 7AU)

June 3–5, **A festive International Exposition for the Minicomputer Industry** (MINIFEST 74 Secretary, Polytechnic of Central London, 115, New Cavendish Street, London W1M 8JS)

June 3–5, **Applications of Marine Geodesy** (G. W. Lennon, Institute of Coastal Oceanography and Tides, Bidston Observatory, Birkenhead, Cheshire, L43 7RA, UK)

June 3–6, **25th Annual Meeting of the Tissue Culture Association** (Dr Vincent J. Cristofalo, Chairman, TCA Publicity Committee, The Wistar Institute, 36th Street at Spruce, Philadelphia, Pennsylvania 19174)

June 3–7, **International Symposium on N_2 Fixation** (Dr William E. Newton, Charles F. Kettering Research Laboratory, 150, East South College Street, Yellow Springs, Ohio 45387)

June 4, **Using Minicomputers and Programmable Calculators for Stress Analysis and Design** (The Meetings Officer, The Institute of Physics, 47, Belgrave Square, London SW1X 8QX)

June 4–7, **Minerals and the Environment** (Institute of Mining and Metallurgy, 44, Portland Place, London W1N 4BR)

June 6–7, **7th Geodesy/Solid-Earth and Ocean Physics** (GEOP) Research Conference (American Geophysical Union, 1707, L Street N.W., Washington DC 20036)

June 7, **Symposium on Mathematical Economics** (Secretary and Registrar, The Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend-on-Sea, Essex SS1 2JY, UK)

June 10–13, **8th Annual Conference on Trace Substances in Environmental Health** (Dr Delbert Hemphill, 426, Clark Hall, University of Missouri, Columbia, Missouri 65201)

June 10–14, **Haploids in Higher Plants: Advances and Potential** (University of Guelph, Ontario, Canada)

June 11, **Symposium on Mathematics in Educational Planning** (The Secretary and Registrar, The Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend-on-Sea, Essex SS1 2JY, UK)

June 11–14, **The Electrodynamics of Substorms and Magnetic Storms** (R. A. Hoffman (Chairman), NASA, Code 646, GSFC, Greenbelt, Maryland 20771)

June 11–14, **8th International Congress for Noise Abatement** (Secretariat Pro Aqua-Pro Vita, ACIB, P.O. Box, CH-4021 Basle, Switzerland)

June 12, **Changing your Computer** (Registration Secretary, BIS-Brandon Training, 80, Blackfriars Road, London SE1 8HB)

June 12, **Glass: An Engineering Material** (The Meetings Secretary, Institution of Metallurgists, Northway House, Whetstone, London N20 9LW)

June 12–14, **International Conference on Submillimeter Waves and their Applications** (K. J. Button, MIT National Magnet Laboratory, Cambridge, Massachusetts 02139)

June 12–15, **Analysis of Lipids and Lipoproteins** (1974 AOCS Lipids Conference, American Oil Chemists' Society, 508, South Sixth Street, Champaign, Illinois 61820)

June 14–22, **17th International Seed Testing Congress** (ISTA Secretariat, Box 68, N 1432 AS-NLH Norway)

June 16–20, **11th Informal Conference on Photochemistry Honoring Professor Francis E. Blacet** (T. W. Martin, Conference Chairman, Box 1506/B, Vanderbilt University, Nashville, Tennessee 37235)

June 17–19, **Velikovsky and the Recent History of the Solar System** (Stephen L. Talbott, Editor *Pensee* Magazine, P.O. Box 414, Portland, Oregon 97207)

June 17–21, **5th International Liquid Crystal Conference** (Conference Secretariat, c/o Stockholm Convention Bureau, Strandvagen 7c, S 114 56 Stockholm, Sweden)

June 19, **26th Annual CIBA Foundation Lecture** (Personal Assistant to the Director, The Ciba Foundation, 41, Portland Place, London W1N 4BN)

June 21–26, **3rd International Congress of Plant Tissue and Cell Culture** (The Secretariat, IAPTC Congress, Botanical Laboratories, University of Leicester, Leicester, LE1 7RH, UK)

June 22–27, **Congress Tertius Societatis Radiologicae Europaeae** (The British Institute of Radiology, 32 Welbeck Street, London W1M 7PG)

June 23–26, **1st International Meeting of the Society "Environment and Health"** (The Secretariat, The National Research Foundation, 48, Vassileos Constantinou Avenue, Athens 501/1, Greece)

June 23–30, **Workshop on Medical Ethics** (Robert M. Veatch, Associate for Medical Ethics, Institute of Society, Ethics and the Life Sciences, 623, Warburton Avenue, Hastings-on-Hudson, New York 10706)

June 24–26, **2nd International Symposium on Mass Spectrometry in Biochemistry and Medicine** (Secretariat c/o Instituto di Ricerche Farmacologiche "Mario Negri" 20157 Milan, Via Eritea, 62, Italy)

June 24–29, **Paris 1974 Immuno-Oncology Week** (Secretariat of the Institut de Cancerologie et D'Immunogenetique, 14, Avenue Paul-Vaillant-Couturier 94800 Villejuif, France)

June 24–28, **Recent Advances in the Assessment of the Health Effects of Environmental Pollution** (The Secretariat, Health Protection Directorate, 29, Rue Aldringen Luxembourg (Grand Duchy))

June 24–28, **Enzymes and their uses in Analysis and Clinical Diagnosis** (Director of the Summer Session, Room E19-356, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139)

June 24–28, **2nd International Symposium on Sex Education** (The Organising Committee, Second International Symposium on Sex Education, P.O.B. 16271, Tel Aviv, Israel)

June 24–28, **9th International Symposium on the Chemistry of Natural Products** (Executive Secretary, 9th International Symposium on the Chemistry of Natural Products, c/o National Research Council of Canada, Ottawa, Ontario K1A 0R6, Canada)

June 25–July 6, **10th International Symposium on Mathematical Geophysics** (Dr J. A. Hudson, Department of Applied Mathematics and Theoretical Physics, Silver Street, Cambridge, UK)

June 27–29, **Gene Regulation in Mammals** (Dr Andrew A. Kandutsch, The Jackson Laboratory, Bar Harbour, Maine 04609)

July 1, **Automated Sequencing for Peptides and Proteins** (Chairman, J. I. Harris, MRC Cambridge, UK)

July 1–3, **549th Biochemical Society Meeting** (The Meetings Officer, The Biochemical Society, 7 Warwick Court, Holborn, London WC1R 5DP)

July 1–5, **6th Symposium on Organic Sulphur Chemistry** (The Secretariat, School of Physical and Molecular Sciences, University College of North Wales, Bangor, LL57 2UW, UK)

July 1–6, **Land use and Society** (The Secretary, S_2A_3 Congress, c/o Rhodes University, Grahamstown, South Africa)

July, 2–4, **The Emergence of Science in Western Europe in Different National Contexts** (The British Society for the History of Science, 47 Belgrave Square, London SW1X 8QX)

July 7–12, **9th International Congress of Neuropsychopharmacology** (Professor J. R. Boissier, General Secretary, Organising Committee, Unite de Neuropsychopharmacology, 2 Rue d'Alesia, 75014 Paris, France)

July 8–10, **Recent Advances in NMR Spectroscopy** (Miss J. M. Usher, The Chemical Society, Burlington House, London W1V 0BN)

July 8–11, **2nd International Conference on the Biology and Pharmacology of Cyclic AMP** (Dr G. I. Drummond, Department of Pharmacology, Faculty of Medicine, University of British Columbia, Vancouver 8, Canada)

July 8–12, **International Dynamic Mass Spectrometer Symposium** (Dr D. Price, Department of Chemistry and Applied Chemistry, University of Salford, Salford M5 4WT, Lancashire, UK)

July 8–12, **Micro 74** (The Administrator, Royal Microscopical Society, Clarendon House, Cornmarket Street, Oxford OX1 3HA, UK)

July 9–12, **Kinetics of Chemical Reactions in Heterogeneous Systems** (Dr Troyanowsky, Secretaire general, Societe de Chimie physique, 10 rue Vauquelin, 75231 Paris Cedex 05, France)

July 9–12, **Interrelation of Structure, Properties and Applications of Polymers** (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX)

July 10–12, **Environmental Pollution: Mutation and Selection** (Professor M. A. Ferguson-Smith, Institute of Genetics, Church Street, Glasgow G11 5JS, UK)

July 11–12, **Recent Advances in Vibrational Spectroscopy** (Miss J. M. Usher, The Chemical Society, Burlington House, London W1V 0BN)

July 11–12, **Trace Elements and Nutrition** (Dr J. D. Sutton, Hon. Programmes Secretary, Nutrition Society, National Institute for Research in Dairying, Shinfield, Reading RG2 9AT, UK)

July 12–16, **8th International Congress on Animal Reproduction and Artificial Insemination** (Professor Stefan Wierzbowski, 32-083 BALICE/Krakow, Poland, Institute of Zootechnics, Department of Animal Reproduction and AI)

July 14–20, **5th International Congress of Radiation Research** (Dr W. K. Sinclair, Secretary-General, Argonne National Laboratory, Argonne, Illinois 60439, USA)

July 15, **Workshop Course on Gradient Centrifugation** (Dr E. Reid, Wolfson Bioanalytical Centre, University of Surrey, Guildford GU2 5XH, UK)

July 15–19, **Dynamic Studies with Radioisotopes in Clinical** Medicine **and Research** (International Atomic Energy Agency, Karntner Ring 11-13, P.O. Box 590, A-1011 Vienna, Austria)

July 15–19, **Course in Modern Microscopy** (The Administrator, Royal Microscopical Society, Clarendon House, Cornmarket Street, Oxford OX1 3HA, UK)

July 15–19, **Centenary Celebrations of the Society for Analytical Chemistry** (The Secretary, Society for Analytical Chemistry, 9/10 Savile Row, London W1X 1AF)

July 16–19, **John Innes Symposium: Modifications of the Information contents of Plant Cells** (Miss Justine Speed, Symposium Secretary, John Innes Institute, Norwich NOR 70F, UK)

July 17, **Society for Drug Research, Anti-Obesity Drugs** (Dr J. R. Cavalla, c/o John Wyeth and Brother Ltd., Huntercombe Lane South, Taplow, Maidenhead, Berkshire, UK)

July 21–26, **3rd International Congress of Plant Tissue and Cell Culture** (Secretariat, Third International Congress of Plant Tissue and Cell Culture, Botanical Laboratories, University of Leicester, Leicester LE1 7RH, UK)

July 21–27, **The Applications of Heterogeneous Catalysis** (Centre for Extension Studies, University of Technology, Loughborough, Leics., LE11 3TU, UK)

July 22–26, **Enzyme and Fermentation Biotechnology Course** (The Assistant Secretary, The Society of Chemical Industry, 14 Belgrave Square, London SW1)

July 22–26, **Course in Photomicrography** (The Administrator, Royal Microscopical Society, Clarendon House, Cornmarket Street, Oxford OX1 3HA, UK)

July 22–26, **International Conference on Urban Development Models** (Mrs M. Houston, Land Use and Built Form Studies, University of Cambridge, 16 Brooklands Avenue, Cambridge, UK)

July 22–August 9, **Origin and Abundance of the Chemical Elements** (Professor Martin Rees, Institute of Astronomy, Madingley Road, Cambridge CB3 0HA, UK)

July 23–31, **8th International Congress on Acoustics** (The Administrative Secretary, Eighth International Congress on Acoustics, 47 Belgrave Square, London SW1X 8QX, UK)

July 25–27, **29th Annual Calorimetry Conference** (Harry Watts, Resarch and Development Department, Dow Chemical of Canada, Limited, P.O. Box 1012, Sarnia, Ontario N7T 7K7, Canada)

July 29–August 9, **Energy: A Unified View** (Director of the Summer Session, MIT, Room E19-356, Cambridge, Massachusetts 02139, USA)

August 1–2, **International Symposium on Microwave Acoustics** (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX)

August 2–8, **International Committee on Avian Anatomical Nomenclature** (Dr John McLelland, Secretary of ICAAN Department of Anatomy, Royal School of Veterinary Studies, Edinburgh EH9 1QH, Scotland, UK)

Erratum

In the article "Speeding up astronomical photography" (*Nature*, **249**, 22; 1974) by P. M. Corben, V. C. Reddish and M. E. Sim, the letters *a* and *b* on Fig. 2 were unfortunately interchanged during preparation of the illustrations for printing.

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■ **Sampling and analysis of various toxic materials in factories** ■ **Limited amount of travel assisting Chemical Inspection on visits to factories** ■ **Development of new analytical methods may also be involved.**

□ Degree, HNC in appropriate subject □ Final year students may apply □ Knowledge of microscopy, X-ray diffraction and florescence, gas chromatography, mass spectropgraphy, atomic absorption and emission spectroscopy advantageous □ Ability to drive □ Age under 27 □ Appointment as Scientific Officer (£1545-£2439 under review) □ Ref: SB/5/DB.

□ Application forms (for return by 17 June 1974) from Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, telephone Basingstoke 29222 ext. 500 or London 01-839 1992 (24 hour answering service).

Science group
CIVIL SERVICE

(2006)

Scottish Health Service

COMMON SERVICES AGENCY

SCOTTISH BLOOD TRANSFUSION CENTRE

Applications are invited for the post of **PRINCIPAL SCIENTIFIC OFFICER** in the above Department. The successful applicant will be responsible to the Regional Director for the day to day management of the scientific, logistic and technical aspects of the Blood Bank and Blood Component Divisions in which 35 technical and scientific staff are employed.

The post is on the Principal Biochemist salary scale, and NHS terms and conditions of service will apply. Further particulars may be obtained from the National Administrator, Scottish Blood Transfusion Service, 17 Rothesay Terrace, Edinburgh EH3 7SF. Applications with the names of 2 referees should be forwarded to the Secretary, Common Services Agency for the Scottish Health Service, 17/18 Rothesay Terrace, Edinburgh EH3 7SF not later than 8th June, 1974.

(1935)

Antarctic Expedition

OBSERVATORY AND IONOSPHERIC PHYSICISTS, ELECTRONIC TECHNICIANS AND ELECTRONIC ENGINEERS

Qualifications: According to post from Honours Degree to O.N.C. or appropriate Armed Service training.

Thorough training is given in all cases and the tour of duty covers two Antarctic winters involving an absence from the United Kingdom of about 30 months. Some categories will be required to work on their field data for up to a year after their return from Antarctica.

Free messing, clothing and canteen. Low income tax.

Applicants must be single and 22 - 30.

If you are interested in seeing a largely unknown, remote and fascinating part of the world please write to:

The Establishment Officer,
British Antarctic Survey,
30, Gillingham Street,
LONDON, SW1V 1HY
Tel: (01) 834 3687

NATURAL ENVIRONMENT RESEARCH COUNCIL

(2035)

UNIVERSITY OF DURHAM

DEPARTMENT OF BOTANY

A POSTDOCTORAL RESEARCH ASSISTANTSHIP is available for three years, to start as early as possible, to work in collaboration with Professor Boulter, on the biochemistry of protein synthesis in peas and beans. Chemists, biochemists and botanists may apply.

Salary will be at an appropriate point on the scale £1,866 to £2,586 per annum (under review), with F.S.S.U.

Applications (3 copies) naming three referees, should be sent by May 27, 1974 to the Registrar and Secretary, Science Laboratories, South Road, Durham, DH1 3LE, from whom further particulars may be obtained. (1963)

THE UNIVERSITY OF NEWCASTLE UPON TYNE CHAIR OF BACTERIOLOGY

Applications are invited for the post of Professor of Bacteriology which will become vacant on October 1, 1974, following the retirement under the age limit of the present holder, Professor C. A. Green. The post carries with it, initially, the Headship of the Department of Microbiology. It is not a condition of appointment that the candidate should hold a registrable medical qualification. Salary in accordance with the appropriate Professorial Scale (Clinical or Non-Clinical), with membership of F.S.S.U.

Further particulars may be obtained from the Registrar of the University, 6 Kensington Terrace, Newcastle upon Tyne NE1 7RU, with whom applications (15 copies), giving the names of not more than three referees, must be lodged not later than June 8, 1974. (Applicants from outside the British Isles may submit one copy only). (1966)

UNIVERSITY OF NOTTINGHAM SCHOOL OF AGRICULTURE

Sutton Bonington, Loughborough

Applications are invited from students expecting to gain a good Honours degree in either Nutrition, Physiology or Biochemistry for an M.R.C. studentship in the Department of Applied Biochemistry and Nutrition to work with Dr R. J. Neale, Lecturer in Human Nutrition. Research interests are those of amino acid metabolism in undernutrition, iron availability from plant proteins and intestinal transport and metabolism.

Applications in writing to be received by June 10, 1974. (1969)

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT

A postdoctoral research assistant is required for a project financed by the Wellcome Trust for work with Dr. R. J. Mayer on "Studies on the turnover of monoamine oxidase with implications for migraine". Experience in membrane enzymology and immunochemical techniques would be extremely valuable for the work. The appointment is for three years beginning on October 1, 1974, starting salary from £2,118 to £2,412 depending on age and experience.

Applications, including curriculum vitae and names of two referees should be sent to Dr R. J. Mayer, Department of Biochemistry, The Medical School, University of Nottingham, Nottingham NG7 2RD. (1971)

JUNIOR TECHNICAL OFFICER

required in the laboratories of the INSTITUTE OF CANCER RESEARCH, Sutton, Surrey. The successful candidate will work in a department concerned with the biochemical pharmacology of anti-tumour agents, and should possess either 'A' levels in science subjects (chemistry and biology), H.N.C. (pharmacology and/or biochemistry), or equivalent qualifications. Salary in the range £1,299 to £2,199, according to age, qualifications and experience. Apply in duplicate with the names of 2 referees to the Secretary, 34 Sumner Place, London, S.W.7, quoting ref. 301/B/517. (1972)

JUNIOR TECHNICAL OFFICER

required by INSTITUTE OF CANCER RESEARCH, Fulham Road, S.W.3, to assist with the work in the Division of Molecular Biology. Experience in the field of protein chemistry would be an advantage as initially the successful candidate would be engaged in amino acid analyses. Candidates should have at least 2 G.C.E. 'A' level passes in Chemistry, Physics or Mathematics. Starting salary according to qualifications and experience approx. £1,300 to £1,550 in scale extending to £2,200. Apply in duplicate to the Secretary, 34 Sumner Place, S.W.7, with the names of two referees and quoting ref. 301/B/515. (1973)

UNIVERSITY OF EDINBURGH

DEPARTMENT OF VETERINARY

ANATOMY

RESEARCH ASSISTANTSHIP

The Agricultural Research Council has awarded a research assistantship to the Department of Veterinary Anatomy for a project concerned with the mechanism of motility in mammalian spermatozoa. The work will depend principally on advanced methods in electron microscopy, for which training will be given.

Applications are invited from students who have, or expect soon to have, a good Honours degree in Zoology or an associated discipline. The salary of the assistant will be at a level approximately equivalent to the value of an S.R.C. postgraduate studentship. The appointment will be for three years, commencing October 1, 1974, and the successful applicant will be expected to register for the degree of Ph.D. (for which the fees will be paid).

Applications, together with the names of two referees, should be sent as soon as possible to Dr D. M. Woolley, Department of Veterinary Anatomy, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh EH9 1QH. Please quote reference number 5018. (1979)

JUNIOR TECHNICAL OFFICER

required in BIOPHYSICS DEPARTMENT OF INSTITUTE OF CANCER RESEARCH, Sutton, Surrey, for work concerned with the action of chemotherapeutic agents on normal and tumour tissues. The work involves various cell transplantation and counting techniques. Applicants should preferably have a degree or equivalent in a biological subject or alternatively considerable experience in relevant experimental work. Good canteen and social facilities. Salary according to M.R.C. scales in the range £1,300 to £2,200. Apply in duplicate with names of two referees to the Secretary, 34 Sumner Place, London SW7 3NU, quoting ref. 301/B/516. (1974)

UNIVERSITY OF HULL

DEPARTMENT OF EDUCATIONAL STUDIES

Applications are invited for appointment as Research Officer or Research Assistant to work under the direction of Dr H. B. Miles in an investigation of factors associated with 'A' level performance. Familiarity with methods of multivariate analysis and computer use necessary; research experience in education desirable. The post may interest someone with qualifications in Statistics, Mathematical Statistics or Operational Research.

Salary on the scale: £2,883 to £3,378 (Research Officer), or £1,518 to £2,223 (Research Assistant), with placing according to qualifications and experience. Plus F.S.S.U. benefits.

Applications (6 copies) giving details of age, qualifications and experience together with the names of three referees should be sent by May 31, 1974, to the undersigned from whom further particulars may be obtained. F. T. Mattison, Registrar. (1975)

UNIVERSITY OF EDINBURGH

DEPARTMENTMENT OF VETERINARY

ANATOMY

RESEARCH ASSISTANTSHIP

DEPARTMENT OF VETERINARY

awarded a three-year research assistantship, commencing October 1, 1974, to the Department of Veterinary Anatomy for a project investigating the sensory mechanisms in the avian respiratory tract and involving the use of techniques in electron microscopy and cytochemistry. Applications are invited from students who have, or expect to obtain shortly, a good Honours degree in Zoology, Physiology or Biochemistry. The salary of the assistant will be at a level approximately equivalent to the value of an S.R.C. Postgraduate studentship. The successful applicant will be expected to register for the degree of Ph.D.

Applications, consisting of a brief curriculum vitae and the names of two referees, should be sent to Dr J. McLelland, Department of Anatomy, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh EH9 1QH, as soon as possible. Please quote reference number 5019. (1980)

Scientist for Drug Metabolism Studies

We have a vacancy in the Drug Metabolism section of our Biochemistry Department for a scientist to participate in a varied programme of work on the metabolism and pharmacokinetics of new and established drugs. Applications are invited from candidates with a good honours degree in chemistry, biochemistry or pharmacy and several years post-graduate experience leading to a higher degree, in a relevant field of research.

This is an excellent opportunity for someone wishing to pursue a career in this important specialised area of applied research, to join an experienced and enthusiastic group of scientists working in new well-equipped laboratories. While postgraduate and postdoctoral experience in drug metabolism work would be an advantage, we would consider applications from those with experience of bioanalytical techniques, separation methods and isotopic tracer work who believe that their experience could be applied usefully to the study of drug metabolism.

Roche Products Limited is part of one of the world's largest and most successful research-based pharmaceutical companies and is itself one of the leaders in the UK. Working conditions and Conditions of Service are excellent. In appropriate cases some substantial assistance with re-location will be available. Please write in confidence quoting reference RB for a personal history form, further information on this post and a Company booklet to the Welwyn Personnel Manager.

ROCHE

Roche Products Limited
Welwyn Garden City Hertfordshire AL7 3AY

(2101)

The Netherlands Cancer Institute
Antoni van Leeuwenhoekhuis, Amsterdam.
Applications are invited for position of a

Senior Research Geneticist

preferably with experience in immunogenetics for research on histocompatibility with relation to cancer in animals. •

Request for further information and applications can be addressed to: •

Prof. O. Mühlbock,
Biology Department,
Sarphatistraat 108,
Amsterdam-Holland.

(2071)

Natural Environment Research Council
Higher Scientific Officer/Senior Scientific Officer Vacancies
University Support Division

The Council provides selective support for research and postgraduate training in the natural environmental sciences. The duties of the University Support Division include the assistance of Council in the formulation of its policies for university support and the implementation of these policies.

Two Higher Scientific Officers/Senior Scientific Officers are required in the Division at the Council's Headquarters in London, which are due to move to Swindon within the next few years.

DUTIES:

The successful applicants will be expected to assist in all the activities of the Division; they will each have special responsibility as Secretary of one of the Council's grants committees covering the field of the Aquatic Life Sciences and Terrestrial Life Sciences respectively. Duties will include the servicing of these Committees with the assistance of executive and clerical staff and maintaining liaison with research workers at universities and other research institutes.

QUALIFICATIONS:

An appropriate first or second class honours degree (or equivalent) and at least two years relevant post-graduate experience, preferably in research or administration in the life sciences.

SALARY SCALES

HSO £2,396–£3,029

SSO £2,973–£4,070

Starting salaries may be above the minimum.

Non-contributory superannuation arrangements. Paid annual leave of 4 weeks and 2 days, plus public and privilege holidays.

Application forms may be obtained from Establishments Division, NERC Headquarters, Alhambra House, 27/33 Charing Cross Road, London WC2H 0AX. (Quoting reference E2/G2/105 and stating for which post you are applying). Closing date: 14th June, 1974.

(1977)

PAISLEY COLLEGE

Applications are invited for three posts as LECTURER (Grade I or II) in the DEPARTMENT OF BIOLOGY.

i. **LECTURER IN BIOLOGY.** Preference may be given to a candidate with experience in the area of cell biology and genetics.

ii. **LECTURER IN PLANT ECOLOGY.** Preference may be given to a candidate with interests in algology. The person appointed will also be expected to assist with the teaching of plant physiology.

iii. **LECTURER IN ENVIRONMENTAL BIOLOGY.** Preference may be given to a candidate with experience of applied ecology in relation, for example, to natural resource management, or to animal behaviour in the field.

Candidates should have a good Honours degree and either a second degree or other research experience. Teaching experience is also desirable. The successful candidates will be asked to share in the teaching of M.I.Biol., BSc. degree and HND students. They will be encouraged to develop research in their own fields.

Salary scales: Grade I — £1,905–£4,173
II — £1,773–£3,750

Application forms and further particulars from Establishment Section, Paisley College of Technology, High Street, Paisley PA1 2BE (Tel. 041-887 1241) quoting ref. N/11/1 (indicating post i, ii, or iii). Closing date 12th June, 1974.

(1986)

JUNIOR TECHNICAL OFFICER

MEDICAL RESEARCH COUNCIL
GASTROENTEROLOGY UNIT

Research assistant needed to work on projects involving immunological and biochemical technique. Interesting work, contact with patients. Experience of immunology desirable, but not essential. Qualifications: B.Sc. or H.N.C. Salary in the range of £1,173 to £2,073 plus £126 London Weighting. Please apply, with curriculum vitae, to The Director, M.R.C. Gastroenterology Unit, Central Middlesex Hospital, Park Royal, London NW10 7NS.

(1981)

SEALE-HAYNE COLLEGE

DEPARTMENT OF FOOD TECHNOLOGY

Applications are invited for the newly created position of HEAD OF DEPARTMENT. The successful candidate will have the opportunity to develop the Higher National Diploma courses in General Food Technology and Food Technology with a Dairying Specialisation, both courses are mounted jointly with the Bristol Polytechnic. The post will be at the Head of Department Grade II level and within the present salary range of £3,345 to £2,753 per annum (under review). Further details may be obtained from The Principal, Seale-Hayne College, Newton Abbot, Devon TQ12 6NQ, to whom applications should be sent as soon as possible.

(1982)

HEALTH SCIENCES CENTRE

Winnipeg, Manitoba, Canada

Applications are invited from suitably qualified and experienced individuals for the position of

DIRECTOR OF CLINICAL CHEMISTRY

in the largest Teaching Hospital affiliated with the University of Manitoba.

The incumbent will direct the laboratory which supports clinical activities of the medical staff in a wide range of disciplines, co-operates in providing support for education and has an internal research programme, as well as supporting the research of others in the Centre.

Enquiries, accompanied by curriculum vitae, should be directed, in the first instance, to:

The Vice-President (Medical),
Health Sciences Centre,
A143, General Centre,
700 William Avenue,
Winnipeg, Manitoba,
R3E 0Z3.

(1990)

NATURAL ENVIRONMENT RESEARCH COUNCIL

INSTITUTE OF GEOLOGICAL SCIENCES

SCIENTISTS

are required in the Institute's Field Survey, Engineering Geology, Applied Geophysics, Continental Shelf and Mineral Assessment Units. The majority of staff are based on London, others on Leeds, Edinburgh or Belfast. Most staff are expected to undertake field work. Opportunities for service overseas. Appointment will be for a three-year period which may in some cases be extended. There are prospects for a limited number of staff to transfer to permanent posts.

Qualifications: Scientific Officer: Degree, H.N.C. or equivalent; for some posts a 1st or 2nd class honours degree is preferred (Final year students may apply). Age normally under 27. Higher Scientific Officer: Degree, H.N.C. or equivalent (with at least 5 years' postgraduate experience); for some posts a 1st or 2nd class honours degree (with at least 2 years' postgraduate experience) is preferred. Age normally under 30. Senior Scientific Officer: 1st or 2nd class honours degree or equivalent and at least 4 years' relevant postgraduate or other approved experience. Age normally at least 25 and under 32.

Salaries (under review): Scientific Officer £1,435 to £2,329; Higher Scientific Officer £2,221 to £2,854; Senior Scientific Officer £2,798 to £3,895. Starting salaries may be above the minima. Salaries £175 more in London. Promotion prospects.

The staff of the Council are not Civil Servants although their pay and conditions of service are similar to those of Scientists in the Civil Service.

For full details and an application form (to be returned by June 21, 1974), write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, telephone Basingstoke 29222, ext. 500 or London 01-839 1992 (24-hour answering service), quoting S/168/74.

(1985)

SEALE-HAYNE COLLEGE

HEAD OF THE DEPARTMENT OF AGRICULTURE

Applications are invited for the above position which will become vacant on September 1, 1974. The person appointed will be responsible to the Principal for the Higher and Ordinary National Diploma courses in Agriculture. There will be opportunities for research. The appointment will be made in the Head of Department Grade II level and within the salary range of £3,345 to £3,753 per annum (under review). Further details may be obtained from The Principal, Seale-Hayne College, Newton Abbot, Devon TQ12 6NQ, to whom applications should be sent as soon as possible. (1983)

TECHNICIAN

with H.N.C. or equivalent qualification required to work on problems in chemical carcinogenesis mainly using cultured cells. Tissue culture or microbiological experience preferred. Salary on Whitley Council Scales in the range £1,566 to £1,971 p.a. according to age and experience. Applications, together with the names of two referees, to Dr J. P. Roscoe, School of Pathology (N), The Middlesex Hospital Medical School, Riding House Street, London W1P 7LD. (1989)

UNIVERSITY COLLEGE

GALWAY, EIRE

HUMAN CYTOGENETICS UNIT

TECHNICIAN

Applications are invited for the above post of Technician in the Human Cytogenetics Unit in the Microbiology Department, commencing salary within the scale £1665 x 72 - £1,953 (under review).

Qualifications: Technician's Certificate of Department of Education, Science Laboratories Technician's Certificate of City and Guilds, Intermediate examination of the Institute of Medical Laboratory Technology, National Certificate in Medical Laboratory Sciences or equivalent.

Experience of Human Cytogenetics or photography desirable but not essential.

Full details and application form from Secretary, Department of Microbiology, University College, Galway, Eire. (1999)

HARVARD MEDICAL SCHOOL

Applications are invited for two new positions as Assistant Professors in the Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Mass. The applicants should have either an M.D. or Ph.D. degree and have demonstrated capacity for independent research in animal cell viruses or aspects of macromolecular control in animal cells. Responsibilities include teaching of graduate and medical students. Write Dr Jon Beckwith, Dept. of Microbiology and Molecular Genetics, Harvard Medical School, 25 Shattuck Street, Boston, Massachusetts 02115 and include a complete curriculum vitae with publications and the names and addresses of three references.

An Equal Opportunity, Affirmative Action Employer. (1991)

CHAIR OF ANATOMY

Medical College and large teaching hospital in major medical center is considering applications for the chair of anatomy. Rush-Presbyterian-St. Luke's Medical Center is an equal opportunity/affirmative action employer. For further information please contact Dr. J. Francis Hartmann, Chairman of the Anatomy Search Committee, Rush-Presbyterian-St. Luke's Medical Center, c/o Department of Neurology, 1753 West Congress Parkway, Chicago, Illinois 60612 U.S.A. (1995)

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT

A postdoctoral research assistant is required for a project financed by the Wellcome Trust for work with Dr. R. J. Mayer on "studies on the turnover of monoamine oxidase with implications for migraine". Experience in membrane enzymology and immunochemical techniques would be extremely valuable for the work. The appointment is for three years beginning October 1st, 1974, starting salary from £2118 to £2412 depending on age and experience.

Applications, including curriculum vitae and names of two referees should be sent to Dr. R. J. Mayer, Department of Biochemistry, The Medical School, University of Nottingham, Nottingham, NG7 2RD. (1997)

NEW ZEALAND

Department of Scientific and Industrial Research

Scientist

The Cement and Concrete Section of the Chemistry Division, Lower Hutt, has a vacancy for an Analytical Chemist with experience in the analysis of ceramic, cementitious or non-metallic materials and in the application of modern instrumental techniques. Duties would be to develop and apply analytical methods and work with a group conducting investigation into the effects of chemical properties on the physical behaviour of concrete and allied products.

Qualifications: minimum of a good Honours degree.

Salary: up to NZ$8568 p.a. dependent on qualifications and experience.

Further details of the position and passage assistance etc., may be obtained from the

Scientific Liaison Officer,
New Zealand House, Haymarket,
London SW1Y 4TQ.

Applicants should quote Vacancy No. 2/279/2079 and forward applications to the High Commissioner for New Zealand by July 23, 1974 (1996)

Public Service Canada **Fonction publique Canada**

This competition is open to both men and women

Research Statistician

St. John's, Newfoundland

Salary up to $24,262

DUTIES: The Newfoundland Biological Station requires a scientist to undertake research studies in statistical theory and methodology in response to problems arising in fisheries research; act as statistical consultant to scientific and professional staff; direct the operation of a computer terminal; to develop specifications for statistical and mathematical computer programs; to supervise the operation of a data processing unit.

QUALIFICATIONS: Include a Doctorate degree, or a lesser degree with related independent research experience equivalent to that required for a Doctorate degree, with specialisation in an area related to the duties of the position. Knowledge of the English language is essential.

To ensure consideration, please forward "Application for Employment" (Form PSC-367-401) available at Post Offices, Canada Manpower Centres, Public Service Commission of Canada offices, or a detailed resumé before JUNE 28, 1974, quoting reference number 73-100-124-N and send it to:

Sciences and Technology Program,
Public Service Commission of Canada,
Tower "A", Place de Ville,
Ottawa, Ontario. K1A 0M7.

Appointments as a result of this competition are subject to the provisions of the Public Service Employment Act. (2007)

BIOMETRICIAN

Applications are invited for a permanent and pensionable post in the Biometrics Division of the Department of Agriculture, Northern Ireland.

The successful candidate will be concerned mainly with the following:

(1) design and analysis of laboratory and field experiments and surveys;

(2) the statistical assessment of genetical studies on poultry, cattle and herbage plants;

(3) the development and application of computer based systems for handling scientific and other data associated with research and development in Agriculture.

The successful candidate may be required to undertake teaching duties in the Faculty of Agriculture, The Queen's University, Belfast.

Appointment to the post may be at Senior Scientific Officer, Higher Scientific Officer or Scientific Officer level, the qualifications for which are:

SSO Over 25 and under 32 years of age with a first or second class Honours Degree or equivalent in mathematics or biology with specialisation in and/or experience of statistics with special reference to the design and analysis of experiments and surveys. Candidates at this level should have at least 4 years relevant post-graduate experience.

HSO Under 30 years of age with an Honours Degree as above and at least 2 years relevant post-graduate experience.

SO Under 27 years of age with an Honours Degree as above.

Grading and commencing salary will be related to qualifications and experience on the following scales:

SSO £2,798-£3,895 HSO £2,221-£2,854 SO £1,435-£2,329

Please write or telephone for an application form, quoting Ref. SB 136/74/N to the Secretary, Civil Service Commission, Clarendon House, Adelaide Street, Belfast BT2 8ND (telephone 0232-44300, ext. 26). Completed forms must be returnd to arrive not later than 30th May, 1974.

(1988)

NORTHERN IRELAND CIVIL SERVICE

Agricultural Research Council
POULTRY RESEARCH CENTRE

Applications are invited for the following posts within the Scientific Officer class in the Nutrition Section of the Poultry Research Centre:-

Post "A" The duties will initially be studies on the value of non-conventional foods for market turkeys and ducks and cost/effectiveness studies on egg production. Candidates should have a good Honours degree (and preferably a Ph.D.) in a biological subject.

Post "B" The duties will initially be to assist with studies to assess the vitamin requirements of poultry and to correlate growth and biochemical criteria with vitamin status. Candidates should have a pass degree, HNC or equivalent in a chemical subject.

Post "C" The duties will initially be to assist with studies on the feeding of broiler and turkey breeders and on the cost/effectiveness of poultry meat production. Candidates should have a pass degree, HNC or equivalent in a biological subject.

Salary: **Post "A"** Higher Scientific Officer £2221 - £2854. Senior Scientific Officer £2798 - £3895. Starting salary according to qualifications and experience. At least 2 years post qualifying experience is required for appointment at HSO, and 4 years for SSO.

Posts "B" & "C" Scientific Officer £1435 - £2329, starting salary according to qualifications and experience.

All posts are superannuable, with a non-pensionable allowance to offset personal contributions.

Application forms, which may be obtained from the Secretary, Poultry Research Centre, King's Buildings, West Mains Road, Edinburgh EH9 3JS, and stating the post for which the application is made, should be completed and returned to him not later than the 31st May 1974.

(1993)

BIOCHEMIST

Required to join group studying male reproduction and fertility control. Previous experience in hormone assays of advantage. Knowledge of German not required. Salary range: DM 2000—3000 per month. Apply to: Dr. E. Nieschlag, 2. Medical Clinic of the University, Moorenstrasse 5, 4 Düsseldorf (Germany). (1994)

THE UNIVERSITY OF MANCHESTER
INSTITUTE OF SCIENCE AND TECHNOLOGY
DEMONSTRATOR IN BIOCHEMISTRY

Applicants with some experience of biological research and a degree or equivalent qualification are invited to apply for the above vacancy in the Department of Biochemistry, which becomes available from 1st October, 1974.

Commencing Salary: £1,764 per annum with F.S.S.U. rising to £1,839 per annum for the second and any subsequent years.

Letters of application stating qualifications and experience, together with the names and addresses of two referees should be forwarded to Professor A. A. Eddy, Department of Biochemistry, U.M.I.S.T., Sackville Street, Manchester M60 1QD. (1998)

UNIVERSITY OF NATAL
Durban
DEPARTMENT OF BIOLOGICAL SCIENCES

Applications are invited from suitably qualified persons for appointment to the two posts of

1. LECTURER

2. SENIOR LECTURER

Preference will be given to applicants in the field of ecology (preferably marine ecology) and in the field of whole-organism biology. Applications will be considered from persons with predominantly zoological or botanical teaching experience, but an interest in the presentation of integrated biology courses is essential.

The salary scales attached to the posts are:

Lecturer; R4800 x 300 - R6900
Senior Lecturer; R6600 x 300 - R8100
+ 15% pensionable allowance.

The commencing salary will be dependent upon the qualifications and experience of the successful applicants. The senior post may be awarded in either of the required fields. An annual vacation savings bonus up to R260 for married men and R130 for women and single men is payable subject to Treasury regulations.

Application forms, further particulars of the post and information on pension, medical aid, staff bursary, housing loan and subsidy schemes, long leave conditions and travelling expenses on first appointment are obtainable from the Registrar, University of Natal, King George V Avenue, Durban, to whom applications should be submitted by 15th July, 1974, quoting Reference Adv. 67/74. (2000)

BIO-RESEARCH LABORATORIES LTD.
CANADA

Applications are invited from qualified Veterinary Pathologists interested in joining the Department of Toxicology in the capacity of Staff Pathologist. Responsibilities will include technical supervision of Autopsy Room and Histology Laboratory personnel and activities. Principally, however, the successful candidate will be responsible for the evaluation of histological specimen material arising out of toxicological research in a variety of laboratory animal species and for the preparation of reports appropriate to the research in question. Salary will be commensurate with qualifications and experience.

Bio-Research Laboratories Ltd. is a Canadian research company operating in the life sciences field. Applications, curricula vitarum and requests for further details may be directed to Dr. C. I. Chappel, Director, Bio-Research Laboratories Ltd., 265 Hymus Blvd, Pointe Claire, Quebec, Canada H9R 1G6. (1799)

THE UNIVERSITY COLLEGE OF WALES
Aberystwyth
DEMONSTRATOR IN BIOCHEMISTRY

Applications are invited for the post of Demonstrator in the Department of Biochemistry and Agricultural Biochemistry. Applicants should hold an Honours Degree or equivalent qualification in Biochemistry, Chemistry or related disciplines.

Salary: £1,758 to £1,923 p.a. Appointment for one year, renewable up to three years.

Further particulars and application forms can be obtained from the Registrar. Closing date: June 7, 1974. (2015)

JUNIOR TECHNICAL OFFICER

required at Pollards Wood Research Station of INSTITUTE OF CANCER RESEARCH, Chalfont St. Giles, Bucks, to take part in a research programme on mutagenic effects of carcinogens. B.Sc. or H.N.C. in biological subject. Experience in genetics, cytology or cell biology desirable. Starting salary £1,400 p.a. in a scale extending to £2,073, according to qualifications, age and experience. Apply in duplicate with the names of two referees, to the Secretary, 34 Sumner Place, London SW7 3NU, quoting ref. 301/B/518. (2017)

ST. BARTHOLOMEW'S HOSPITAL
West Smithfield, London EC1A 7BE

ELECTRONICS ENGINEER

Applications are invited from honours graduates in Electronic Engineering, or Electrical Engineering or Physics for the above research post in the Department of Medical Electronics. The research programme involves the application of electronic techniques to clinical medicine in ultrasonics, transducer design and signal processing. The successful applicant will be expected to be involved in circuit design for the research programme carried out in the Department as well as initiate his own research. Suitable research work may be considered for a higher degree. Previous hospital experience is not essential although industrial experience would be an advantage.

The appointment will be on the scale £1,764 to £4,299.

Applications in writing naming two referees, to the Personnel Office, quoting ref. R/4488/N. (2018)

UNIVERSITY OF LIVERPOOL
Chair of Clinical Pharmacology

Applications are invited for the Chair of Clinical Pharmacology which will become vacant on September 30, 1974, following the retirement of Professor A. Wilson.

The salary of the Professor will be within the range approved for clinical Professorial salaries and initially will depend on the qualifications and experience of the successful candidate.

Applications (16 copies), together with the names of three referees, should be received not later than June 3, 1974 by the undersigned, from whom further particulars may be obtained. (Candidates overseas may send one copy only, by airmail). Quote ref. RV/N/276015.

H. H. Burchnall,
Registrar,
The University,
P.O. Box 147,
Liverpool L69 3BX. (1872)

UNIVERSITY OF OXFORD
DEPARTMENT OF BIOCHEMISTRY

M.R.C. IMMUNOCHEMISTRY UNIT

A vacancy exists for a Junior Technical Officer/Technical Officer in the above unit. Applicants should be qualified to Higher National Certificate standard and have several years' experience in biochemical/analytical techniques, including spectrophotometry, chromatography and radiochemistry. Some knowledge of protein chemistry and an ability to work with small animals is desirable, but not essential.

Salary, according to age and experience, on the scale £1,854 to £2,073 (bar) £2,184 to £3,057 (under review. Applications, including full details of education and career to date, and the names of two referees, to: The Administrator, Department of Biochemistry, South Parks Road, Oxford OX1 3QU. (1891)

UNIVERSITY OF LONDON
King's College

RESEARCH ASSISTANT IN PHARMACOLOGY

Applications are invited from graduates in the biological sciences for the above post in connection with a research project on nicotine dependence. Applicants should have at least two years experience in postgraduate research and preferably have some knowledge of neurotransmitter estimation or radio immunoassay techniques.

The post is for a period of three years. Salary approximately £2,100 rising by yearly increments of £125 plus F.S.S.U. benefits.

Applicants should apply in writing, including a curriculum vitae and names of two academic referees, to Dr. J. M. Littleton, Department of Pharmacology (N), King's College, Strand, London WC2R 2LS. (2022)

Department of Agriculture

Applications are invited for a permanent and pensionable post in the Crop and Animal Husbandry Division, Northern Ireland.

The successful applicant will undertake work on pig production with special reference to increased efficiency, and will be required to undertake teaching duties in the Faculty of Agriculture, Queen's University, Belfast.

Appointment to the post may be at Senior Scientific Officer, Higher Scientific Officer or Scientific Officer level, the qualifications for which are:

SSO Over 25 and under 32 years of age with a 1st or 2nd Class Honours Degree in Agriculture and at least 4 years post-graduate experience, preferably in relation to pig production.

HSO Under 30 years of age with an Honours Degree as above and at least 2 years relevant post-graduate experience.

SO Under 27 years of age with an Honours Degree as above.

Grading and commencing salary will be related to qualifications and experience on the following scales:

SSO £2,798-£3,895 HSO £2,221-£2,834 SO £1,435-£2,329

Please write or telephone for an application form and further information, quoting Ref. SB 135/74/N to the Civil Service Commission, Clarendon House, Adelaide Street, Belfast BT2 8ND (telephone 0232-44300, ext. 26). Completed forms must be returned to arrive not later than 28th May, 1974. (1987)

NORTHERN IRELAND CIVIL SERVICE

Pathologist

Our Toxicology and Drug Safety Unit is expanding and has recently moved into new, well-equipped laboratories; an additional pathologist is now required to assist in the assessment of the safety of novel pharmaceutical compounds.

His (or her) main duty will be in the interpretation of histopathology but candidates should display an interest in the development of specialised procedures in related fields.

The successful applicant is likely to possess a degree in veterinary medicine or in a relevant Biological science and ideally would have prior experience in histology or histopathology; however, further training can be arranged where necessary.

A competitive starting salary will be paid, together with the fringe benefits which one associates with a large, British-based company. The Medicinal Research Centre is located one mile from Harlow New Town and Development Corporation property may be available to suitable candidates at an economic rent.

If you would like to be considered for this post, please write, outlining your career to date, to :–

BRL

John Atkinson
Site Personnel Officer
Beecham Research Laboratories
The Pinnacles
• Harlow
• Essex

(2014)

THE UNIVERSITY OF CALGARY

THE FACULTY OF MEDICINE

Applications and nominations are invited for the positions of Professor and Head, Division of

MORPHOLOGICAL SCIENCE

and Professor and Head, Division of

PHARMACOLOGY & THERAPEUTICS

The successful applicants will be expected to have an interest in undergraduate and graduate medical education and to be a well-established and active investigator.

The positions are to be filled Sept. 1/74 or as soon as possible. Salary will be according to qualifications and experience.

Nominations and/or applications will be received by Dr. L. E. McLeod, Dean, Faculty of Medicine, The University of Calgary, Calgary, Alberta, T2N 1N4, Canada, until July 1, 1974. Applications are to be accompanied by a detailed curriculum vitae.

THE UNIVERSITY OF CALGARY

(2027)

N.M.R. SPECIALIST

We are seeking a person, preferably at post-doctoral level, who is familiar with the use of a variety of modern N.M.R. instruments and techniques such as Fourier Transform equipment, and highly skilled in the interpretation of spectra. He must be interested in the application of these techniques to problem-solving in synthetic organic chemistry. An interest in the application of N.M.R. to biological problems related to medicinal chemistry would also be an advantage.

The job will involve supervision of the N.M.R. laboratory within the Analytical Department of the Scientific Development Group and the successful candidate will give advice on future expansion of this laboratory in line with current developments in the N.M.R. field.

We are a major international pharmaceutical company, part of the AKZO organisation and our laboratories are situated on the main M8 Edinburgh-Glasgow road. We offer a salary based on qualifications and experience, commensurate with the responsibility of the position. Conditions of employment are in keeping with the best modern practice.

Assistance with any relocation expenses will be given.

Write with details of age, qualifications and career to date to:—

Personnel Manager,
Organon Laboratories Limited,
Newhouse, ML1 5SH, Lanarkshire.

Organon

(2028)

QUEEN MARY COLLEGE
University of London
ORGANIC ELECTROCHEMISTRY

Applications are invited for an S.R.C. supported Postdoctoral Research Fellowship for collaboration with Dr. J. H. P. Utley on the development of aspects of cathodic synthesis. A versatile organic or physical-organic chemist is required. Appointment is for 2 years from October 1, 1974. Salary within range £2,118 to £2,412 p.a. plus £162 London Allowance.

Applications (giving age, qualifications and names and addresses of 2 referees) to The Registrar (N), Queen Mary College, Mile End Road, London E1 4NS. (2021)

DALHOUSIE UNIVERSITY
Nova Scotia, Canada
DEPARTMENT OF ANATOMY
TWO ASSOCIATE OR ASSISTANT PROFESSORS

Applications are invited from persons with a Ph.D. or a professional qualification for the above positions. Duties may include the teaching of Anatomy to Medical, Dental or Paramedical students. An ability to teach gross anatomy and a research interest in the nervous system would be an advantage. Salary (minimum $15,000) and rank commensurate with experience. Applications together with curriculum vitae and the names of two referees should be addressed to Dr. D. G. Gwyn, Department of Anatomy, Dalhousie University, Halifax, Nova Scotia, Canada by June 20, 1974. (1881)

COMMONWEALTH AGRICULTURAL BUREAUX
Vacancy for
SCIENTIFIC INFORMATION OFFICER
at the
COMMONWEALTH BUREAU OF SOILS
Rothamsted Experimental Station
Harpenden, Hertfordshire

Duties: Preparing abstracts for the abstracting journal "Soils and Fertilizers", which covers world scientific literature on soils and soil-plant relationships, and dealing with scientific enquiries within these fields.

Qualifications: A degree in soils or in agricultural science, or in sciences basic to these. Ability to write good and concise English essential. The successful candidate may be required to learn a foreign language. Experience or training in information work, including subject indexing, would be an advantage.

Salary: In scale £1,435 to £2,592 (bar) £2,798 to £3,478 (bar) £3,756 to £4,004, plus a compensatory allowance (taxable but not superannuable) of 4½% to offset personal contribution to F.S.S.U. Starting salary according to qualifications, experience and age.

Application forms: and full particulars from the Secretary, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Slough SL2 3BN.

Closing date for applications: June 14, 1974. (1888)

UNIVERSITY OF SURREY
Guildford, Surrey GU2 5XH
DEPARTMENT OF CHEMISTRY

Applications are invited for a Science Research Council Postdoctoral Assistantship for work with Professor J. A. Elvidge and Dr. J. R. Jones on a study of tritium exchange in heterocyclic compounds. The appointment is available from 1/10/74 and will be for either one or two years. Salary £2,058 by £165 plus F.S.S.U. Applications together with curriculum vitae and the names and addresses of two referees should be sent as soon as possible to one of the above-named persons. (1882)

UNIVERSITY OF EAST ANGLIA
SCHOOL OF ENVIRONMENTAL SCIENCES

Applications are invited for the position of Senior Research Associate to conduct research on a NERC supported project to investigate the chemistry of the sea surface microlayer. The post is suitable for someone with first degree training in chemistry and relevant post-graduate experience in chemistry or oceanographic/ atmospheric sciences. Knowledge of gas chromatographic techniques would be an advantage. The appointment is for one year initially, but is renewable for a further year. Salary will be on the scale £1,929-£2,223 per annum plus FSSU.

Further information is available from Dr. P. S. Liss, School of Environmental Sciences, University of East Anglia, Norwich, NOR 88C, to whom applications, quoting two referees, should be sent to arrive as soon as possible, but not later than 13 June 1974. (2046)

NATURE CONSERVANCY COUNCIL

Scientific Officers/Higher Scientific Officers

SALARY RANGE £1435 - £2854

Applications are invited for the following posts in the Nature Conservancy Council:

Scientific Officers/Higher Scientific Officers, Geology & Physiography Section.

The work of the Section includes the inspection, evaluation, selection and conservation of areas of geological importance, the provision of relevant advice and liaison with Government, University and other geologists and geographers and with land owners. The duties do not include geological and physiographical research.

Candidates for Scientific Officer posts should be under 27 years of age with a degree (or equivalent) in Geology and/or Physical Geography; for Higher Scientific Officer posts candidates should be under 30 years of age with the above qualifications and at least two years relevant post graduate experience. A current driving licence is essential.

Starting salary according to qualifications and experience.

Application forms and further particulars are available from Establishments (S), Nature Conservancy Council, 19 Belgrave Square, London SW1X 8PY. Please quote reference number E2/03/02.

Closing date for completed application forms: 14 June 1974. (2034)

UNIVERSITY OF KEELE

There will be two vacancies for Demonstrators in Geology from October 1, 1974. Applicants should possess good Honours degree and be keen to carry out research. Preference given to those particularly interested in mineralogy, petrology, or structural geology though others will be considered. Salary £1,626 per annum. Further particulars and application forms from the Registrar, The University, Keele, Staffs, ST5 5BG, to whom completed applications should be returned by June 20, 1974. (2062)

UNIVERSITY OF BIRMINGHAM ADMINISTRATIVE POSTS

CENTRE OF WEST AFRICAN STUDIES

to assist Director of a multi-disciplinary Centre—duties include student admissions, the administration of the Centre, the Centre Library: salary in Administrative III up to £2,412.

SCHOOL OF BIOLOGICAL SCIENCE

to assist the Chairman of an undergraduate School of six departments—duties include admissions, examinations, committee work, financial records: salary in Administrative III.

DEPARTMENT OF PHYSICS

to assist the Head of a large science department (c. 150 non-academic personnel): duties may include committee work, budgeting, graduate admissions, personnel work, conference organisation. Salary in Administrative II or III.

Candidates should be graduates preferably with previous administrative experience. Salary scale (after August) Administrative III £1,953 to £3,285; Administrative II £2,580 to £3,990 p.a. with F.S.S.U.

Further particulars and application forms available from Senior Assistant Secretary, University of Birmingham, P.O. Box 363, Birmingham B15 2TT to whom forms should be returned by June 7. (2063)

Postdoctoral Research Assistant

A Postdoctoral Research Assistant is required for the Division of Cellular Biology for research into the cellular biochemical response of synovial cells to phagocytosis of immune complexes. The appointment is for one year in the first instance. The work involves maintenance organ culture and microdensitometry and is part of a programme of work on the biochemistry of human rheumatoid synovium. Applications, including the names of two referees, to The General Secretary, Kennedy Institute of Rheumatology, Bute Gardens, Hammersmith, London W6 7DW. (2044)

Cardiovascular Research

Section Head, £4000+

Our cardiovascular teams have proved themselves capable of identifying new approaches to the treatment of heart disease. It is intended that these teams will form the basis of a new section in the department of Pharmacology and Biochemistry.

We need an established pharmacologist, someone with a minimum of 4 years' post-doctoral experience, capable of giving excellent leadership to this section. Good managerial potential is expected since the section will probably expand.

Future growth of cardiovascular research in Fisons will depend largely on your drive and creative ability. A contribution to other fields of drug research will also be expected through interaction with senior scientists in other departments.

Starting salary will be over £4000; benefits, including contributory pension, welfare schemes and expenses towards relocation are excellent.

Please write or telephone for an application form, quoting reference 355R:
John Mills, Personnel Manager,
Fisons Limited, Pharmaceutical Division, Bakewell Road,
Loughborough, Leics.
Tel: Loughborough 66361

FISONS

(2085)

EDITOR

required by chartered Society for monthly journal and news-sheet, annual review, Colour Index, text books and other publications on chemistry and technology of dye and pigment manufacture and application, all with world-wide circulation.

Salary depending on qualifications and experience, but not less than £2,500.

Apply in writing to Honorary Secretary, Society of Dyers and Colourists, Perkin House, P.O. Box 244, 82 Grattan Road, Bradford, Yorkshire BD1 2JB, marking envelope 'Confidential'. (2094)

UNIVERSITY OF SIERRA LEONE

Njala University College

Applications are invited for SENIOR LECTURESHIPS/LECTURESHIPS IN THE DEPARTMENT OF PHYSICS. Applicants must possess a good first degree in Physics, i.e. first or second class honours and must have some teaching experience in a University. Preference will be given to applicants with a higher degree. Appointees will be expected to teach any branch of Physics at general degree level. Salary scales: Senior Lecturer Le4,550 to Le5,400 p.a.; Lecturer Le2,400 to Le4,740 p.a. (£1 sterling = Le2). The British Government may supplement salary of Senior Lecturers by £1,600 p.a. (sterling) for married appointees or £1,000 p.a. (sterling) for single appointees (normally free of all tax) and provide children's education allowances and holiday visit passages. These supplementation rates are currently under review. This supplementation is unlikely to be applied to appointments at Lecturer level. Superannuation scheme or gratuity; various allowances, family passages; biennial overseas leave. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail, not later than June 10, 1974, to the Secretary, University of Sierra Leone, Private Mail Bag, Freetown, Sierra Leone. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90-91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address. (2065)

SUNDERLAND POLYTECHNIC

SCHOOL OF PHARMACY

LECTURER I or II IN PHARMACOLOGY AND PHYSIOLOGY

Preference will be given to graduates in Pharmacy but applications from graduates in Pharmacology will be considered. Applicants should have relevant postgraduate qualifications and/or experience.

The appointee will be required to participate in the teaching programme of the Department which includes honours degree courses in Pharmacy, Pharmacology and Physiology.

Appointment will be from September 1, 1974 or as soon as possible thereafter. Research and consultancy are encouraged.

Salary will be in accordance with the Burnham Technical Scale (at present under review).

Lecturer Grade I—£1,660 to £2,847.

Lecturer Grade II—£2,515 to £3,243.

The Education Authority is prepared to help the successful candidate(s) with removal expenses, and temporary lodging allowance.

Further particulars and an application form may be obtained from the Personnel Officer, Sunderland Polytechnic, Chester Road, Sunderland SR1 3SD, and should be returned not later than June 6, 1974. (2075)

CSIRO

AUSTRALIA

RESEARCH SCIENTIST

Commonwealth Meteorology Research Centre Melbourne, Vic.

The Commonwealth Scientific and Industrial Research Organization has a broad charter for research into primary and secondary industry areas. The Organization has approximately 6,500 employees—2,100 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

FIELD Dynamic Meteorology

(Atmospheric/Pollution/Chemistry)

GENERAL:
The Commonwealth Meteorology Research Centre is a joint venture by the Commonwealth Scientific and Industrial Research Organization Division of Atmospheric Physics and the Commonwealth Bureau of Meteorology carrying out research in dynamical meteorology.

Current activities involve the development and use of general circulation models with major emphasis being placed on their use for geophysical experiments and the attainment of an improved understanding of atmospheric behaviour. In addition a large effort is devoted to the development and operational implementation of weather forecasting models and research into the problems of obtaining and assimilating initial data into such models.

DUTIES:
To undertake research in the General Circulation Group on dynamical problems associated with atmospheric composition and pollution. Several numerical general circulation models are available and the appointee will be expected to use these to investigate the possible effects of pollutants on the climate, and to develop expertise in relevant atmospheric compositional problems with a view to incorporating appropriate chemistry into the model.

QUALIFICATIONS:
A Ph.D degree or equivalent qualifications, and demonstrable research ability, preferably in the field of meteorology or fluid dynamics. Some experience in Atmospheric Chemistry would be an advantage.

SALARY:
The appointment will be made within the salary ranges of Research Scientist or Senior Research Scientist: $A9,380-$A14,290 p.a. Outstanding applicants may be offered appointment at a higher level.

TENURE:
Either a fixed term or an indefinite appointment may be negotiated. An indefinite appointment carries Australian Government Superannuation benefits.

Applications stating full personal and professional details, the names of at least two professional referees, and quoting Reference Number 424/15 should reach:—

The Personnel Officer,
Australian Scientific Liaison Office,
64-78, Kingsway,
London,
WC2B 6BD

by the 21st June, 1974

Applications in U.S.A. and Canada should be sent to
The Counsellor (Scientific),
Embassy of Australia, 1601 Massachusetts Avenue, N.W.,
Washington, D.C. 20036, U.S.A.

(2086)

UNIVERSITY COLLEGE CARDIFF

Applications are invited for the following vacancy:-

TUTORIAL FELLOW

in the Department of Geology (Caledonian geology and/or Geophysics). Salary Range £1311 - £1473. Duties to commence as soon as possible. Closing date for applications 15 June 1974.

Applications, together with the names and addresses of two referees, should be forwarded to The Registrar, University College, P.O. Box 78, Cardiff, CF1 1XL from whom further particulars may be obtained. Please quote ref. 0566. (2058)

UNIVERSITY OF SIERRA LEONE

Fourah Bay College

Applications are invited for LECTURESHIP IN GEOGRAPHY, in any branch of the discipline, although preference will be given to those with a teaching and research interest in (a) Geomorphology; (b) Climatology/Hydrology; or (c) Social Geography. Appointee will be expected to teach both General and Honours degree students and conduct research in the chosen field. Salary scale: Le2,400 to Le4,740 p.a. (£1 sterling=Le2). The British Government may supplement salary in range £950-£1,300 p.a. (sterling) for married appointee or £350-£700 p.a. (sterling) for single appointee (normally free of all tax) and provide childrens education allowances and holiday visit passages. The supplementation rates are currently under review. F.S.S.U. Family passages; various allowances; regular overseas leave. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail, not later than June 11, 1974 to the Secretary, University of Sierra Leone, Private Mail Bag, Freetown, Sierra Leone. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address. (2066)

UNIVERSITY OF LONDON

King's College

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for the post of LECTURER IN BIOCHEMISTRY, tenable from September 1, 1974. Candidates should be interested in teaching dental students and preferably have had relevant experience. The successful applicant will be primarily responsible for the planning and organisation of a new biochemistry course for dental students starting next session. He will be expected also to contribute to the teaching of medical undergraduates and Honours B.Sc. students.

From October 1, 1974, salary will be on the new scale for lecturers (£2,118 to £4,896 plus £162 London Allowance) with F.S.S.S.U. benefits.

Application forms and further particulars of the appointment are available from the Registrar, University of London King's College, Strand, London WC2R 2LS and should be returned to him by June 14, 1974, quoting reference N24/5. (2068)

UNIVERSITY OF IBADAN

Nigeria

Applications are invited for posts of (a) PROFESSOR and (b) SENIOR LECTURER and (c) LECTURER IN THE INSTITUTE OF APPLIED SCIENCE AND TECHNOLOGY. These posts are in the Engineering and Technology disciplines of Agriculture or Electronic Instrumentation or Food or Forestry or Petroleum or Wood. Candidates for (a) should hold a Ph.D. or equivalent in their field of specialisation as well as possessing widely recognised teaching, industrial and research experience. Administrative experience or ability for administrative duties are highly desirable qualities. Candidates for (b) should hold a Ph.D. or equivalent in their field of specialisation as well as significant teaching, industrial and research experience. Candidates for (c) should hold a Ph.D. or equivalent in their field of specialisation as well as the potential to perform effectively in teaching, industrial liaison and research. Salary scales: (a) N6,600 p.a. (b) N5,030 to N5,750 p.a.; (c) N2,760 to N4,830 p.a. (£1 sterling=N1.54). The British Expatriates Supplementation Scheme is unlikely to be applied to these appointments. Family passages; various allowances; superannuation scheme; biennial overseas leave. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by air mail, not later than June 11, 1974 to the Registrar, University of Ibadan, Ibadan, Nigeria. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address. (2067)

AHMADU BELLO UNIVERSITY
Nigeria

Applications are invited for posts of (i) READERS/SENIOR LECTURERS and (ii) LECTURERS IN PHARMACEUTICS AND PHARMACOGNOSY in the Department of Pharmacy and Pharmacology. Candidates for (i) should have experience in teaching in Pharmaceutical Technology, Physical Pharmacy or Pharmaceutical Microbiology. A good higher degree and research publications in the field of specialisation are essential. Candidates for (ii) should have a good honours degree and a higher degree in their field of specialisation. Salary scales: Reader N6,100 p.a.; Senior Lecturer N5,030 to N6,050 p.a.; Lecturer N2,760 to N4,830 p.a. (£1 sterling=N1.54). The British Government may supplement salaries of Readers and Senior Lecturers in range £1,050 to £1,150 p.a. (sterling) for married appointees or £500 to £600 p.a. (sterling) for single appointees (normally free of all tax) and provide children's education allowances and holiday visit passages. For those appointed to clinical duties, supplementation may be in range £1,200 to £1,250 p.a. (sterling—if married) or £650 to £700 p.a. (sterling—if single). These supplementation rates are currrently under review. This supplementation is unlikely to be applied to appointments at Lecturer level. Family passages; various allowances; superannuation scheme; biennial overseas leave. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be forwarded by airmail, not later than June 10, 1974 to the Registrar, Ahmadu Bello University, Zaria, Nigeria. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address. (2069)

UNIVERSITY OF EDINBURGH
AND
LOTHIAN HEALTH BOARD
Teaching and Research Centre
Western General Hospital
ADMINISTRATIVE ASSISTANT

Applications are invited from young men and women, preferably with a Degree in Science and some practical or research experience, for a post as Administrative Assistant at the Teaching and Research Centre, Western General Hospital. The post is tenable from July 1, 1974 or such later date as may be arranged. This post will appeal to those wishing to take up a career in Administration in a scientific organisation. It is hoped that the person appointed will have an interest and preferably some experience in the development of Audio-Visual Aids.

The initial salary will be at a suitable point on the scale £1,929 to £2,553 per annum (under review). Superannuation provision will be under F.S.S.U.

Further particulars may be obtained from the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh EH8 9YL to whom applications (6 copies) giving the names of three referees, should be sent not later than June 8, 1974. Please quote reference number 4003. (2073)

UNIVERSITY OF KENT
At Canterbury
Research Assistant in Biology

A RESEARCH ASSISTANT is required from October 1, 1974 to work on the growth kinetics of mixed populations of micro-organisms using continuous culture techniques. Applicants should have, or expect to obtain, a good Honours degree in microbiology, preferably with an interest in mathematical modelling of biological systems. Salary £1,593. Applications giving details of qualifications and experience with names of two referees to the Assistant Registrar, Natural Sciences, Chemical Laboratory, The University, Canterbury, Kent CT2 7NH by June 10. Please quote ref. A52/74. (2072)

ARMAGH OBSERVATORY
Northern Ireland

Applications are invited for the post of Astronomer. Salary £2,388 x £165 - £3,213 with the equivalent of F.S.S.U., the starting point of the salary scale will depend on the experience and qualifications of the successful candidate. The post is a research one and the candidate's main interest should be in Stellar Astronomy. Armagh Observatory shares the facilities of the Boyden Observatory. Three copies of application should be sent not later than 15th July, 1974 to the Secretary, Armagh Observatory. (2077)

GLASSHOUSE CROPS RESEARCH INSTITUTE

Plant Physiology Department

A. PHYSICIST (CROP PROCESSES)

required to develop a biophysics research programme on the definition of plant structure in quantitative terms and on its relation to within-plant physical functions, especially transport processes. Appointee will collaborate with physiologists and biometricians in a major programme of mathematical modelling of glasshouse crop processes. Applicants should have a good honours degree and at least four years' research experience, preferably in experimental physics, biophysics, or a related subject; experience of biological work desirable. Appointment as SENIOR or PRINCIPAL SCIENTIFIC OFFICER, depending on experience. Closing date 31st July.

B. INSTRUMENTATION PHYSICIST

required for Design & Development Section, to join a team developing scientific equipment to meet special research problems. Applicants should have a sound background in applied physics and measurement theory, aptitude and experience in instrument design and construction, and a degree, HNC or equivalent qualification, in an appropriate subject. Appointment as

SCIENTIFIC OFFICER or HIGHER SCIENTIFIC OFFICER, depending on experience. At least five years' post qualifying experience is normally required for appointment in the higher grade. Closing date 28th June.

Salaries:

Scientific Officer	£1,435 — £2,329
Higher Scientific Officer	£2,221 — £2,854
Senior Scientific Officer	£2,798 — £3,895
Principal Scientific Officer	£3,715 — £4,895

Starting salary depending in each case on qualifications and/or experience. Contributory superannuation scheme for both posts, with addition non-pensionable allowance to offset contributions.

Further particulars (state post A or B) from the Secretary to the Institute, Worthing Road, Rustington, Littlehampton, Sussex, to whom applications should be sent by appropriate date. (2095)

LEEDS AREA HEALTH AUTHORITY (TEACHING)
EASTERN DISTRICT

PHARMACEUTICAL ANALYST

Applications are invited from pharmacists or graduate chemists for the above post based at St. James's Hospital, Leeds.

The person appointed will be accountable to the Pharmacist in charge of the Laboratory but will be responsible for the work of the other technicians. The work involves some routine analysis with investigations into quality control techniques and analytical methods.

Salary will depend on age, experience and qualifications but will be in the range £1,680–£2,916.

Application forms are available from the Personnel Manager, St James's Hospital, Leeds 33144 Ext. 203/293 (2091)

SENIOR TEACHING FELLOWSHIP—ORGANIC CHEMISTRY
(Ref F11N)

Applicants should have interests in physical organic chemistry or in the chemistry of natural products. The appointee will have a particular responsibility for part of the first year laboratory. The appointment will be from February 1st 1975.

The research interests of the School are in the areas of solid state physics, chemistry and biochemistry.

Salaries: Senior Lecturer $A12,268 - $A14,308. Senior Teaching Fellow $A7,270 - $A8,698.

For further details of these posts and for the method of application, write to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London, WC1H 0PF. The closing date for applications is **June 24th 1974.** (2082)

UNIVERSITY OF STRATHCLYDE
DEPARTMENT OF BIOLOGY
RESEARCH ASSISTANT

Applications are invited from graduates for the post of Research Assistant in the Marine Laboratory, Garelochhead.

The successful candidate will work on problems concerned with the biological effects of waste disposal in the Solway Firth. A considerable amount of field work is undertaken and the ability to drive is essential.

Salary within the rate £1401 - £2001 with placing according to qualifications and experience. Appointments for a period of up to three years.

Applications (quoting R15/74) to Dr. E. J. Perkins, Department of Biology, Royal College, University of Strathclyde, 204 George Street, Glasgow, G1 1XW. (2074)

FLATFORD MILL FIELD CENTRE GRADUATES

(females) wanted who would be interested in the post of Temporary Help (cooking) at Flatford Mill Field Centre whilst searching for permanent science appointments. Could attend interviews and leave at short notice if necessary. Would suit two friends. Write to The Warden, Flatford Mill Field Centre, East Bergholt, Nr. Colchester, Essex or telephone (reverse charges) East Bergholt (020-629) 283. (2078)

ROYAL POSTGRADUATE MEDICAL SCHOOL

University of London

OSTI on-line information retrieval project

Applications are invited for a post of Research Assistant in the Wellcome Library to be responsible for its participation, as a biomedical user centre, in an on-line bibliographical retrieval project which is being financed by the British Library, Research and Development Division (formerly OSTI). The systems principally involved will be the MEDLARS on-line retrieval systems, MEDUSA and MEDLINE and the person appointed will act as intermediary in on-line search formulation and evaluation.

Applicants should have relevant academic and library/information science qualifications or experience; no computer knowledge is required. The salary will be on a scale £1929 to £2553 per annum (plus £162 London Allowance) according to qualifications and experience. The post is available from 1st July and will extend until December 1975.

Further details from The Librarian, Miss E. M. Read, Telephone 01-743 2030 Ext 97.

Applications to the Secretary, Royal Postgraduate Medical School, Hammersmith Hospital, DuCane Road, London W12 0HS by 7th June 1974. (2081)

GRIFFITH UNIVERSITY

Brisbane, Australia

SCHOOL OF SCIENCE

Griffith University will admit its first undergraduate students in March 1975. Its organisation is on a broad school of study basis and it has a commitment to interdisciplinary teaching. Applications are now invited from men and women for the following positions:

SENIOR LECTURESHIP—CHEMICAL PHYSICS

(Ref F10N)

Applicants should have expertise in one of the areas at the interface of physics and chemistry. The appointee will have a key role in the second year teaching programme, in which emphasis will be placed on areas of overlap between chemistry and physics. The appointment will be from March 1st 1975.

VICTORIA UNIVERSITY OF WELLINGTON

New Zealand

CHAIR OF APPLIED MATHEMATICS

In view of the impending retirement of Professor C. J. Seelye in May 1974 from the Chair in Applied Mathematics, applications for this Chair are invited from persons with suitable qualifications in the branch of classical or modern applied mathematics. There are three Chairs in the Department, covering the range of applied, pure and statistical mathematics.

The Mathematics Department works in close cooperation with the Department of Information Science and the Applied Mathematics Division of D.S.I.R., which are both situated in the same building as the Mathematics Department.

Salary range: NZ$13,863 to NZ$17,645 p.a. Superannuation on the FSSU basis and allowance for travel expenses.

Further particulars and application procedure available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close **31 July 1974.** (2083)

DEPARTMENT OF ANATOMICAL SCIENCES expects to have two faculty positions open for neurobiologists with active research programs in the development of the vertebrate nervous system or in the structure and function of mammalian nervous system.

Teaching responsibilities will include neuroscience for medical and dental students and graduate student training. Rank and salary will be determined by qualifications. Reply to Maynard M. Dewey, Chairman, Department of Anatomical Sciences, Health Sciences Center, SUNY, Stony Brook, N.Y. 11790, by June 30, 1974. Applications from women and members of minority groups are encouraged. (2032)

UNIVERSITY OF SHEFFIELD

DEPARTMENT OF PSYCHOLOGY

APPROACHES TO THE STUDY OF EARLY BEHAVIOURAL DEVELOPMENT

Nato Advanced Study Institute, Catania, Sicily, 1-10 September 1974. Topics covered include genotype-environment interaction. Growth of brain and nervous system, language development, cognitive growth. Perception and skills. Social integration.

Further information from Professor K. J. Connolly, Department of Psychology, University of Sheffield, Sheffield S10 2TN, U.K. (2031)

PLYMOUTH POLYTECHNIC

SCHOOL OF ELECTRICAL ENGINEERING

RESEARCH ASSISTANTSHIPS

Vacancies exist in the School for Research Assistants to work on projects in the following fields from September 1974.

1. Digital Techniques applied to Television.
   Qualifications:- Degree in Electronic or Electrical Engineering or Physics.
2. Computer-Assisted Learning Systems in Electrical Engineering.
   Qualifications:- Degree in Electronic or Electrical Engineering or Physics or Mathematics.
3. The application of Thin Film Technology in the Teaching of Engineering and Science.
   Qualifications:- Degree in Electronic or Electrical Engineering or Physics or Materials Science.

Applicants should hold or be expecting to obtain, a good honours degree, or an equivalent qualification in a relevant discipline such as those listed, and the successful applicant will be expected to register for a higher degree.

Further details and application forms are available from, The Establisment Officer, Plymouth Polytechnic, Plymouth PL4 8AA, and must be returned by 7th June, 1974. (2053)

MEDICAL RESEARCH COUNCIL

TISSUE CULTURE

Applications are invited for an appointment (Scientific Staff scale) to join a group studying the effects of inhaled substances on the lung. The team requires a member to undertake studies in cell culture with particular emphasis on the effects of particles on macrophages. Applicants should have post-doctoral experience in the relevant field. Duties to commence on 1 October 1974.

Apply giving details of age, qualifications, and the names of two refrees to: Director, MRC Pneumoconiosis Unit, Llandough Hospital, Penarth, Glamorgan, CF6 1XW. (2052)

TRAINEE HOSPITAL BIOCHEMIST

Applications are invited from science graduates (or those expecting to graduate this year) for a 2 year appointment leading to an M.Sc. in Clinical Biochemistry starting 1st October 1974. The successful applicant will be required to attend the University of Surrey (Guildford) for a total of three terms. Training in clinical biochemistry, which includes a research project, will be given within laboratories in the Avon Area, including those of the Southmead Health District and the Bristol Health District which constitute the clinical teaching hospitals of the University of Bristol.

Salary will be on the scale for Hospital Biochemists in the range £1497 - £2259.

Applications (including the names of two referees) should be submitted before 19th June to the Personnel Officer, Southmead Hospital, Westbury on Trym, Bristol BS10 5NB. (2010)

PORTSMOUTH POLYTECHNIC

RESEARCH ASSISTANTS

BioMolecular Research Group

One assistant for research on the synthesis, modification and function of chromosomal proteins. The project, which is supported by the Science Research Council and the Cancer Research Campaign, uses the naturally synchronous division cycle in the true slime mould Physarum polycephalum and a specific gene isolated from the same organism.

The research will be largely biochemical in nature.

Crystallographic Research Group

One assistant to work on structural problems of biological interest. The present programme of work includes the investigation of the molecular configuration of substrates of enzymes in the glycolytic pathway.

Crystal Growth Research Group

One assistant to work on crystal growth from high temperature solutions and on studies of crystal perfection and surface topography.

Applicants must have, or expect to have, a god honours degree in a science subject. There will be opportunity to register for a higher degree.

Salary Scale: £1,230-£1,330 per annum (under review)

Application forms and further particulars may be obtained from the Staff Officer, Portsmouth Polytechnic, Alexandra House, Museum Road, Portsmouth PO1 2QQ, to whom completed applications should be returned by 10th June 1974.

Please quote ref. S12. (2051)

UNIVERSITY OF SOUTHAMPTON LECTURER IN THEORETICAL PHYSICS

Applications are invited for the above post in the Department of Physics. Candidates should have an active research interest in Quantum Physics. It is expected that the successful candidate will have experience in the use of fields and groups, and will interact effectively with the Theoretical High Energy Physics group in the Department. Salary scale: £2118 - £4896. The initial salary will depend on qualifications and experience. Further particulars may be obtained from the Deputy Secretary's section (ext. 2400). The University, Southampton SO9 5NH, to whom applications (7 copies from United Kingdom applicants) should be sent not later than 14th June 1974, quoting reference number 229/A/Na (2002)

UNIVERSITY OF DURHAM

DEPARTMENT OF ENGINEERING SCIENCE

Applications are invited for the post of **TEMPORARY LECTURER IN ENGINEERING SCIENCE** for one year from 1 October 1974. Applicants should have teaching, research or professional experience in either electrical or civil engineering. The undergraduate course in Engineering Science consists of a unified course in civil, mechanical and electrical engineering followed by specialisation in the final year.

The salary will be on the scale £1929 - £4548 (under review) per annum with FSSU.

Applications (3 copies) naming three referees should be sent by Monday, 10 June 1974 to the Registrar and Secretary, Science Laboratories, South Road, Durham, DH1 3LE. (2004)

UNIVERSITY OF EAST ANGLIA

SCHOOL OF MEDICAL SCIENCES

Applications are invited for a S.R.C. Post-Doctoral Research Assistantship for one year in the first instance from 1 October 1974, to work on the magnetic circular dichroism of matrix-isolated molecules. A novel apparatus for this purpose has recently been constructed and is now operational. The investigation is to be extended to the study of the magnetic and optical properties of unstable molecules generated photochemically. Salary will be on a scale starting at £1929 per annum plus F.S.S.U., according to previous experience.

Applications as soon as possible either to Dr. R. Grinter or Dr. A. J. Thomson, School of Chemical Sciences, University of East Anglia, Norwich, NOR 88C. (2005)

YOUNG SCIENCE GRADUATE wanted to assist in the laboratory investigation of patients with degenerative arterial disease. Please write for application form to Mr. Dormandy, Surgical Unit, Clare House, St. George's Hospital, Tooting, SW17. (2008)

JUNIOR TECHNICIAN required for Department of Histopathology to assist in research on multiple sclerosis and immunity in relation to the nervous system. O.N.C. or Science A levels an advantage. Apply in writing to the Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT, giving details of age, qualifications and experience, if any, and quoting Ref. H.P.1. (2013)

THE UNIVERSITY OF MANCHESTER

DEPARTMENT OF BIOCHEMISTRY

Applications are invited from graduates with good Honours degrees or from students graduating this June in either Biochemistry or Chemistry to undertake research leading to a higher degree. The research is in the field of Biophysical Chemistry and is concerned with the study of the properties of copolypeptides in aqueous solution. This is a CASE studentship in conjunction with Unilever Ltd. Applications, including the names of two refrees should be addressed to Dr. M. N. Jones, Department of Biochemistry, The University, Manchester, M13 9PL. (2047)

UNIVERSITY OF SOUTHAMPTON

DIRECTOR

University Computing Service

Applications are invited for the new full-time post of Director of the University Computing Service. The salary will be at an appropriate point within the University's professorial range and will consequently not be less than £5973 per annum. F.S.S.U. Further particulars may be obtained from the Academic Registrar, The University, Southampton SO9 5NH and applications (ten copies from applicants in the United Kingdom and one from others must be submitted before 30 June, 1974. (2009)

UNIVERSITY OF BRISTOL

DEPARTMENT OF PHYSICAL CHEMISTRY

Applications are invited for a temporary Research Assistantship at postdoctoral level on a two year project supported by the Science Research Council to investigate the structure of the electrical double layer in D.O. The post will involve both experimental and theoretical work and some experience in electrochemistry or related field is desirable. Commencing salary up to £2553 per annum (£2757 per annum after 1 October 1974).

Further information can be obtained from Dr. R. Parsons, School of Chemistry, Cantock's Close, Bristol BS8 1TS, to whom application giving curriculum vitae and the names of two referees should be sent as soon as possible. (2011)

UNIVERSITY OF BRISTOL

DEPARTMENT OF PATHOLOGY

REPRODUCTIVE IMMUNOLOGY RESEARCH GROUP POST-DOCTORAL RESEARCH APPOINTMENT

Applications are invited from Science or Medical Graduates for a post of Research Assistant or Research Associate within a group investigating immunological aspects of mammalian reproduction. Preference may be given to candidates with previous experience in this field but applications from those with a suitable background in immunology, reproductive physiology or developmental biology will be considered.

The post is available immediately and for up to 3 years in the first instance, with a salary on the scale £1,929-£3,048 depending upon qualifications and experience. F.S.S.U. subject to the accord of academic status.

Applications (2 copies) should include details of name, age, education, qualifications, research experience, publications, and the names of two referees and be submitted not later than June 14th 1974 to Dr. W. D. Billington, Department of Pathology, Medical School, University Walk, Bristol BS8 1TD, from whom further particulars may be obtained. (2042)

UNIVERSITY OF NEWCASTLE UPON TYNE

DEPARTMENT OF ORAL PHYSIOLOGY

Junior Research Associate

Applications are invited from graduates with an Honours Degree preferably in Biochemistry or Chemistry for a post as Junior Research Associate to assist in research into the fluoride of dental plaque with special reference to the prevention of tooth decay being undertaken in the Department of Oral Physiology. The appointment will be for a period of up to 3 years and provides an opportunity to work for a higher degree in the case of those who do not already hold such a qualification. Salary will be according to qualifications and experience on the scale £1,569-£1,818 per annum.

Applications should be submitted within three weeks of the appearance of this advertisement to Professor G. N. Jenkins, Department of Oral Physiology, The Dental School, The University, Northumberland Road, Newcastle upon Tyne, NE1 8TA. (2045)

UNIVERSITY OF BRISTOL

MATHEMATICAL STATISTICS

Applications are invited from probabilists and statisticians for the post of LECTURER IN STATISTICS in the School of Mathematics. The person appointed will have the responsibility for teaching undergraduates in a combined mathematics and statistics degree programme and for supervising the research of postgraduate students in Probability and Statistics. His own research interests should lie in the same fields. Ideally the candidate should be prepared to take up his duties from the autumn term 1974, but candidates whose commitments preclude their availability before January 1975 will be considered.

The salary will be on the revised scale £2,118-£3,813 per annum.

Further particulars may be obtained from the Secretary, University Senate House, Tyndall Avenue, Bristol BS8 1TH, to whom applications should be sent by 7th June 1974 (please quote reference EB). (2041)

UNIVERSITY OF BRISTOL

DEPARTMENT OF PATHOLOGY

A vacancy exists for a Research Technician, Grade 3, (with O.N.C. or equivalent) to join a small new team studying cellular hypoxia; experience in tissue culture is essential, and in histology desirable. The duties will involve the preparation and maintenance of brain-cell and fibroblast cultures, plus some histological and general preparative work. This is a 'funded' post, tenable at the School of Veterinary Science, Langford, and it carries a salary within the scale £1,650 x £54 to £1,920 p.a. The present grant, which is renewable, runs to December 1977 initially. Applications in writing, giving the names and addresses of two referees to Professor I. A. Silver, Department of Pathology, Medical School, University Walk, Bristol BS8 1TD. (2043)

THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF METALLURGY

POST-DOCTORAL RESEARCH ASSISTANT

Applications are invited for the post of RESEARCH ASSISTANT under an S.R.C. grant to Dr. H. A. Davies for research on the formation, structures and properties of NON-CRYSTALLINE ALLOYS PRODUCED BY ULTRA-RAPID QUENCHING OF MELTS. This is an interesting and established programme of research on a new class of materials that promise to find important commercial applications in the near future, for instance, as high strength reinforcing filaments. Candidates should possess or expect to obtain shortly a Ph.D. degree in Metallurgy, Materials Science or Physics. Experience in electron microscopic, X-Ray diffraction or calorimetric/D.T.A. techniques would be an advantage. Tenable for one year in the first instance, renewable for a further period to 30 June 1977. Starting salary in the range £1,929-£2,223 (under review), with F.S.S.U. provision. Further particulars from the Registrar and Secretary, The University, Sheffield S10 2TN to whom applications should be sent by 7 June 1974. Quote Ref. R.74/G. (2030)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF BOTANY

Lecturer in Ecological Genetics

Applications are invited for the above post commencing on 1st October, 1974. Applicants should possess a good honours degree and post-graduate experience in the ecological genetics of plants, plant breeding or plant population biology. Interest and experience in quantitative or mathematical approaches would be an advantage.

There will be excellent opportunities for collaborating with an active teaching and research group in evolution, population biology and plant breeding.

Initial salary within the range £2,118 - £2,412 per annum on a scale rising to £4,896 per annum.

Applications together with the names of three referees should be received not later than 22nd June, 1974, by the Registrar, The University, P.O. Box 147, Liverpool, L69 3BX, from whom further particulars may be obtained. Quote ref RV/276028/N. (2040)

TECHNICIAN

required to assist in immunological research in gastrointestinal disease. Laboratory experience involving immunological and histological methods advantageous. Commencing salary according to experience and qualifications within scale £1,440 to £1,509 plus £126 p.a. L.W. Apply, The Secretary, St. Mary's Hospital Medical School, Paddington, London W2 1PG quoting Ref. MUW. (2070)

THE UNIVERSITY OF LANCASTER DEPARTMENT OF BIOLOGICAL SCIENCES

PLANT PHYSIOLOGIST

Applications are invited for a 3-year post-doctoral appointment supported by N.E.R.C. The research will consist of a study of the responses of plants to SO_2 and NO_2 pollution. The post is suitable for post graduate students in whole-plant physiology or ecologists with experience of physiological methods who are expecting to graduate this year. Salary: £2,118 to £2,247 to £2,412.

Further particulars may be obtained (quoting reference L.828/C) from the Establishment Officer, University House, Lancaster LA1 4YW, to whom applications (5 copies), naming three referees, should be sent not later than June 8, 1974. (2100)

UNIVERSITY OF NEW SOUTH WALES

PROFESSOR OF GEOGRAPHY

This is an additional chair in the School of Geography within the Faculty of Applied Science. The new professor will share in the further development of this recently established school. He will be responsible for undergraduate and post-graduate courses in economic geography and in human geography generally, and will be expected to foster and supervise research in economic geography by staff and postgraduate students. Experience in applied economic geography is desirable. The present head of the School is Professor J. A. Mabbutt. Professor M. Chaikin, Dean of the Faculty of Applied Science, will be in England during June. Anyone who wishes to see him should write to the University Appointments Office (P.O. Box 1, Kensington, N.S.W. 2033, Australia) by 24 May 1974.

Salary: $A19,102 per annum. Subject to the consent of the University Council, professors may undertake a limited amount of higher consultative work. The University reserves the right to fill any chair by invitation.

Details of appointment, including superannuation, study leave and housing scheme, may be obtained from the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close in Australia and London on **28 June 1974.** (2036)

UNIVERSITY OF NEW BRUNSWICK

DEPARTMENT OF BIOLOGY

SCANNING ELECTRON MICROSCOPY TECHNICIAN

Required to operate and maintain a Cambridge S4-10 scanning electron microscope and related equipment. Duties will include specimen preparation, photography, and possibly some transmission electron microscopy. Most work will be with biological material.

Previous experience of scanning electron microscopy would be advantageous, but training will be given where necessary.

Starting salary up to $9,000 depending on qualifications and experience.

Application with names of two referees to Dr W. D. Seabrook, Department of Biology, University of New Brunswick, Fredericton, N.B., Canada. (2097)

McGILL UNIVERSITY

DEPARTMENT OF BIOLOGY

CHAIRMAN

Applications and nominations are invited for the abovementioned position which will be open on June 1, 1975. The Department of Biology consists of 51 full-time and affiliated academic staff representing most areas of biology with emphasis on Cell, Molecular and Developmental Biology, Ecology, Human Genetics and Neurobiology.

Qualifications sought include evidence of scholarship in research, skill in teaching, and administrative experience. Inquiries, nominations and applications (accompanied by a curriculum vitae and names of at least three referees) should be directed to:

Dean E. J. Stansbury
Faculty of Science
McGill University
P.O. Box 6070
Montreal H3C 3G1
CANADA (2096)

KING'S COLLEGE, LONDON
JUNIOR TECHNICAL OFFICER

An assistant is required to help with electron microscopy and diffraction studies in a laboratory which investigates macromolecular structure and function in muscle and other contractile systems. Previous experience in electron microscopy though desirable, is not essential. Applicants should have H.N.C. or two Science or Maths A levels, or equivalent. Salary is in the range of £1,173 to £2,073 (+ £126 London allowance), according to age and qualifications. Please write, giving full personal details, to Mr. J. Cribbin, Department of Biophysics, King's College, 26-29 Drury Lane,WC2.
Closing date June 14. (2099)

BIOCHEMIST

Applications are invited for a postdoctoral research position for work on the plasma membrane of **Dictyostelium discoideum** during aggregation and differentiation. A background in either membrane biochemistry or carbohydrate biochemistry would be helpful but is not essential. The appointment is available September 1, 1974 at a salary of $8,700.

Applications with full curriculum vitae and the names of three referees should be sent to Dr G. Weeks, Department of Microbiology, University of British Columbia, Vancouver, B.C., V6T 1W5, Canada.

Further details available on request. (2098)

SENIOR BIOCHEMIST

required by a leading British pharmaceutical Research Organisation, to initiate and direct research projects in the areas of pharmacokinetics, drug metabolism, molecular pharmacology and enzymology in association with the design and development of new medicines. Persons with appropriate experience are invited to apply in writing for further details to The Secretary, Biorex Laboratories Ltd., Biorex House, Canonbury Villas, London N1 2HB, marking the envelope CONFIDENTIAL.
(2090)

TOXICOLOGISTS/ PHARMACOLOGISTS

Honours Graduates/Post-Doctoral Fellows, with experience, required for Research and Short and Long Term Toxicological Studies of compounds of potential therapeutic importance. Excellent opportunities for advancement in modern, well-equipped laboratories. Pension and Assurance Scheme. Application forms from The Secretary, Biorex Laboratories Ltd., Biorex House, Canonbury Villas, London N1 2HB. (2089)

PHARMACOLOGISTS

Graduates/Post-Doctoral Fellows, with or without experience, required for Research and the Screening of Compounds of potential therapeutic importance and in the elucidation of their mode of action and toxicity. Vacancies are open for both Senior and Junior Pharmacologists. Excellent opportunities for advancement in modern, well-equipped laboratories. Application forms from The Secretary, Biorex Laboratories Ltd., Biorex House, Canonbury Villas, London N1 2HB. (2088)

FELLOWSHIPS AND STUDENTSHIPS

Liverpool Polytechnic
SCHOOL OF PHARMACY

SRC Studentship in Medicinal Chemistry

Applications are invited from candidates with a good honours degree in Pharmacy or Chemistry, or Grad. R.I.C. to work for a Ph.D. degree on the project "4-phenylpiperidine derivatives as potential non-addicting analgesics". The work will involve organic synthesis and stereochemical studies.

The project is a CASE award and the co-operating body is Allen and Hanburys Ltd., usual S.R.C. stipend applies which may be supplemented by payment for teaching duties and income during the industrial period.

Enquiries and applications to Dr. A. F. Casy, School of Pharmacy, N. Liverpool Polytechnic, Byrom Street, Liverpool, L3 3AF. (2033)

Lakehead University

DEPARTMENT OF CHEMISTRY
Post-Doctoral Research Fellowship

Several Post-Doctoral Fellowships are available at present for work in the following fields:

Kinetic and thermodynamic studies of gas/solid reactions (R. A. Ross)
Polymer photochemistry; dielectric studies of polymers (N. A. Weir)
Dielectric relaxation studies in organic liquids and solids (J. Crossley)

The fellowships will carry salaries up to $8,500 per annum. Applicants should state their preferred field of interest and forward a full curriculum vitae including the names of three referees to:

Mr. D. E. Ayre, Secretary, Lakehead University, THUNDER BAY "P", Ontario, Canada. P7B 5E1.
(2057)

THE UNIVERSITY OF LEEDS
DEPARTMENT OF COLOUR CHEMISTRY AND DYEING
WOLFSON ORGANIC POWDERS RESEARCH UNIT

Research Studentship Research Assistantship
Post-Doctoral Fellowship

Applications are invited for the above research posts to work on polymorphism in organic solids and the stabilisation of metastable organic dispersions. Current research in the Unit includes studies on the nucleation and growth of dye and pigment particles, their physico-thermal stability and the influence of conditions of precipitation on application properties. The research may be extended to include the study of pharmaceutical preparations and organic intermediates used in colour and related industries.

Applicants for the RESEARCH STUDENTSHIP should possess, or expect to be about to be awarded, a good honours degree in Physics or Chemistry. The terms will be equivalent to a Research Council Studentship and the holder will register for a Ph.D. degree. (Ref. No. 39/2/D).

Applicants for the RESEACH ASSISTANTSHIP should have similar qualifications but may not necessarily wish to read for a higher degree. This post will be initially for one year, with possible renewal, on the scale £1449 - £1818. (Ref. No. 39/3/D).

The appointment to the POST-DOCTORAL FELLOWSHIP will be for one year in the first instance, with possible renewal, at an initial salary of £1929 (under review) with F.S.S.U. (Ref. No. 39/4/D).

Applicants should give previous experience and the names and addresses of two referees. Forms of application and further particulars from the Registrar, The University, Leeds LS2 9JT (Please quote appropriate reference number). Closing date 1 July 1974. (2026)

RESEARCH STUDENTSHIPS IN PHARMACOLOGY

Applications are invited from graduates in pharmacology, biochemistry or physiology for Research Scholarships, to work on projects in neuropharmacology and immunopharmacology. Successful applicants will be expected to register for a higher degree.

Applicants should send their c.v. and names and addresses of two referees to Professor J. L. Mongar, Department of Pharmacology, UNIVERSITY COLLEGE LONDON, Gower Street, London WC1E 6BT. (1913)

UNIVERSITY OF LEICESTER
GEOLOGY
N.E.R.C. STUDENTSHIPS 1974/5

Applications are invited for three Postgraduate Studentships in the following three topics:

(1) Chemistry of diagenetic carbonate cements (Dr. J. D. Hudson).

(2) Geochemistry and mineralogy of stalactitic mineral deposists (Dr. T. D. Ford).

(3) Geophysical studies of the Gregory Rift, Kenya (Dr. M. A. Khan).

Successful applicants will be expected to register for a higher degree.

Enquiries should be sent to Dr. C. H. James, Department of Geology, The University, Leicester LE1 7RH. (1957)

UNIVERSITY OF SOUTHAMPTON
DEPARTMENT OF MATHEMATICS
Semiconductor Theory

Applications are invited for RESEARCH STUDENTSHIPS (C.A.S.E.) supported by the Science Research Council, which are available for theoretical work to investigate recombination and related problems by use of quantum mechanics and statistical mechanics. There will be contact with experimental work at Ferranti Limited and with government research laboratories. Posts are tenable for up to three years, and the work will be supervised by Professor P. T. Landsberg.

A good honours degree in Mathematics, Physics, Engineering Science or similar qualifications is essential and registration for an M.Phil. or a Ph.D. degree is encouraged.

Please apply as soon as possible giving the name of a referee to the Academic Registrar, The University, Southampton SO9 5NH. (1958)

UNIVERSITY OF SOUTHAMPTON
DEPARTMENT OF PHYSICS
POSTDOCTORAL RESEARCH FELLOW

Applications are invited from suitably qualified candidates for the above post, to join the magnetism group in an experimental investigation of the thermal expansion of magnetic materials near the critical temperature.

The post is financed by the Science Research Council and will be tenable for two years from October 1, 1974. The initial salary will be up to £2,223

Further details may be obtained by writing to the Deputy Secretary, The University, Southampton SO9 5NH to whom applications, giving brief curriculum vitae and the names of two referees should also be sent Please quote reference number: 226/R/Na. (1959)

UNIVERSITY OF SOUTHAMPTON
DEPARTMENT OF PHYSIOLOGY AND BIOCHEMISTRY
RESEARCH STUDENTSHIPS

Application are invited from graduates, or from those who expect to graduate this summer, for Research Studentships to undertake research work for higher degrees. The Department has a wide research interest in several areas of physiology and biochemistry.

Further details and application forms obtainable from Postgraduate Admissions Office, Department of Physiology and Biochemistry, Medical and Biological Sciences Building, The University of Southampton, Bassett Crescent East, Southampton SO9 3TU. (1961)

UNIVERSITY OF SOUTHAMPTON
DEPARTMENT OF CHEMISTRY

Applications are invited for the post of Lecturer in the Department of Chemistry. Candidates should have interest in any branch of Physical Chemistry. Salary scale: £2,118 to £4,896. The initial salary will depend on qualifications and experience. Further particulars may be obtained from the Deputy Secretary's Section (Ext. 2400), The University, Southampton SO9 5NH, to whom applications (7 copies from United Kingdom applicants) should be sent not later than June 14, 1974. Please quote reference number: 193/A/Na. (1962)

HERIOT-WATT UNIVERSITY - EDINBURGH
Postgraduate Research Studentships in Department of Physics
Prof. S. D. Smith **Tel. 031-449 5111**

Applications are invited for SRC, NERC and University Research Studentships tenable for 3 years from September 1974. The principal research interests of the Department are in solid state and laser physics, non-linear optics, tunable laser spectroscopy and satellite meteorology. Active and substantial projects are current on applications of the spin-flip Raman laser, opto-acoustic spectroscopy with tunable dye lasers and the remote measurement of atmospheric temperature and pressure from satellites. (1851)

THE UNIVERSITY COLLEGE OF WALES
Aberystwyth
DEPARTMENT OF BIOCHEMISTRY
RESEARCH STUDENTSHIP

The Science Research Council is prepared this year to offer to a suitable candidate a C.A.S.E. Research Studentship, tenable at this College. The studentship will be jointly supervised by Dr. P. W. Trudgill of the Department and Dr R. E. Cripps of Shell Research Ltd.

The successful candidate will be expected to spend about three months of each year at the Shell laboratories and for the remainder of the year will be based at Aberystwyth.

The project, a study of the metabolism of aromatic organophosphate insecticides by microorganisms, including pathway studies and the purification and characterization of associated mixed function oxygenases, is expected to lead to a higher degree.

Candidates should either have or expect to obtain this year a Class I or II (i) degree in Biochemistry. The studentship will commence on October 1, 1974 and will be tenable for up to three years. The value of the award is currently £695 per annum plus approved fees.

Enquiries and applications should be made as soon as possible to Dr P. W. Trudgill, Department of Biochemistry, University College of Wales, Aberystwyth. (Tel. 0970 3111, Ext. 100). (1967)

UNIVERSITY OF NOTTINGHAM
DEPARTMENT OF PHYSIOLOGY AND ENVIRONMENTAL STUDIES
S.R.C. C.A.S.E. POSTGRADUATE STUDENTSHIP

Applications are invited for a Research Studentship for studies on the role of hormone receptor proteins in the regulation of nuclear R.N.A. synthesis in plants. Candidates should hold or expect to gain a First or Upper Second Class Honours degree in Biochemistry, Botany or a related subject. The successful applicant will be expected to register for the degree of Ph.D.

Those interested should write to Dr D. Grierson, Department of Physiology and Environmental Studies, University of Nottingham, School of Agriculture, Sutton Bonington, Loughborough LE12 5RD as soon as possible. (1968)

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL
DEPARTMENT OF BIOCHEMISTRY
M.R.C. RESEARCH STUDENTSHIP

Applications are invited for an M.R.C. studentship which is available from October 1974. Candidates should hold a good Honours degree in chemistry or a biological science. The successful candidate will pursue a course of research, leading to a Ph.D. degree, involving a study of changes which occur in the protoplast membrane of *Bacilus amyloliquefaciens* during the secretion of extracellular enzymes with particular regard to the metabolism of membrane phospholipids.

Applications and enquiries should be made to Dr G. Coleman or Dr D. White, Department of Biochemistry, University of Nottingham Medical School, University Park, Nottingham NG7 2RD. Telephone 0602 56101. (1970)

RESEARCH STUDENTSHIP

Applications are invited for a three-year research studentship to study aspects of the vitamin requirements of the Golden hamster (**Mesocricetus auratus**). Applicants should have or expect to attain this summer, first or upper second class honours degree in nutrition, or a relevant biological discipline. The successful candidate who will register for further degree, will receive a grant plus a small travel and book allowance, and fees will also be paid.

Applications with names of two referees and short curriculum vitae should be made to Dr. D. J. Ford, Medical Research Council, Laboratory Animals Centre, Woodmansterne Road, Carshalton, Surrey SM5 4EF, from whom further details may be obtained. (2019)

UNIVERSITY OF EDINBURGH
RESEARCH FELLOW

A science or clinical graduate (Lecturer scale) is required for a research fellowship tenable for two years in the first instance in the Clinical Immunology Laboratories in the Royal Infirmary and No. 2 Forrest Road, Edinburgh, under the direction of Dr. W. J. Irvine. The successful applicant would be involved in the study of immune complexes in the sera of patients with cancer. A post-doctoral fellow is preferred, but applications from others are welcome.

Applications giving full details of age, qualifications and previous experience should be sent as soon as possible to the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh EH8 9YL. Please quote reference number 5020. (1978)

UNIVERSITY OF ABERDEEN
UNIT FOR RESEARCH ON ADDICTIVE DRUGS

A S.R.C. Studentship (Co-operative Award in Science and Engineering, C.A.S.E.), will become available on October 1, 1974. It is tenable for 3 years in co-operation with the Pharmaceutical Division of Reckitt and Colman Ltd., Hull, and is open to First and Upper Second Class Honours Graduates in Pharmacology, Biochemistry or Physiology. The research project is concerned with the action of newly-synthesized morphine-like compounds on neurotransmitter release and on behavioural patterns in normal and morphine-dependent animals.

Applications (3 copies) to be submitted to Professor H. Kosterlitz, from whom further particulars may be obtained, University of Aberdeen, Marischal College, Aberdeen AB9 1AS, not later than May 31, 1974. (1844)

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL
DEPARTMENT OF PHYSIOLOGY
Cardiovascular Research

Applications are invited from honours graduates or prospective honours graduates in physiology, pharmacology or a related discipline, for a studentship for training in research methods tenable from October 1974. The holder will be eligible for registration as Ph.D. student of the University. The value of the award and conditions of appointment will be those of M.R.C. Studentships.

Facilities are available for the postgraduate student to join a group working on several aspects of autonomic nervous control of the cardiovascular system in animals and in man, under the supervision of Dr. P. H. Fentem. Applicants should write to Dr. Fentem giving a brief curriculum vitae and the names and addresses of two referees, at the Department of Physiology, The Medical School, University of Nottingham, Nottingham NG7 2RD. (1820)

UNIVERSITY OF LONDON
King's College
RESEARCH STUDENTSHIP IN BIOCHEMISTRY

An S.R.C. research studentship is available for three years from October 1974 leading to a Ph.D. degree. The successful candidate will work with Dr. C. J. Chesterton on a project in eukaryotic molecular developmental biology.

Applicants should hold, or expect to attain, a first or upper second class honours degree in biochemistry or an allied subject.

Applications (naming two referees) should be sent to Professor H. R. V. Arnstein (N), Department of Biochemistry, King's College, Strand, London WC2R 2LS. (2020)

UNIVERSITY OF KEELE
RESEARCH STUDENTSHIP IN CHEMISTRY

Applications are invited for a Co-operative award in Science and Engineering (C.A.S.E.) for a graduate to carry out an investigation of volatile pheromones in insects (Dr E. D. Morgan) jointing with Rothamsted Experimental Station, Harpenden, Herts. The usual regulations for S.R.C. studentships apply. Information and application forms from Dr Morgan, Chemistry Dept., Keele University, Keele, Staffs. ST5 5BG. (1976)

THE DENTAL SCHOOL
UNIVERSITY OF BIRMINGHAM

Applications are invited from graduates in Biochemistry or Chemistry for a Research Studentship available in the Department of Oral Pathology in the Dental School. The project, which will be suitable for registration for a Ph.D., is on the subject of the composition and properties of the organic components of biological calcifications. The investigation will have particular reference to dental plaque and its mineralisation but a comparative approach involving study of microbial calcification and both normal and pathological calcifications is envisaged. No specialised dental background is required.

The award is of £695 p.a. (subject to review) plus the payment of fees for Ph.D. registration.

Further details may be obtained from the Assistant Registrar, The Dental School, St. Chad's Queensway, Birmingham B4 6NN, to whom applications, including curriculum vitae and names of three referees, should be sent. (1984)

UNIVERSITY OF EDINBURG
RESEARCH STUDENTSHIPS IN CHEMISTRY

Applications are invited for CASE studentships, financed by the Science Research Council, and tenable for 3 years from 1st October, 1974 for research in catalytic oxidation, colloidal stability, reactions of excited oxygen, or heterocyclic cyclo-addition reactions, and for another studentship for heterogeneous caavsis.

Applicants should write before 15th June, 1974 giving brief curriculum vitae and the names of two referees to Professor C. Kemball, Department of Chemistry, University of Edinburgh, West Mains Road, Edinburgh, EH9 3JJ. (1992)

QUEEN ELIZABETH COLLEGE
(University of London)
Atkins Laboratories
Campden Hill, London W8 7AH

Applications are invited for two S.R.C. C.A.S.E. Studentships for research into the chemistry of degradation and stabilisation of polymers. The research would involve work in one or more of the following areas: synthetic organic chemistry, organometallic chemistry, polymer chemistry and isotopic tracer techniques; for which excellent facilities are available. There will be a close collaboration with the Research Department of I.C.I. Plastics Division, Welwyn Garden City, where the students will spend a short time each year. Applications, together with the names of two referees should be forwarded to the Secretary, Chemistry Department (N), as soon as possible. (2023)

UNIVERSITY OF
NEWCASTLE UPON TYNE
SCHOOL OF CHEMISTRY
SCIENCE RESEARCH COUNCIL C.A.S.E. STUDENTSHIPS

Applications are invited from suitably qualified candidates for three C.A.S.E. studentships to be held in the Department of Organic Chemistry from October 1974 for research leading to the Ph.D. degree.

The usual conditions of eligibility and stipend for S.R.O. Studentships apply.

One award associated with Imperial Chemical Industries, Pharmaceuticals Division, will be concerned with a study of metal complexes of physiologically active molecules. Another award associated with Bush Boake Allen Ltd., will be for the study of novel methods of terpene synthesis using organometallic and other intermediates. The third award associated with Beecham Research Laboratories will be concerned with the synthesis of penicillin and cephalosporin analogues.

For further details and application forms write to Mr. S. J. Hall, The School of Chemistry, The University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU. (2016)

IMPERIAL COLLEGE OF
SCIENCE AND TECHNOLOGY
DEPARTMENT OF BOTANY
RESEARCH FELLOWS

One or more Research Fellows, expected to assist in undergraduate teaching preferably in plant physiology, or cytology and ultrastructure, or phylogeny and taxonomy, and to undertake independent research, are required from October 1, 1974.

Applicants should normally hold a Ph.D. degree. Given this qualification, the initial salary will be in the range of £2,118 to £2,412 plus £162 London allowance. Appointments will be renewable annually up to a maximum of 3 years.

Further details available from Professor A. J. Rutter, Department of Botany, Imperial College of Science, Prince Consort Road, London, S.W.7, to whom applications, giving details of education experience and research interests, and the names of two academic referees, should be made by June 14. (2025)

UNIVERSITY OF KENT
AT CANTERBURY
RESEARCH FELLOW IN CHEMISTRY

Applications are invited for a post-doctoral research fellowship, initially for one year with the possibility of extension, to work with Professor R. F. Hudson on Radical Rearrangements and e.s.r. Spectroscopy. Salary £2118 (1974/75 scale). Application forms and particulars from the Assistant Registrar, Chemical Laboratory, The University, Canterbury, Kent CT2 7NH. Closing date 7th June. Please quote ref. A35/74. (2001)

UNIVERSITY OF IBADAN
Nigeria

Applications are invited for the following posts:-

1. LECTURER/MEDICAL RESEARCH FELLOW IN NUTRITION in the Food Science and Applied Nutrition Unit. Candidates must be registered medical practitioners with post-graduate training on one or more of the following: Nutrition, Public Health, Child Health. Preference will be given to those with field experience in any or all of these fields in an African environment. There are possibilities of further training in the field of Nutrition. Duties of appointee, will include co-operation with members of staff in research of Public Health Nutrition, including the assessment of nutritional status of communities and the planning and execution of applied nutritional programmes; teaching of public health nutrition to medical students and other paramedical staff and the awakening of awareness of nutrition in the general population.

2. LECTURER IN MEDICAL BACTERIOLOGY in the Department of Medical Microbiology. Candidates must be medically qualified, have a Diploma in Bacteriology or equivalent and have appropriate experience in teaching, research and diagnostic work. Interest and experience in the clinical application of the subject in the tropics would be an advantage, particularly in the field of Microbiology of Venereal Diseases. Appointee (1) must be experienced in advising clinical colleagues on the treatment of patients, when requested to do so and (2) organise and supervise diagnostic service for the Venereal Diseases and Treponematosis Research Unit. The post carries honorary Consultant appointment at the University College Hospital, Ibadan.

Salary scales: Lecturer (Consultant Status) N4,880 - N5,800 p.a. Research Fellow (Medical Clinical) N3,960 - N5,800 p.a. Lecturer (Non Consultant) N3,960 - N4,830 p.a. Lecturer/Research Fellow (Medical Pre-Clinical) N2,760 - N4,830 p.a. (£1 sterling=N1.54). The British Expatriates Supplementation Scheme is unlikely to be applied to these appointments. Family passages; various allowances; superannuation scheme; biennial overseas leave. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail, not later than 12 June 1974 to the Registrar, University of Ibadan, Ibadan, Nigeria. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London, W1P 0DT. Further particulars may be obtained from either address. (2085)

UNIVERSITY OF KENT
AT CANTERBURY
Tutorial Fellowship in Biochemistry

Applications are invited for the post of Tutorial Fellow in Biochemistry. Preference will be given to candidates with an interest in mammalian biochemistry of physiology. The appointment would be for 3 years and the salary, depending on age and qualifications, inthe range £2,118 to £2,412 (1974/75 scale). Application forms and particulars may be obtained from the Assistant Registrar, Faculty of Natural Sciences, Chemical Laboratory, The University, Canterbury, Kent CT2 7NH. Closing date for applications: June 14, 1974. Please quote ref. A51/74. (2024)

UNIVERSITY OF YORK
DEPARTMENT OF PHYSICS
POSTDOCTORAL RESEARCH FELLOW

Applications are invited for the above post from as early a date as possible for a period of two years. The successful applicant will work on the construction and evaluation of a scanning Auger electron microscope. The project is sponsored by the R. W. Paul Fund of the Royal Society and the research wil involve modifying a UHV scanning electron microscope equipped with a field emitting source so as to present images derived from the output of a concentric hemispherical electron energy analyser. Experience in these or related topics would be an advantage.

Salary £2118 - £2247, with FSSU.

Three copies of applications, naming two referees, should be addressed by 15 June 1974 to the Registrar, University of York, Heslington, York, Y01 5DD, from whom further details are available. Please quote reference number 11/6017. (2003)

UNIVERSITY COLLEGE CARDIFF
Department of Applied Mathematics and Astronomy

Applications are invited from those possessing or expecting to have a first or Upper Second Class Honours Degree in Mathematics or Physics for a CASE studentship in collaboration with the Royal Radar Establishment, Malvern. The work involves the calculation of energies of formations of vacancies using Greens' function methods and applicants will be expected to register for a Ph.D. Applications to Dr. T. Lukes, Dept. of Applied Mathematics and Astronomy, University College, P.O. Box 78, Cardiff, CF1 1XL. Please quote ref. 0563. (2012)

UNIVERSITY OF STRATHCLYDE
DEPARTMENT OF PHARMACEUTICAL CHEMISTRY
DRUG METABOLISM UNIT PROSTGRADUATE STUDENTSHIPS

Applications are invited from Honours graduates and students in their final year of undergraduate study in Pharmacy, Chemistry or Biochemistry for postgraduate studentships available for research in the Drug Metabolism Research Unit leading to a higher degree. Current research includes investigation of metabolic pathways, analytical methodolgy in pharmacokinetics, protein-binding and drug interactions, and studies on conjugating and desulphurating enzymes.

Studentships will provide for the payment of fees and a maintenance allowance of not less than £675 for students living in hostel or lodgings.

Applications to Professor J. B. Stenlake, Department of Pharmaceutical Chemistry, Royal College, University of Strathclyde, 204 George Street, Glasgow, G1 1XW. (2060)

HERIOT-WATT UNIVERSITY, EDINBURGH
DEPARTMENT OF BREWING & BIOLOGICAL SCIENCES

Applications are invited for a S.R.C. CASE research studentship for studies on the role of polysaccharides in the molecular architecture of cereal grains. The co-operating body is the Brewing Industry Research Foundation.

Applicants must hold a first or upper second class honours degree in chemistry or biochemistry.

Further details may be obtained from Professor D. J. Manners, Heriot-Watt University, Chambers Street, Edinburgh, EH1 1HX. (2056)

UNIVERSITY OF STRATHCLYDE
DEPARTMENT OF BIOCHEMISTRY
RESEARCH STUDENTSHIP

An M.R.C. research studentship is available for the Department of Biochemistry, for work in the field either of Biochemical Pharmacology (including pharmacokinetics), or in the Biochemistry of Reproduction, including ovarian, pituitary and uterine metabolism. Extensive facilities are available for work in these areas.

Applicants, who should possess or expect to possess a good Honours Degree in Biochemistry, Pharmacology, or a relevant subject, should submit applications to Professor P. J. Heald, Department of Biochemistry, University of Strathclyde, Royal College, 204 George Street, Glasgow, G1 1XW from whom further information can be obtained. (2059)

UNIVERSITY OF ST. ANDREWS
DEPARTMENT OF BIOCHEMISTRY
S.R.C. Postdoctoral Research Fellowship

Applications are invited from candidates with a Ph.D. for the above post tenable for two (possibly three) years from July, 1974. The work will involve the development of immobilised enzyme structures and the evaluation of these materials as analytical reagents in automated analysis. Starting salary £1929; F.S.S.U. Applications, with the names of 2 referees, should be sent to Dr. W. E. Hornby, Department of Biochemistry, University of St. Andrews, Fife by 7th June, 1974. (2055)

THE UNIVERSITY OF ASTON IN BIRMINGHAM
DEPARTMENT OF BIOLOGICAL SCIENCES

Applications are invited for RESEARCH STUDENTSHIPS in:—

1 The metabolic, nutritional and endocrine aspects of fish culture.
2 The physiology and biochemistry of isolated islets of Langerhans.

Candidates will be expected to have at least a 2(1) degree in Biology, Biochemistry or Physiology.

Further details are available from Professor A. J. Matty, Department of Biological Sciences, The University of Aston, in Birmingham, Gosta Green, Birmingham B4 7ET. (2048)

UNIVERSITY OF ABERDEEN
GENETICS DEPARTMENT
RESEARCH STUDENTSHIPS

Applications are invited for Postgraduate Studentships leading to a Ph.D. Degree. Research interests in this Department include chromosome polymorphism, comparative study of DNA organization, mutagenesis and repair, recombination, plant biochemical genetics, ecological genetics of plant and animal populations, human cytogenetics, etc.

Applications, with names of two refrees, should reach Professor F. W. Robertson, Department of Genetics, Zoology Building, 2 Tillydrone Avenue, Aberdeen, AB9 2TN, not later than June 7th, 1974. (2054)

UNIVERSITY OF ESSEX
N.E.R.C. RESEARCH STUDENTSHIP—Geological Sciences

Applications are invited for postgraduate studies into (i) mechanisms of deformation and recovery in carbonate rocks and (ii) the microstructure of stony meteorites. Electron microscopy, a new technique to the geological sciences, will be central to both projects.

The studentship, tenable in the Physics Department, is open to students with good honours degrees and has a value of £695 plus fees (under review).

Applications should be made to Dr. D. J. Barber, Physics Department, University of Essex, Wivenhoe Park, Colchester C04 3SQ. (2050)

UNIVERSITY OF BRISTOL
Long Ashton Research Station
POST DOCTORAL FELLOWSHIP

Applications are invited for a post-doctoral fellowship, sponsored by a consortium of wine companies, to investigate the anti-microbial effects of sulphur-dioxide and sorbic acid on wine spoilage yeasts, in studying the quantitative effect of inhibitors on micro-organisms. Experience in the use of isotopes

Applicants should have a doctorate and experience would be an advantage.

The appointment will be for two years, the starting date is by agreement. Salary £2,413 -£2,497 per annum.

Applications to the Secretary, Research Station, Long Ashton, Bristol, BS18 9AF, **by 17th June, 1974,** from whom application forms and further particulars can be obtained. (2080)

THE AUSTRALIAN NATIONAL UNIVERSITY
RESEARCH SCHOOL OF CHEMISTRY
Fellows or Senior Fellows

The School proposes to make up to three appointments in research areas primarily concerned with organic chemistry, and one appointment in the area of physical chemistry.

The appointments would be at the level of Fellow or Senior Fellow, for five years in the first instance with the probability of re-appointment to retiring age. These classifications, which are particular to the Australian National University, span in terms of salary and status the categories lecturer to reader in other Australian Universities.

Applications in the following fields are of particular interest to the School:

- Physical organic chemistry
- Synthetic organic chemistry, with emphasis on photochemical, electrochemical or other novel procedures.
- Theoretical chemistry related to organic applications and
- Physical aspects of the photochemistry of organic solids.

Applicants for the post in the physical-theoretical group should have interests complementary to photochemical research already represented in the School in visible and ultraviolet spectroscopic photochemical studies (Dr. J. Ferguson, Professorial Fellow), EPR and NMR (Dr. R. Bramley, Fellow), and theoretical work (Professor D. P. Craig). Possible areas are structoral and other aspects of defective solids in relation to photochemistry and topochemistry.

The organic group of the School works under the guidance of Professor A. J. Birch, Mr. R. W. Rickards (Professorial Fellow), and Dr. J. K. MacLeod (Senior Fellow).

The appointees would principally conduct independent research, with some support by post-doctoral workers and Ph.D. students, and would be expected to assist with other aspects of post-graduate training and School administration. The School is non-departmental, and opportunities exist for co-operation with other groups in the School.

The School is well equipped to contemporary standards, especially in optical, NMR and EPR spectroscopy, mass spectrometry, X-ray crystallography and cryogenics. There are comprehensive workshop facilties. In computing there is access to the School PDP 11 and the University Univac 1108 systems.

Salary on appointment will be in accordance with qualifications and experience within the ranges: Senior Fellow $14,308 - $16,462 per annum, Fellow $10,432 - $14,288 per annum. All amounts are in Australian currency. Current exchange rates are approximately $A1 : 67 NP : $US 1.49.

Reasonable travel expenses are paid and assistance with housing is provided for an appointee from outside Canberra. Superannuation is on the FSSU pattern with supplementary benefits.

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

There is no application form. Applicants should supply by **15 July 1974** a curriculum vitae, list of publications, a statement of research interests, and the names and addresses of three academic referees together with tow passport-sized photographs.

G. E. Dicker
Acting Academic Registrar

P.O. BOX 4,
Canberra, ACT, 2600
Australia. (2037)

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL
DEPARTMENT OF BIOCHEMISTRY
S.R.C. CASE Studentship

Applications are invited from graduates who hold Honours Degrees in Chemistry, Biochemistry, Biology or related subjects at the First or Upper Second Level, and from those who hope to have such qualifications by October, 1974, for a C.A.S.E. Studentship concerned with research into sterol ester metabolism in mammalian skin. This will be carried out mainly at the University of Nottingham and in part at the Environmental Safety Division, Unilever Research Laboratory, Colworth House, Bedford, and should lead to a higher Degree.

Research will entail a study of sterol ester metabolism in relation to other lipids in skin, with particular emphasis on prostaglandins, and will also involve fractionation of mammalian skin into its constituent components and cell types, subcellular fractionation of skin, and the effect of environmental change upon sterol ester metabolism in skin.

Further details and application forms may be obtained from Professor J. N. Hawthorne, Department of Biochemistry, The Medical School, The University of Nottingham, Nottingham NG7 2RD. (2092)

AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited for the following:

RESEARCH SCHOOL OF PHYSICAL SCIENCES
Research Fellow/Postdoctoral Fellow in Theoretical Physics

The Department of Theoretical Physics has an academic staff of fifteen headed by Professor K. J. Le Couteur, FAA. For this appointment preference will be given to applicants interested in statistical mechanics. The permanent staff members in this area are Dr. K. Kumar and Dr R. J. Baxter. The Department also has research interests in the general field of nuclear physics, elementary particle physics and solid state physics.

Closing date: **14 June 1974.**

SALARIES: Salary on appointment will be in accordance with qualifications and experience within the ranges: Research Fellow $A8,698 - $11,901 per annum, Postdoctoral Fellow $A8,698 - $11,901. Current exchange rates are approximately $A1 : 67NP : $US1.49.

OTHER CONDITIONS—Tenure: Research Fellow normally for three years in the first instance with possible extension to a maximum of five years; Postdoctoral Fellow for not less than one year and not more than two years.

Reasonable travel expenses are paid and assistance with housing is provided for an appointee from outside Canberra. Superannuation is on the FSSU pattern with supplementary benefits.

Travel expenses from overseas (except New Zealand) are not paid for posts at the level of Senior Tutor but assisted passage can be considered for a person offered appointment from the United Kingdom who intends to settle permanently in Australia

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should write to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF for further particulars before applying. (2038)

UNIVERSITY OF BATH
S.R.C. STUDENTSHIPS IN BIOCHEMISTRY

ENZYME CHEMISTRY AND TECHNOLOGY RESEARCH STUDENTSHIP

The project will involve the study of active sites of dehydrogenases using fluorinated substrates. This research is part of a general programme using ^{19}F n.m.r. to study enzyme—substrate interactions.

C.A.S.E. STUDENTSHIP IN CONJUNCTION WITH UNIGATE LTD.

Membrane surface glycoproteins of the mammary cell will be studied using the milk fat globule membrane as a convenient large scale source of membrane material. The major membrane glycoprotein will be isolated and structurally characterised. Its contribution to surface antigenic effects will be examined using immunochemical techniques.

Applications for the above are invited from candidates who have or expect to receive a first or upper second class Honours Degree in Biochemistry or Chemistry. Full particulars should be included in a letter addressed to Registrar (S), The University, Bath BA2 7AY, quoting reference 74/85. (2061)

CHELSEA COLLEGE
University of London

Applications are invited for the post of POST-DOCTORAL RESEARCH FELLOW as member of an S.R.C. supported team investigating THE INTERACTION OF THE HUMAN THERMO-REGULATORY AND CARDIOVASCULAR CONTROL SYSTEMS in collaboration with the Engineering in Medicine Laboratory at Imperial College.

The successful candidate will probably be a Physiologist with an interest in human experimentation and data analysis, but applications from candidates with other backgrounds but a real interest in the project will be welcomed.

The appointment will be for three years commencing as soon as possible. Salary £1,746 to £1,920 to £2,091 per annum. Superannuable. Application forms from the Personnel Office, Chelsea College, Manresa Road, London SW3 6LX. (2064)

UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF CHEMISTRY
Atmospheric photochemistry of sulphur dioxide

Applications are invited for a S.R.C. supported post-doctoral RESEARCH FELLOWSHIP. The work will involve both kinetic and spectroscopic techniques in the near and vacuum ultra-violet regions.

Salary: £2,118 to £2,412 plus F.S.S.U.

Further details may be obtained from Drs J. A. Kerr and J. P. Simons (021-472 1301) or the Assistant Registrar (S), P.O. Box 363, Birmingham B15 2TT, to whom applications (3 copies) naming 3 referees by June 21, 1974. Please quote reference: NC4. (2087)

UNIVERSITY OF WARWICK
POSTDOCTORAL RESEARCH FELLOW IN MOLECULAR BEAM SCATTERING

Applications are invited for an SRC Postdoctoral Research Fellowship in the Department of Molecular Sciences to work on the investigation of four centre ion-molecule reactions. This will include experimental work using an existing crossed molecular beam apparatus at the Thornton Research Centre and the calculation of potential energy surfaces and scattering calculations. The applicant should have experience in high vacuum work and preferably in molecular beams. The post is available immediately and will be for one year in the first instance with the possibility of renewal for a further two years. The starting salary will be in the range £2,118-£2,580 p.a. with FSSU. Application forms and further particulars may be obtained from the Academic Registrar, University of Warwick, Coventry CV4 7AL, quoting Ref. No: 42/Q/74; completed applications should be returned as soon as possible. (2029)

UNIVERSITY OF GLASGOW
DEPARTMENT OF CHEMISTRY
RESEARCH STUDENTSHIPS

Applications are invited for SRC CASE Research Studentships for work on the following topics: Prostaglandin Synthesis using Optically Active Precursors (directed by Dr. A. J. Baker in collaboration with Reckitt & Colman Ltd.); Synthetic Reactions involving Stabilised Carbanions (Dr. E. W. Colvin; I.C.I. Ltd., Pharmaceuticals Division); β-Lactam Derivatives related to Cephalosporins (Dr. E. W. Colvin; Pfizer Ltd.) and; Sulphur-Bridged Bicycle Systems (Dr. P. H. McCabe; I.C.I. Plant Protection Ltd.). Applications, including a brief curriculum vitae and the names of two referees, should be sent to Professor G. W. Kirby, Chemistry Department, University of Glasgow, Glasgow, G12 8QQ.

In reply please quote Ref. No. 3466M (2039)

MSc in Biodeterioration of Materials

Applications are being considered now for the 1974/75 session starting in September. SRC Advanced Course Studentships are available to suitable candidates.

Details and application form from
Administrative Assistant
Department of Biological Sciences
Portsmouth Polytechnic
Park Road, Portsmouth
Tel. Portsmouth (0705) 27681 Extn. 322

(2049)

BRUNEL UNIVERSITY
SCHOOL OF BIOLOGICAL SCIENCES
Part-Time Masters Courses

Applications are invited for admission to the following 2-year part-time postgraduate courses beginning in October.

1. M.Sc. in Applied Immunology
2. M.Sc. in Brain Studies
3. M.Sc. in Applied Cell Science and Virology
4. M.Tech in Weed Biology
5. M.Tech. in Environmental Pollution Science

Applicants for these courses should normally possess an honours degree in an appropriate subject.

Further information can be obtained from The Secretary, School of Biological Sciences, Brunel University, Uxbridge, Middlesex, U.K. (2079)

LECTURES AND COURSES

FEBS Advanced Course No. 32

Ion Transport and Mitochondrial Function

Budapest 31 August—1 Sept. 1974

This Advanced Course will take the form of a colloquium in which a panel of experts will lead discussions on (1) Transport of Substrate Anions: Mechanisms and Physiological Implications; (2) Relationship between Proton Transport and Mitochondrial Function; and (3) Transport of Cations: Mechanisms and Physiological Implications.

Participants may be able to present 10-minute communications on relevant results.

Fee: Swiss Fr. 30.00.

Enquiries as soon as possible to Dr. A. Fonyo, H-1082 Budapest, Ülloi ut 78/a, Hungary. (1842)

'ETTORE MAJORANA' CENTRE FOR SCIENTIFIC CULTURE

INTERNATIONAL SCHOOL OF THEORY AND APPLICATION OF COMPUTERS

3rd Course: DATA. Erice, Sicily. 2–21 September 1974

TOPICS: Transmission and Networks; Storage and Access; The Rôle of Small Computers; Special Processors.

PARTICIPANTS on the average will have a few years' experience in one of these topics and will wish to extend their knowledge in the wider field.

COST (fees plus board and lodging) 1,000 Swiss Francs.

Further details/applications to: Dr T. Massam, CERN 1211 Geneva 23, Switzerland. (2093)

GRANTS

LARGE TELESCOPE USERS

Further to the preliminary announcement regarding applications for time on the Anglo-Australian Telescope, it is still not yet known exactly what auxiliary equipment will be available during the first half of 1975, but some observations at the prime focus and the Cassegrain focus will be possible. Applications for time from January to June 1975 inclusive are invited, and should reach Mr. C. A. Murray at the Royal Greenwich Observatory, Herstmonceux Castle, Sussex by June 10, 1974. (2076)

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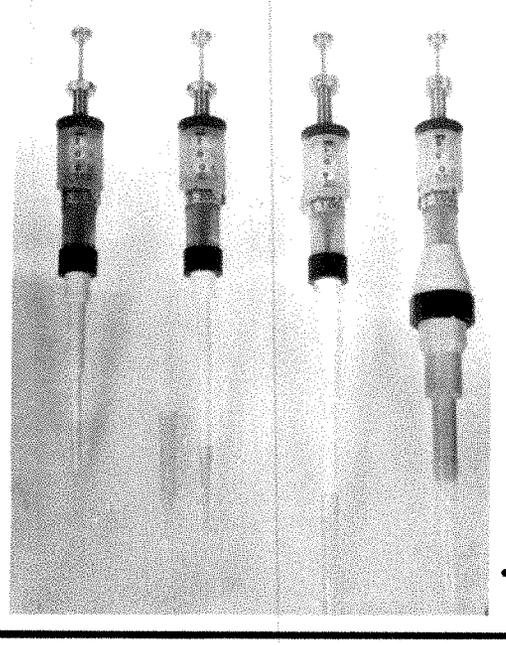
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Vol. 3
A. E. Needham
The Significance of Zoochromes

By **Arthur E. Needham,** Department of Zoology, South Parks Road, Oxford, Great Britain
With 54 figs. XX, 429 pp. 1974. Cloth DM 68,–; US $26.20
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After considering the molecular basis of color, the chemical classes of zoochrome, their distribution among animals and within their bodies, the author concentrates on the functions of zoochromes. He deals first with physiological functions, those based primarily on physical properties, and then with biochemical functions, based primarily on chemical properties. In the rest of the book he seeks to assess the extent to which all zoochromes have functional significance. This involves considering their relationships to excretory metabolism and to pathological, reproductive, and developmental conditions, the sophistication of physiological and genetic controls of chromogenesis, and the evolution of biochromes. A further aim is to test the unity and validity of "chromatology" as a subject. The book represents a new departure and is addressed to zoology teachers and students, zoologists, physiologists, and biochemists. It will also appeal to chemists with an interest in biology.

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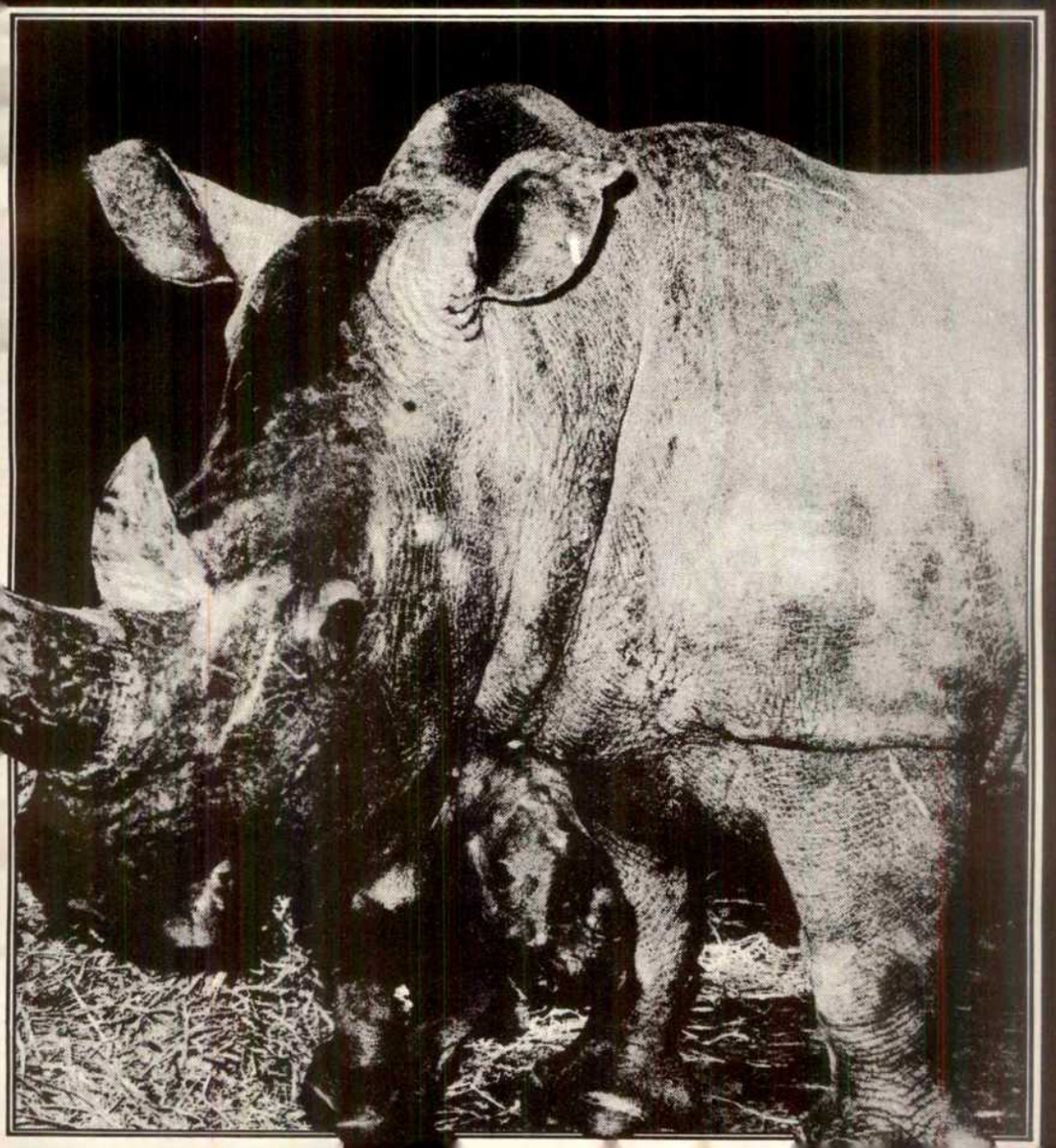
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Monthly — Volume 1 1974

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Mungo: a white rhino born at Whipsnade last year. Zoological Society's annual report: page 401.

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Nuclear hypocrisy in India

'NOTHING will better serve the interests of those who are hostile to us than for us to lose our sense of perspective and to undertake measures which would undermine the basic progress of the country. We believe that to be militarily strong, it is equally important to be economically and industrially strong'. Thus Mrs Gandhi in 1968 in declaring that India would develop her atomic energy programme exclusively for peaceful purposes. In the strictest sense, the recent detonation by India of a nuclear device does not violate any of the carefully worded statements of the past. But if the Indian government believes it can explain away its possession of nuclear devices by careful wording of statements, and can justify a peaceful-purposes programme on economic grounds it is guilty of wicked hypocrisy. Whatever the celebrations in India, scientists there should know that they gain no increased respect from scientists in other countries for producing a nuclear explosive, rather they lose it for being party to such a transparent deception.

The explosion was detonated in the Rajasthan desert—a mere 90 miles from the Pakistan border—and had a yield of 10 or so kilotons. It was fired sufficiently close to the surface (perhaps no deeper than 100 metres) so that it produced a large crater, the purpose of which, apparently, is water storage. There are no immediate reports of radioactive leakage so the experiment must have been done with some delicacy. There have been several cratering shots both in the United States and the Soviet Union and the literature on the subject is relatively open.

Can a nuclear explosives programme be purely peaceful? There is nothing in the science and technology which differentiates between peaceful and military devices, so the distinction can only be in the mind of the firer. Whatever Mrs Gandhi may say about her own intentions she is not going to dismantle the devices when she resigns from office, and her successor could well have much more aggressive intentions. Even in Mrs Gandhi's term of office a political situation could easily arise in which the Government 'with the greatest reluctance' was forced to consider the option of commandeering peaceful devices for warlike purposes. Thus India possesses all the psychological and physical ingredients for a nuclear deterrent and deceives none of its neighbours by talking about making holes and mining copper. The means of delivery are also there in the form of Canberra bombers; despite recent pronouncements India does not need missiles to become a military nuclear power to be reckoned with.

Can India afford it? It costs several hundred million pounds to develop nuclear devices. The cost of the hole in the desert and of any future project could if produced in more conventional ways hardly amount to this—and could employ and feed large numbers of people. If the programme is to be justified on its peaceful benefits, and is not to 'undermine the basic progress of the country' the government has a lot of explaining to do to its people, particularly the hungry ones. For a country with a GNP per capita of £40 there are surely more effective economic ventures than this.

What will be the impact on other potentially nuclear countries? The test is bound to trigger concern in Pakistan, but a more important question is the reaction of many other countries with nothing to fear from India. There are several abstainers from the Non-Proliferation Treaty who are bound to feel increasing pressure as a result of India's action, notably Israel and South Africa. India has compounded the seriousness of the situation by showing not only that even a poor country can make nuclear devices but also that there is a way in which they can be given some sort of flimsy justification in terms of peaceful uses.

Two golden opportunities have been lost. If India had been prepared to press her need for peaceful nuclear explosives in international circles, it is not out of the question that she could have been responsible for establishing some sort of agency which would have removed peaceful explosions from their present national basis. And if the present nuclear countries had busied themselves more over nuclear arms control in the past 5 years, they would have a better moral platform from which they could harangue proliferators.

100 years ago



AN excellent device has been forwarded to us for use in field-club excursions. It is designed to promote an interest in common flowers, and can of course be varied and worked without a prize. It consists of a large envelope, with a description, but not the name, of a plant, and directions as to what ought to be done with the plant when found. The particular envelope, forwarded to us by Mr. Higgins of the Liverpool Naturalist's Club, contains the following on its back :—

EXTRA PRIZE.

DESCRIPTION OF PLANT.

Leaves opposite, Sessile, Lanceolate, Acuminate.
Sepals 5, half as long as the 5 deeply-cleft Petals.
Stamens 10, Styles 3, height about 12 in.

Members finding a plant answering to this description should take it to the President or Botanical Referee, with their name signed at the foot of this slip. When correct the slips will be initialed and handed to the Secretary. The finder should be prepared to answer questions on the description; but the name of the plant will not be officially announced till after tea.

A Prize or Prizes will be awarded at the end of the Season to those most successful.

Signal, _____

From *Nature* **10**, 95, June 4, 1874.

Nature Vol. 249 May 31 1974

European space looks forward to the 1980s

This survey is by Adam Dattner, Assistant Director of ESRO.

THE new unified European Space Agency (ESA) is scheduled to come into being next month. It will carry on with the execution of various space programmes which have already been decided on and initiated in the framework of ESRO (the European Space Research Organisation).

The accomplishments of ESRO so far have been mainly in the field of scientific space missions, and the seven scientific satellites launched between 1968 and 1972 have all been remarkably successful. This record of technical success has resulted in a number of scientific achievements, mainly in magnetospheric physics and astronomy but also in solar physics and aeronomy. In particular, remarkable ultraviolet observations of stars have been made with high spectral resolution, as well as one of the first attempts at measuring celestial γ rays.

Four of the satellites launched are still in orbit and operating successfully. In particular two highly eccentric orbit satellites, HEOS A1 and HEOS A2, which measure the properties of magnetospheric plasma, solar wind and the interplanetary magnetic field out to some 30 Earth radii. ESRO IV, in a near-Earth orbit, measures a variety of charged and neutral particles, their temperature, density and composition–particles typical of the upper ionosphere as well as those of solar origin. TDIA, apart from its detectors for solar, X and γ rays, as well as celestial X and γ rays and primary cosmic rays, has two ultraviolet telescopes aboard for high resolution stellar spectroscopy and celestial scanning.

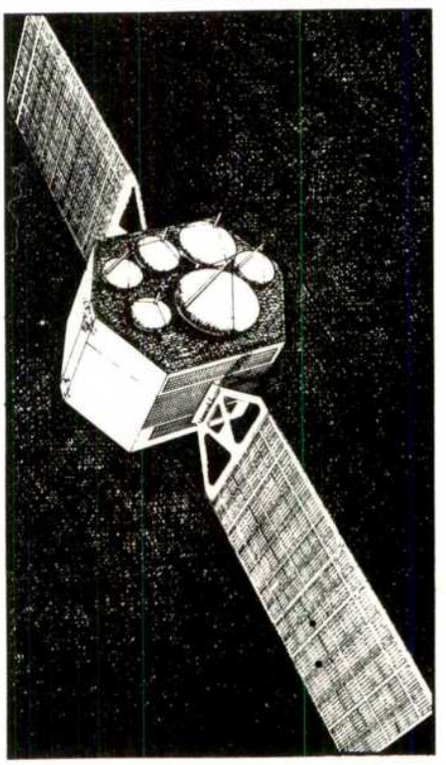
The development programme at present being carried out and the one planned for the near future include sophisticated missions which will pursue the exploration of the Earth's environment with investigation of the magnetosphere, using the GEOS spacecraft (a geostationary satellite to be launched in 1976) and IME (International Magnetospheric Explorer to be launched in 1977 as a part of a three-spacecraft collaborative mission with NASA).

Both will measure typical magnetospheric parameters but GEOS is characterised by its extremely high data flow and advanced experimental techniques; IME together with the other NASA spacecraft will provide a tool for a synoptic study of the magnetosphere where spatial variation of local parameters is separated from the temporal one.

In the field of astronomy the European community of astronomers is to participate in the joint United Kingdom/NASA IUE (International Ultraviolet Explorer) geostationary mission which is to carry a high resolution ultraviolet telescope and spectrometer to which the European scientists will have access through a Data Acquisition ground facility in Spain. Also in astronomy, but this time not ultraviolet, are the ESRO missions COS-B, carrying a γ-ray telescope and EXOSAT carrying an X-ray telescope; the latter mission will no doubt bring X-ray astronomy a step further, thanks to the excellent angular resolution that can be obtained by using lunar occultation.

Looking to the still more distant future, the scientists are now studying missions of various kinds and one of the dilemmas they will eventually face is brought about by the development of Spacelab. This facility will have features distinctly different from those the scientists have been used to in previous missions: the substantially larger payload capability will enable them to accommodate large instruments and crew but the orbital limitations will make *in situ* magnetospheric measurements beyond, say, 1,000 km altitude impossible. Furthermore the duration of missions will be limited but repetition of them will probably be quite frequent, and man's presence aboard will make the missions more flexible. The requirement for long period monitoring will no doubt remain and automatic satellites in many fields of research will complement the measurements on board Spacelab.

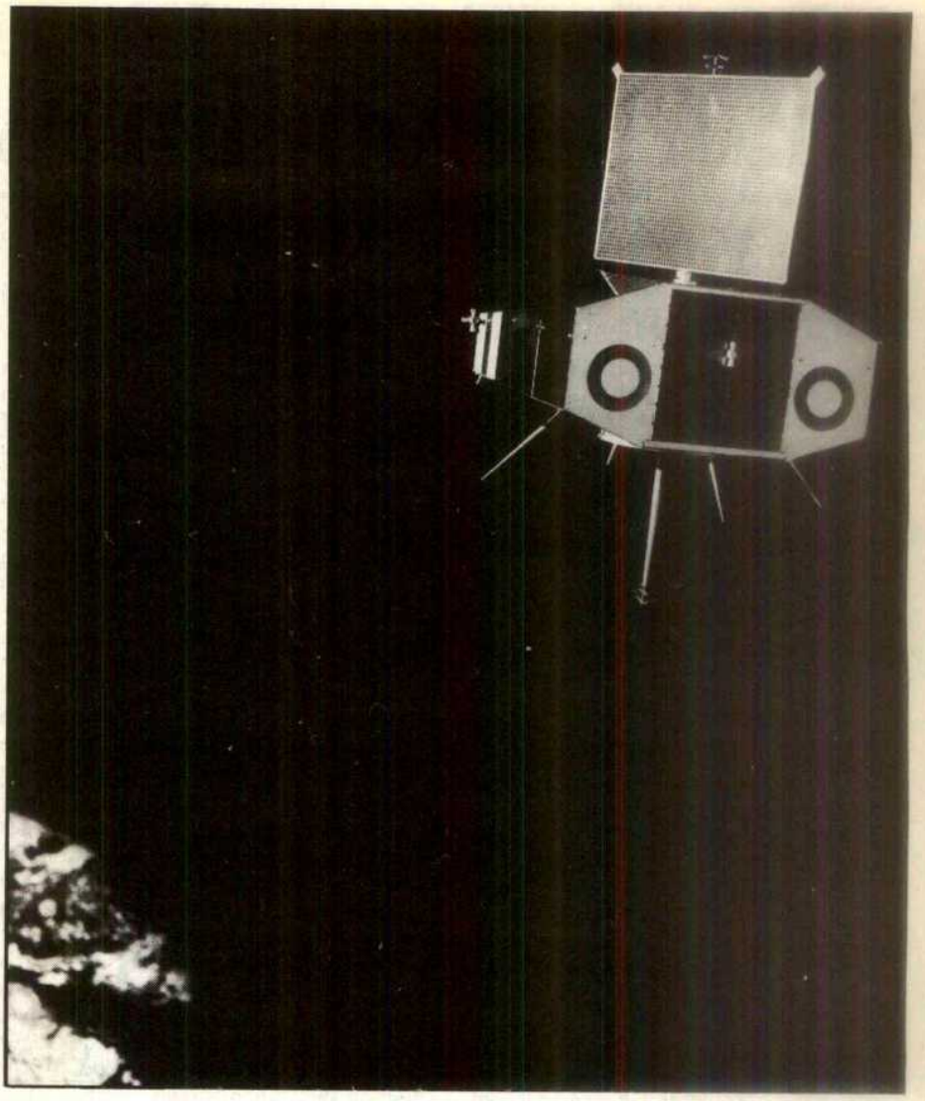
OTS: orbital test satellite



Astronomy remains one of the main areas of interest with continuing investigations in the ultraviolet, X-ray and γ-ray regions of the spectrum and extensions into the infrared.

Future missions being studied aim at infrared observations using either automatic satellites for sky surveys, or a larger Spacelab-borne telescope with good pointing accuracy for measurement of emission and absorption lines. Furthermore advanced missions in ultraviolet and X-ray celestial spectroscopy are envisaged, mainly using Spacelab. Solar physics with high spectral and spatial resolution remains at the centre of interest, using both Spacelab-borne telescopes and automatic satellites, depending whether the misison objective is a detailed, high resolution study of phenomena that cannot be observed from the ground or continuous monitoring of the solar disk. Also a Spacelab-borne facility for magnetospheric, ionospheric and atmospheric research is being considered, as well as automatic satellites for measurements of fields and particles out of the ecliptic plane or far out in the Solar System.

Since 1971 ESRO activities have also been oriented towards space applications on which heavy emphasis has been placed. The Meteosat programme is to be Europe's contribution to the space element of the Global Atmospheric Research Project (GARP) which will be carried out in 1976 and 1977 prior to establishing a worldwide system to be part of the World Weather Watch (WWW). The geostationary Meteosat project will carry a radiometer to provide good resolution photographs of the Earth in the visible and infrared. As from late 1973 the project has been in the development stage and the satellite will be launched at the end of 1976.



EXOSAT : highly eccentric orbit satellite to be launched in 1979, carrying an X-ray telescope in the 0.1–20 keV range, using lunar occultations for high resolution determination of X-ray sources.

A geostationary satellite, the Orbital Test Satellite (OTS), is under development for telecommunications, experiments and pre-operational use, its beam covering western Europe and the Mediterranean. It is to be a precursor of an operational communications satellite system to satisfy the needs of the European Postal and Telecommunications Services in the early 1980s, some four years after the launching of OTS in early 1977.

The increased air-traffic density over nonpopulated areas and the inadequate high frequency communications now used for aircraft over such areas give rise to an obvious application of satellites for air traffic control. Aerosat a collaborative venture of ESRO and the United States aims at establishing a pre-operational system of geostationary satellites, in the first instance over the Atlantic.

Marots, the latest newcomer to the family of ESRO's applications missions, is another example of space technology's potential for communications. The spacecraft technology being developed for OTS is perfectly suitable for maritime communications. The beam of the geostationary satellite will cover a large part of the hemisphere centred on the Greenwich meridian. The spacecraft is going to be launched in mid-1977, about six months after the launching of OTS.

Two other new projects recently included in the ESRO programme are Spacelab and Ariane. The United States shuttle programme, now going ahead at full speed, will permit the placing into orbits of several hundred kilometres altitude of an aircraft-like vehicle roughly the size of the present DC9. The vehicle is recovered at the end of each mission as well as many elements of the associated boosters used for launching. The 'Sortie Module', or as it is now called 'Spacelab' (a fully equipped modular laboratory placed into the vehicle) is being developed by ESRO as part of a collaborative programme with NASA. Spacelab can consist of a combination of modular elements, assembled to either a cylindrical container some 6.5 m long and 4.5 m in diameter, or to other configurations consisting of a smaller Spacelab capsule and external pallets on which experimental equipment, such as astronomical telescopes and other sensors, can be mounted. The large volume available for experiments operated and maintained by humans, the large weight-carrying capability and the power available—all combined with a shirt-sleeve environment, with data processing and other facilities aboard—destine Spacelab to become by early 1980 the most flexible tool for space experimentation and space operations ever built.

The missing element in Europe's space capability has always been heavy launchers. The Ariane launcher (originally the French L3S, now Europeanised) is to fill the gap. A three-stage vehicle, using high energy propulsion in its third stage, will be able to place 750 kg into geostationary orbit when launched from the Kourou range in French Guiana. Four test launches are foreseen between the beginning of 1979 and mid-1980.

The almost simultaneous start of all the programmes described above, and thus the concentration of launches in 1976 and 1977, will put a strain in the next two to three years on Europe's resources devoted to space. In spite of this the interest for other space programmes remains, and several other applications are being actively studied. Data transmission from a satellite seems to offer interesting advantages and television broadcasting seems to be another obvious candidate for space application. The United States' experience in experimental surveys of Earth resources indicates that this application may be one of the more important ones in the future. ESRO is also studying the possibility of such missions.

Recently a possibility of determining, with extremely high accuracy, the position of radio emitters on the ground, on the sea or in the air (using orbiting satellites) attracted attention and a French proposal to undertake a project called Geole is being discussed. The proposal is to use such techniques for cartography, glaciology and also for mineral prospecting and exploitation.

This list is by no means exhaustive and it has been the experience of all those involved in space research that new applications are constantly being found. Space research is only 17 years old—the first Earth satellite was in 1957. It is only in the 1980s that humanity will fully make use of this new and promising field.

international news

Mr. Varley reports on North Sea energy

Eleanor Lawrence

ALL set fair on the energy front was the message that the Secretary of State for Energy, Mr Eric Varley, delivered in his annual report to Parliament on the state of Britain's oil and gas reserves (*Production and reserves of oil and gas in the United Kingdom: a report to Parliament by the Secretary of State for Energy*, HMSO, 32p).

Assuming the forecasts are correct, Britain should be self sufficient in oil by 1980. At present, consumption of oil in the United Kingdom runs at around 100 million tons a year and is exepected to rise in the next 10 years. Oil production could well reach 100–140 million tons a year by 1980 and be sustained at this rate throughout the decade, says the report. Although present proven commercial reserves could not keep this sort of production up, it is confidently expected that further commercial finds will be made in areas already licensed in the North Sea, the Celtic Sea and areas that will be explored when licences are granted. Indeed, as Mr Varley was introducing the report Occidental Oil announced a new find, the Claymore Field near its Piper Field off the Orkneys.

But the estimates for the first oil ashore in 1975 have been downgraded from 25 million tons (last year's estimate) to 5 million tons. This setback is due to delays in obtaining and installing pipeline and production equipment as a result of materials and labour prolems.

On the thorny problem of finding building sites for the massive production platforms, Mr Varley said that he had no plans in hand to bring in legislation. At present the industry is not unduly worried about a shortage of platform building sites, but Mr Varley warned that his department would not hesitate to ask for legislation to speed up present planning procedures if it became necessary.

The first oil ashore is expected to come from the big Forties field controlled by BP. This is now expected to start production in 1975 after delays in installing steel production platforms that were supposed to have been in position in 1973.

Estimated reserves of oil in the North Sea now total 895 million tons from proven sources with a possible total of 2,950 million tons when all possible sources already discovered are taken into account. These figures compare very favourably with last year's estimate of 70–100 million tons with reserves of 800–1,300 million tons to be proved to sustain the forecast production level. The report stresses the difficulties in giving accurate estimates of production levels and reserves, especially as no oil has actually come ashore yet. More realistic figures should be available when the fields have been operating for a few years.

In contrast to oil, the North Sea gas industry has now been in operation for several years. Reserves remaining are estimated to be sufficient to provide between 5,000 and 6,000 million cubic feet a day, about double last year's consumption, in the late 1970s. This rate of production could be sustained with existing reserves until the 1980s but unless new reserves are found production will then slowly decline. Exploration for new reserves is now being concentrated in parts of the North Sea other than the heavily developed area off the south-east coast of England and the northern basin, which includes the Frigg field, and seems extremely promising.

Commenting on the report, Mr Varley said that Britain was better placed for energy in the medium term than many other industrial countries. In the 1980s some crude oil of the heavier grades would have to be imported for certain purposes such as the manufacture of lubricants but otherwise Britain would probably be physically self sufficient for oil. When asked if he planned any further controls to prevent oil companies sending the oil abroad, he said that present regulations ensured that all oil from the North Sea would be landed in Britain and that present export licensing arrangements would be sufficient controls. Self sufficiency in oil means, however, that a great deal of the oil from the North Sea will have to end up as domestic and industrial fuel given current consumption in the United Kingdom. Some people are of the opinion that the high quality North Sea oil is too valuable to burn and that Britain would get better value from her oil if it were chanelled into the manufacture of petrochemicals for export.

Although he regards the turn of Britain's energy fortune as very encouraging, Mr Varley tempered the general air of euphoria with a warning that the oil and gas reserves will not last for ever and that the best possible use must be made of them. To these ends he committed his department to investigating ways of conserving energy although he conceded that this would be extremely difficult to do for the domestic consumer.

Test ban talks draw liberal fire

Colin Norman, Washington

THE recent announcement that the United States and the Soviet Union are trying to negotiate an agreement which will limit the size of underground nuclear explosions (but not ban them entirely) has drawn fire from the Federation of American Scientists (FAS), an intellectually heavyweight organisation which lobbies chiefly for arms control, and from a handful of liberal senators. The federation has warned that such a treaty would probably have little effect on the development of new nuclear weapons and that it would have no effect on nuclear proliferation.

The fact that a so-called threshold treaty is under discussion surfaced earlier this month, before India joined the nuclear club, and it is generally believed that some form of agreement will be ready for President Nixon to sign when he travels to Moscow for a summit meeting at the end of June. It is cynically being viewed in Washington as a device which will allow Nixon to maintain his self-proclaimed image as a peacemaker even though he will be unlikely to bring back from Moscow any major new arms limitation agreement resulting from the strategic arms limitation talks.

A threshold test ban treaty is likely to set a limit on the magnitude of seismic signals which can be sent out by an underground nuclear explosion. In other words, it will enable both the United States and the Soviet Union to carry on testing small nuclear devices provided that they do not send out seismic signals greater than whatever threshold is negotiated.

An FAS statement, released at a press conference last week and endorsed by five former US disarmament negotiators, argued that unless the threshold is set at a very low level it is unlikely that the number of tests will be reduced below their present level. This is particularly true since two major weapons programmes —the developement in the United States of small, low yield tactical nuclear explosives and the building in the USSR of new and improved MIRV warheads—require tests which will probably be below the threshold.

As Dr James Leonard, the US ambassador to the Geneva disarmament negotiations between 1969 and 1971 and now a Vice President of the United Nations Association, put it at the press conference, "A threshold treaty is a reflection of the philosophy that the United States needs to keep testing".

The FAS also emphasised the difficulties that would be involved in making sure that a threshold treaty is not violated. Since the seismic signals sent out by an underground explosion would not necessarily be of the same magnitude at all instrumentation stations, an explosion which in fact is below the threshold could register as being above it. "Thus, not only will the negotiation of an international verification system be extremely difficult", the statement says, "but even with the best of intentions suspicions of cheating are likely to arise".

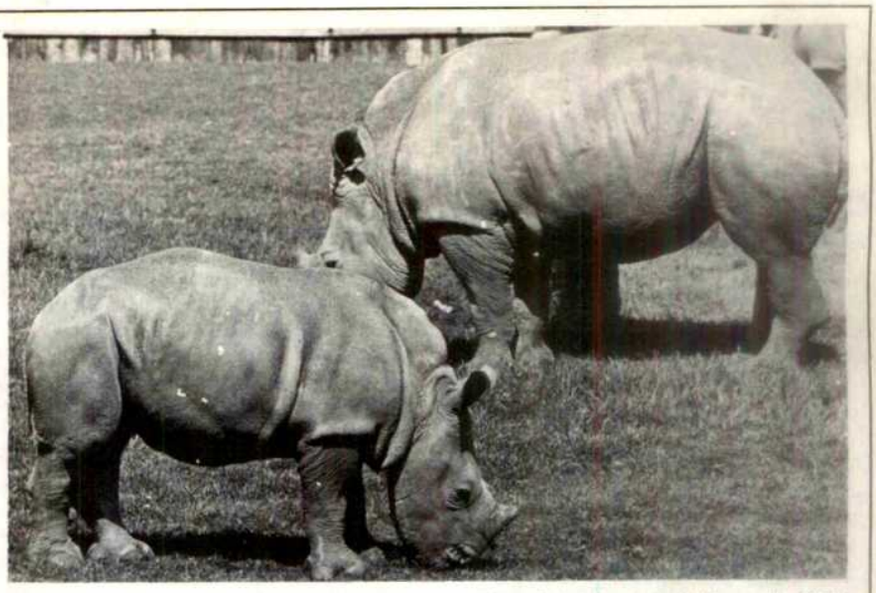
Another serious reservation expressed by the FAS is that a threshold treaty would do little to halt the spread of nuclear weapons—a topic which has suddenly been thrust into the foreground with the announcement of India's first successful nuclear test on May 18. Leonard, for example, suggested that such a treaty "would be seen by others in the world not as a serious disarmament measure but as a sham—a fraud by the United States to protect its right to test". He added that "it would not only be seen that way, it would be used that way".

Since many non-nuclear nations have refused to sign the nuclear non-proliferation treaty unless the super powers limit their development of nuclear weapons, a threshold treaty which allows continued testing would do little to improve the prospects for halting proliferation. Similar sentiments were also expressed last week by Senator Edward M. Kennedy, who warned that a threshold treaty "would be seen by many nations . . . as continued hypocrisy by the super powers".

Kennedy, who has recently returned from a trip to the Soviet Union reports that he is convinced that the time is now ripe to press ahead with negotiations for a comprehensive test ban. He said that from his "discussions with Soviet leaders, I believe that they would be willing to move forward in this area".

Backed by 36 co-sponsors, Kennedy last year introduced into the Senate a resolution calling on the United States and the Soviet Union to seek an immediate moratorium on nuclear testing and to begin serious negotiations toward a comprehensive test ban. That resolution will be on the floor of the Senate in the next couple of weeks and if it is passed it will send a clear signal to the Administration. Since the Senate will have to ratify whatever treaty is negotiated, if it insists on a comprehensive treaty by passing Kennedy's resolution intact according to some observers, it could have one of two effects. Either the Administration will try to negotiate a treaty with as low a threshold as possible, or the hand of those within the Administration who have argued for a comprehensive ban will be strengthened to such an extent that the negotiations will be aimed not at a threshold but at a complete test ban.

One concern voiced both by Kennedy and the FAS is that negotiation of a threshold treaty could forestall completely the achievement of a comprehensive ban. As the FAS statement put it, "We understand very well that the politics of summitry and the politics of impeachment both impel the Administration towards an arms control agreement in June in Moscow but we do not want to see an important element in our national security—the complete test ban—sold out in favour of a much less useful agreement".



White rhino born at Whipsnade, 1973.

DESPITE fears that the higher entrance charges as a result of the introduction of Value Added Tax would discourage visitors, both London Zoo and Whipsnade had even higher attendances last year and finished 1973 in surplus. For the first time since 1968 more than two million people visited the London Zoo in 1973, spending a total of £1 million. Introducing the Annual Report, the Secretary of the Zoological Society, Lord Zuckerman, said that the high attendance, in the absence of an outstanding animal event or the opening of any major building, reflected the very high standard of 'normality' at the Zoo. The cost of the research and educational programmes that the society maintains amounted to more than £0.5 million in 1973. The two research institutes in the society's grounds, the Nuffield Institute of Comparative Medicine and the Wellcome Institute of Physiology cost £189,000 and £49,000, respectively, to run last year and of this the society directly contributed £183,600 from its income from such sources as entrance fees. Throughout the report, the theme of the rôle of the zoo in conservation is apparent. Births in captivity of rare or endangered animals such as white rhinos, cheetahs and orang-utans are given prominence, and this function of the present-day zoo is picked up in another of the society's publications, the *International Zoological Yearbook*, which for the first time has a special section devoted to the ethical and practical problems of trading in and rearing rare wild animals. Geoffrey Schomberg, the Secretary of the Federation of Zoological Gardens of Great Britain and Ireland, writing in the Yearbook asks: "Are endangered species a proper object of retail trade in any circumstances?" Too many zoos, he says, while paying lip service to the ideals of conservation become fiercely competitive in acquiring rare specimens.

Pentagon admits Vietnam rainmaking

Colin Norman, Washington

AFTER years of secrecy, evasion and even outright denials, the Department of Defence has at last admitted that it carried out an extensive weather modification programme during the Vietnam War. The operation, which took place between March 1967 and July 1972, was designed to increase rainfall over the Ho Chi Minh Trail and make it difficult for the North Vietnamese to move troops and equipment into South Vietnam along muddy roads and jungle trails.

The Pentagon's admission came during top secret hearings held by a Senate subcommittee, on March 20, a transcript of which was made public last week. The transcript reveals that the rainmaking operations were so highly classified that even some senior Pentagon officials were unaware of them, and that there is now a deep division of opinion within the Department of Defence about their effectiveness.

According to testimony given at the hearings by Lieutenant Colonel Ed Soyster, a total of 2,602 cloud seeding missions were flown over Laos, Cambodia and North and South Vietnam, and 47,409 cannisters of silver and lead iodide were expended during the five-year programme. The objective was to supplement natural rainfall during the normal rainy season, and to extend its length, thereby causing landslides, washing out bridges and generally turning trails into a sea of mud.

Although it has been rumoured and reported in newspapers for about three years that the United States indulged in rainmaking operations during the Vietnam War, the extent of the programme revealed during the Senate hearings has surprised many observers.

The operations resulted from an extensive test carried out in October 1966 in Laos. According to Soyster's testimony, 56 cloud seedings were conducted, with a success rate of more than 85%, and the Commander in Chief of Pacific Operations concluded that rainmaking over the Ho Chi Minh Trail "could be used as a valuable tactical weapon". Operational cloud seeding missions began on March 20 the following year.

An air force base in Thailand was used for the operation which was carried out with weather reconnaissance aircraft. In short, cloud seeding consists of dropping burning photoflash-type cannisters into an updraft in a cloud mass. As the cannisters burn, silver iodide or lead iodide is produced and this acts as a seeding agent, causing drops of moisture to form in the cloud, which eventually fall as rain. The entire military rainmaking operation cost about $3.6 million a year.

Initially, the area chosen for cloud seeding consisted of South-eastern Laos and a small area of North Vietnam, but later in 1967, operations were extended into North Vietnam and a small region of South Vietnam. Finally, in 1972, portions of Cambodia and a larger region of South Vietnam were included.

All cloud seeding activities over North Vietnam were, however, halted on November 1, 1968, the day that President Lyndon Johnson ordered a bombing halt, and they were never reinstated. And the entire operation was ended on July 5, 1972—just two days after the *New York Times* published a lengthy account of the Pentagon's rainmaking activities.

Soyster told the committee that although it was usually possible to seed every cloud within a target area, priority was given to seeding clouds directly over roads, intersections and river crossings, which indicates an accuracy for the operation far greater than many independent observers believe possible.

How successful was the programme? According to Soyster, the Defence Intelligence Agency calculated that "rainfall was increased in limited areas up to 30% above that predicted for the existing conditions". He added that sensor recordings of movement along the Ho Chi Minh Trail "indicated enemy difficulties from heavy rainfall", and said that in April 1971 there were about 9,000 enemy logistic movers in Laos each week, but by the end of June the number had dropped to 900. The greatest decreases in troop movements came during the first week in June, when a typhoon struck the area, and during the third week in June, when the Pentagon's rainmaking activities were at their height.

The Pentagon's admission that it has indulged in weather modification came after persistent attempts by Senator Claiborne Pell, a Rhode Island Democrat, to get at the facts. Pell, who is Chairman of the Subcommittee on Oceans and International Environment of the Senate Foreign Relations Committee, proposed a resolution which the Senate passed last year by a vote of 82 to 10 calling on the United States Government to launch international negotiations aimed at banning weather modification as an instrument of war, and it was his subcommittee which held the top secret hearings on the military rainmaking operations. The transcript of those hearings was declassified by Secretary of Defence James Schlesinger.

Details of the rainmaking operations were held in strict secrecy, presumably because of the sensitivity of the programme. According to Soyster, individual mission reports were transmitted through special communication channels to the joint chiefs of staff, who made periodic reports to the Secretary of Defence and the White House.

Furthermore, since reports and rumours of the operation appeared in the press, the Pentagon has been more than evasive on the matter. During hearings held by the Senate Foreign Relations Committee in April 1972 for example, then Defence Secretary Melvin Laird was asked whether the military had ever indulged in rainmaking activities. He replied: "We have never engaged in that type of activity over North Vietnam", but in January this year he wrote to the committee to apologise for his mis-statement, saying that he was unaware of the weather modification operations carried out over North Vietnam by the Johnson Administration. He was, of course, fully aware of the rainmaking activities he had authorised over Laos, Cambodia and South Vietnam, but simply evaded any mention of them.

The Pentagon's obsession with the secrecy of the programme was taken to its final extreme in 1972, when it refused even to supply details of the operation to an interagency task force chaired by Herman Pollack, director of the bureau of international scientific affairs in the Department of State, which was trying to develop administration policies on both military and civilian weather modification. Although that study itself was classified, the Pentagon has now admitted that the rainmaking operations were so heavily classified that it refused to turn over any information to Pollack.

Since the skies over South-east Asia were raining high explosives and all manner of unsavoury weapons during the Vietnam war, why did the Department of Defence choose to hide the fact that it was tinkering with the weather? One reason is that the whole area of weather modification is a sensitive one simply because it is a new area of warfare which may eventually open up some frightening possibilities.

As Pell puts it, "We should not open the Pandora's box of harnessing nature, of changing the weather or developing techniques to create typhoons or earthquakes with devastating effects upon foes and neutrals alike. I believe this new kind of weaponry should be eschewed by developed, so-called civilised nations".

Club of Rome associations

David Spurgeon, Ottawa

CANADA is the latest country to have formed a national association inspired by the Club of Rome. The decision was made at a meeting in Toronto early in May, and the venture has the backing of an impressive array of government and business officials.

The association's objects are "to promote study and discussion among all segments of the Canadian public of the nature of world problems and the need to develop new policies, attitudes and courses of action to ensure a stable and viable future for mankind . . . " More specifically, it will attempt to identify Canada's role in the solution of world problems, and the implications for Canada of possible world solutions.

Both the co-founders of the Club of Rome, Dr Aurelio Peccei of Italy and Dr Alexander King, who until recently was with the OECD in Paris, attended the Toronto meeting, which was opened with a welcoming letter from Canada's Prime Minister, Pierre Trudeau. From their accounts it was apparent that the club—largely through the impact of the MIT study it sponsored, *The Limits of Growth*—had made considerable progress in directing attention to world problems since its inception in 1968.

One reason for thinking so, as suggested by Dr Peccei, is the establishment of a series of United Nations Conferences for discussion of world problems such as population, food, energy and materials. Another is the organisation in many nations of groups like the Club of Rome.

As described by Dr King, these included a whole diversity of approaches. The first was set up in Holland, which according to King is not surprising, because of its twin problems of dense population and heavy industrialisation. The Dutch association is a group of private people gathered in a nonparty organisation.

The second—also not surprisingly, for the same reasons—was organised in Japan, on a different basis, backed by and formed as a committee of the techno-economic society. Groups in other countries include some not connected with the Club of Rome but interested in similar problems (for example, Great Britain and Austria), and luncheon clubs and groups of engineers or teachers in France, Denmark, Belgium and Sweden.

Finland has formed a more formal study group to determine what the role should be of a small, progressive country in the modern world; Switzerland, with four Club members, invited four government members to discuss Club of Rome thinking and found them receptive to the points where they decided that both the traditional political organisation and neutrality of the country was obsolete.

But Dr King found some of the keenest interest in an unexpected place: South Africa. I was in South Africa recently, he said, "and, much to my surprise, found the Club of Rome better known in that country than in any other." Some citizens there have formed an independent information group who just meet and talk.

The Canadian Association for the Club of Rome has applied for a charter as a corporation without share capital. Its first directors include the president of the pulp and paper industry's research institute, Dr Pierre R. Gendron; Senator Maurice Lamontagne, whose senate committee carried out one of the most comprehensive studies of national science policy yet made; a retired oil company executive, Mr Ronald Ritchie; the president of the Ontario Research Foundation, Dr William Stadelman; the former Chief Science Adviser to the Federal Cabinet, now Dean of Applied Sciences at Queen's University, Dr Robert J. Uffen; and a senior civil servant with many years experience in Canadian Science policy, Dr J. Rennie Whitehead.



BENEATH the elegant tapestries of the Schlosshotel, Kronberg, Germany a small group of biologists, philosophers and psychiatrists gathered recently to constitute the Boehringer Ingelheim Symposium on the Creative Process in Science and Medicine. Pictured above, listening to Sir John Eccles testify are, in the front row, Sir Hans Krebs, Jacques Monod, Manfred Eigen, Desmond Morris and Sir Karl Popper. What leads to some scientists being creative way above the average, and does the creativity of scientists bear any resemblance to that of artists and composers? Participants were decidedly coy about speaking other than in the abstract, despite Krebs's personal example of trying to piece together how he came to produce new ideas. All seemed agreed that breaking out from conventional thinking was a major ingredient in creativity —the agreement was so complete that it must qualify as conventional thinking and therefore . . . Monod discussed 'subjective simulation'—a process almost of putting yourself in the position of the electron or whatever that you are thinking about. The debt of many participants to Popper was clearly great—an interesting contrast with the world of physical science where one's impression is that there is less understanding of what he is about. Desmond Morris had probably the nicest story to tell—A journalist once asked Picasso: What is creativity? 'I don't know, and if I did I wouldn't tell you.'

correspondence

Canadian science

SIR,—In your issue of March 15, David Spurgeon offered his own interpretation of recently announced changes in the structure of federal scientific activities. After reading his article, I cannot avoid feeling that the author has done a disservice to Canadian scientists.

Spurgeon makes the statement that the press releases of the Ministry of State for Science and Technology served only to confuse in an attempt to provide more detail about the rather general statements made in the throne speech. I submit that he is guilty of that particular offence.

"The question in the minds of some", wrote Spurgeon, "was whether the ministry was finally becoming . . . an all-powerful, monolithic instrument of power in Canadian science policy". He went on to say that a quick sampling of reaction in the science community indicated that the ministry's releases had indeed given this impression. The sample must have been a very quick one because, following the release of the information kit, a survey of Canadian scientific opinion published in the daily press indicated that most of those surveyed were not prepared to condemn the changes out of hand but instead would wait for specific actions before passing judgment.

Spurgeon cites, as perhaps the most confusing element of the announcement, the failure of the ministry to mention anything about possible new reporting relationships for the granting councils. Surely Spurgeon is hung up on the administrative considerations he so vigorously condemns. Reporting relationships are simply provisions for accountability to Parliament. What is important to every scientist are factors such as peer assessment of research proposals, the allocation of funds to the granting councils and the input of the granting councils to the Inter-Council Coordinating Committee. All these factors were clearly dealt with in the ministry press releases. The reporting channels for the existing granting councils have not been altered.

Finally, Spurgeon questions the separation of the granting function of the National Research Council of Canada from the laboratory function. Such a separation, he says, will destroy a vital link between government research and industry. The changes proposed for the NRC affect only the university granting mechanisms. The traditional liaison between the NRC and industry will continue.

Yours faithfully,

AURELE BEAULNES

Ministry of State, Science and Technology,
Ottawa

Journals jungle

SIR,—I am writing in reference to the article "Science Journals in a Prices Jungle," which appeared in the *Nature* dated February 15, 1974. That article discussed price rises of a variety of journals and listed "a record-breaking increase" for *International Pharmaceutical Abstracts* of "261%". I must respond to this kind of reporting since not all the information was included; this makes the information that does appear misleading.

Prior to January, 1973, *IPA* had two subscription prices—an institutional subscription price of $100.00 a year and a subscription price to individuals of $40.00 a year. A two-subscription price system was difficult to administer since many people who should have paid $100.00 were paying only $40.00, and so on. We were faced with having to increase both rates since we were losing money in the situation that existed at that time. The expense of publishing *IPA* was increasing each year and that just could not be balanced by income at existing subscription rates. After a thorough study of our subscribers, we decided to change to one institutional subscription price of $150.00 a year. The increase was from $100.00 to $150.00 and not quite as record breaking as the article implied. We felt, however, that we could not ignore the individual completely and therefore decided to offer an additional copy subscription rate of $30.00 a year (if one subscription exists at the $150.00 rate, as many additional copies as desired can be obtained at the $30.00 rate).

Other facts relating to the change are that we improved and expanded the services offered. All our promotional pieces and literature at that time mentioned that we would be providing greater coverage, most abstracts, and that *IPA* would be more current. This in fact has happened, and in 1973 we published 13% more abstracts than we did in 1972 and *IPA* was on time. We did follow through on our promises of additional services for the change in subscription price—it was not just a price change for the same service. One other fact that seems to have been completely overlooked is that *IPA* is still one of the lower priced secondary publications. Most abstracting-indexing publications are far more than $150.00 a year. Possibly our real problem is that we began at too low a subscription price for *IPA* when it began.

Yours faithfully,

DWIGHT R. TOUSIGNAUT

American Society of Hospital Pharmacists,
4630 Montgomery Avenue,
Washington DC 20014

Kidney transplants

SIR,—Why publish such a rank piece of scientific journalism as "Transplants: the failing machinery" (April 19, 1974)? The subject has always been an emotive one, but this is not an excuse for the lack of reason displayed and the dramatic aura with which the article is surrounded. To use comparisons between kidneys received from and contributed to the national donor pool as an indication of "reluctance . . . to share out kidneys which are not suitable for their own patients" is nonsense unless some measure of the kidneys potentially available within an institution by comparison with the size of its recipient pool is also given. Both St Mary's and Hammersmith are hospitals with a large transplant programme in areas where irremediable cranial trauma is rare. Further St Mary's does not have a neurosurgical service so that we see few patients with intracranial vascular accidents and such patients as we have with progressive primary intracranial malignant disease usually go elsewhere before they die. So we shall always be debtors rather than creditors.

Is there unequivocal evidence that "lack of understanding and cooperation between doctors . . . is largely to blame"? Of course, being human, we are too lazy, too hidebound and too hardworked always to do the right thing in complicated situations, but I do not know of data to suggest that a large number of kidneys are being lost through medical indifference.

Yours faithfully,

H. A. F. DUDLEY

St Mary's Hospital,
London W2

news and views

Super-rotation of the upper atmosphere

OBSERVATIONS of small changes in the orbital inclinations of artificial satellites have shown that the Earth's upper atmosphere (at altitudes of 150–400 km) is rotating about 20–30% faster than the Earth itself. This phenomena has become known as super-rotation. Even though it was discovered by King-Hele in the early 1960s, the cause of this 100 m s^{-1} west to east wind is still a subject of considerable debate. The change in inclination because of super-rotation is only about 0.1° during the satellite lifetime, and so spatial variations and short term effects are very difficult to detect. No clear evidence has been found of year to year variations, such as might be caused by the 11-yr-periodicity in solar activity, but over daily periods the wind seems to maximise between 2100 and 2400 local time.

Possible causes of super-rotation are:

- Horizontal pressure gradients may be set up in the atmosphere as a result of thermospheric heating by solar ionising radiation, and bombardment by energetic particles. The resulting global pressure distribution produces geostrophic and cross-isobaric winds which are probably controlled by ion drag (the motion of the F region ions borne along by the neutral winds is strongly effected by the geomagnetic field). A problem with this theory is that heating of the auroral zone, which takes place during magnetic disturbances, can reverse the mean meridional pressure gradient. This would cause a drastic change in the super-rotation, a change which has not been observed.
- Super-rotation might be caused by ionospheric or magnetospheric motions which couple with the neutral atmosphere by collision processes. Meridional electric fields can move F region ions quite efficiently even in the presence of the geomagnetic field. An average field directed towards the equator could produce the super-rotation, and these fields have been observed in high northern latitudes during negative magnetic bays. The effect should, however, be enhanced during magnetic disturbances. This is contrary to observations.
- The moving flame effect. A heat source acting on a fluid produces convective motion. If the heat source moves (as, for example, the Sun does in a westerly direction with respect to the Earth's atmosphere) the convective cells tilt, producing horizontal and vertical motions which interact to produce a mean horizontal flow. The flow rate is a function of the frequency of the heating function, and of the ratio of the kinematic viscosity and thermal diffusivity of the fluid. Permissible values of such parameters in the case of the Earth's atmosphere unfortunately yield inappreciable amounts of super-rotation; a situation which compares unfavourably with the case of Venus where the upper atmosphere rotates 50–60 times faster than the planet mainly because the heating function has a period of 240 days (the Venusian surface rotation period) as opposed to 1 day on Earth.
- Atmospheric waves may impart momentum to the F region, in which they are dissipated. If transmission efficiency depends on the direction of wave propagation, a net motion can be produced. As wave velocity is an inverse function of density, an imperceptible mean velocity at low levels can produce the observed thermospheric velocity.
- The Scott effect may produce super-rotation: a small brass cylinder suspended in a cylindrical vessel containing a low pressure gas, experiences a torque when a vertical magnetic field is applied, and if a temperature gradient is set up between the cylinder and the vessel. This effect might act on the atmosphere.

At present it seems that none of these theories can explain the phenomena of super-rotation completely. A sixth theory has now been put forward by Ved Mitra (Physics Department, MNR Engineering College, Allahabad, India) in a recent edition of *Planetary and Space Science* (**22**, 559; 1974). In this case, super-rotation is caused by meteoroid deposition. The meteoroids, small interplanetary dust particles which mainly originate from decaying comets, and which move in elliptical orbits around the Sun, carry, on average, a greater amount of angular momentum than that corresponding to the Earth's orbit. When they interact with the Earth's atmosphere the angular momentum must be conserved and therefore the excess orbital angular momentum of the meteoroids becomes manifest as extra spin angular momentum in the atmospheric layers in which they are arrested.

Mitra assumes firstly that the majority of the mass deposited in the atmosphere is in the form of particles with masses 10^{-6}–10^{-3}g. These meteoroids are assumed to have orbits similar to those of the larger meteors which were recorded photographically by super-Schmidt cameras during the Harvard photographic meteor project. The orbits are, in the main, within Jupiter's orbit, with low inclinations, and high eccentricities and direct motion. When these particles collide with the Earth's atmosphere they cause it to 'spin up'.

For example, particles coming from behind the Earth will be accelerated by gravitational attraction, and this increase in velocity will cause them to move away from the Sun and collide with the Earth from outside, giving it a prograde spin. Particles in front of the Earth, in direct orbits, will lose velocity because of gravitational attraction, strike the Earth on the Sunward side and again impart prograde spin. This increase in spin during gravitational accretion is exactly the same process propounded by Alfvén for the origin of planetary rotation in the Solar System.

Mitra calculates the orbital angular momentum per unit mass for the incident meteoroids, and by comparing this with the angular momentum of the Earth finds the influx required to produce the observed super-rotation of 100 m s^{-1} in the atmospheric layers above 150 km. The answer comes to 34 tons a day, a value in excellent agreement with that of 43.5 tons a day obtained by Whipple (*Smithson. Astrophys Inst. Prepr.*, No. 239; 1967), who, however, considered the Earth as a nongravitating sphere; 55 tons a day by Dohnanyi (*Icarus*, **17**, 1; 1972) who used results of satellite-borne penetration experiments, and ground-based visual and radar observations; and 48 tons a day obtained by Zook *et al.* (*Planet. Space Sci.*, **18**, 953; 1970) obtained from observations of micrometeoroid impact on the window of Gemini spacecraft.

Unfortunately, there are two problems with Mitra's argument. The first is minor and concerns the size of

the incident particles. Recent work (for example, Hughes, *Space Res.*, **14**, 789; 1974) indicates that about 95% of the mass incident on the Earth is in the form of particles of mass 10^{-9}–10^{-5}g which have very low geocentric velocities and different orbits from those assumed by Mitra.

The second point concerns Mitra's assumption that "the effective deceleration of the velocity initially possessed by a meteoroid takes place in the 150–400 km region". This is contrary to visual and radar observations of the more massive meteoroids ($> 10^{-3}$g) which have maximum ablation at heights of about 90 km. Either it must be assumed that the super-rotation is produced by the minor amount of ablation and momentum transfer that occurs at heights above 150 km, in which case Mitra's 34 tons a day influx must be increased accordingly, or a process of momentum transfer from the lower layers where the ablation occurs to the regions of super-rotation must be invoked; similar to that propounded for the transfer of atmospheric wave energy.

It seems therefore that the cause of super-rotation still remains a mystery and that the Earth's spinning upper atmosphere is still the happy hunting ground for new theories.

DAVID W. HUGHES

The collagen triple helix

JAMES WATSON, in his Nobel address on the work which led to the Watson–Crick model for DNA, spoke of his fear that this structure might have turned out to be "dull and uninteresting, like collagen". In the event, collagen has turned out to be a most interesting protein which has occupied the attention of workers from a wide range of disciplines.

Recently, attention has been focussed on the intracellular process by which the three polypeptide subunits (α chains) of monomeric collagen form its characteristic triple-stranded helix. The very slow and incomplete *in vitro* renaturation of α chains derived from extra-cellular collagen led to the proposal by Speakman (*Nature*, **229**, 241; 1971) that the α chains as initially synthesised might have peptide extensions. It was postulated that these extensions or 'registration peptides' might serve to bring the three α chains into a conformation which would allow rapid formation of the triple helix. Subsequently much evidence has accumulated to indicate the existence of a precursor of this type (called procollagen) during the biosynthesis of collagen in a wide variety of tissues, and some of the properties of procollagen have been defined (reviewed by Schofield and Prockop, *Clin. Orthopaed. Rel. Res.*, **97**, 175; 1973). Initially considerable controversy existed about the size of these extensions and their amino acid composition, but there now seems to be general agreement that type I and type II procollagens comprise polypeptides of 120,000–125,000 daltons. Moreover, it seems that all pro- α chains contain cysteine, an amino acid not usually found in extracellular interstitial collagen, with the possible exception of a newly discovered form of collagen (type III) recently isolated from several human tissues (Chung and Miller, *Science*, **183**, 1200; 1974).

The demonstration of the presence of cysteine in procollagen suggested that disulphide bonding occurs between the pro- α chains and several groups have now reported that procollagen is secreted as a triple-stranded disulphide-stabilised molecule of molecular weight of about 360,000 daltons. Similar observations have been reported by Monson and Bornstein (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 3521; 1973) who also demonstrated that the procedures used previously in their laboratory for the extraction of procollagen from cranial bones resulted in partial cleavage of the extension peptides by endogenous proteases such that the regions containing the interchain disulphide linkages were lost. This finding reconciles several apparently conflicting reports concerning the occurrence of interchain disulphide bonds in procollagen.

The possibility of a relationship between the process of disulphide bond formation among pro-α chains and that of triple-helix formation was suggested by studies reported by Grant *et al.* (*J. biol. Chem.*, **248**, 7432; 1973) on the biosynthesis of a basement membrane collagen of embryonic chick lens. Procollagen secreted by lens cells was found to be linked by interchain disulphide bonds and in a triple helical conformation. By contrast, intracellular lens collagen was found to be in a random coil conformation, and interchain disulphide bonds between the pro-α chains were also absent. That the formation of the disulphide bonds may be of considerable importance in promoting the formation of the triple helix is also indicated by studies using pulse label and chase experiments in embryonic chick tendon cells (Schofield *et al.*, *Biochemistry*, **13**, 1801; 1974) and cartilage cells (Uitto *et al.*, *Fed. Proc.*, **33**, 617; 1974). A close correlation was observed between the extent of interchain disulphide bonding among pro-α chains and the extent to which the procollagen was in a triple-helical conformation. The rates of these processes, however, seem to vary according to the cell type and may thus influence the time taken for secretion of procollagen from different types of cells.

Further evidence of a role for disulphide bonds in procollagen synthesis comes from the work of Uitto and Prockop (*Biochem. biophys. Res. Commun.*, **55**, 904; 1973). Embryonic chick tendon cells were incubated at 37° C under conditions in which they synthesised and accumulated protocollagen, (unhydroxylated procollagen) which is non-helical above 24° C. When the cells were cooled to 15° C, the intracellular protocollagen, which was shown to contain interchain disulphide bonds, became triple helical within five minutes. But when disulphide bonds in the amino-terminal extensions of protocollagen were reduced by treating the cells with dithiothreitol, the rate of helix formation on cooling was markedly decreased; this suggests that the formation of interchain disulphide bonds is probably a necessary prelude to helix formation. Whether or not this process is mediated by enzymes is unknown, but in tendon cells interchain disulphide bonding of procollagen polypeptides occurs in the rough endoplasmic reticulum soon after release of the completed polypeptides from membrane-bound ribosomes (Harwood *et al.*, *Biochem. biophys. Res. Commun.*, **55**, 1188, 1973).

A further factor in considering the formation of the collagen triple helix is the part played by hydroxyproline. Uitto and Prockop using protocollagen were able to dissociate the disulphide bonding process from any influences exerted by hydroxyproline residues in the polypeptides. It is clear from studies of the denaturation temperatures of procollagen and protocollagen, however, that the absence of hydroxylated prolyl residues markedly decreases the thermal stability of the collagen triple helix (Jimenez *et al.*, *Biochem. biophys. Res. Commun.*, **52**, 106; 1973; Berg and Prockop, *ibid.*, 115). It seems therefore that the major role of hydroxyproline is to maintain the procollagen polypeptides in a triple-helical conformation under physiological conditions, and that one of the chief roles of the amino-terminal extensions is to facilitate chain association through the formation of interchain disulphide linkages leading to the formation of the triple-helical structure in a time which is biologically feasible.

D. S. JACKSON
M. E. GRANT

First official geological survey in the British Isles

from Gordon L. Davies

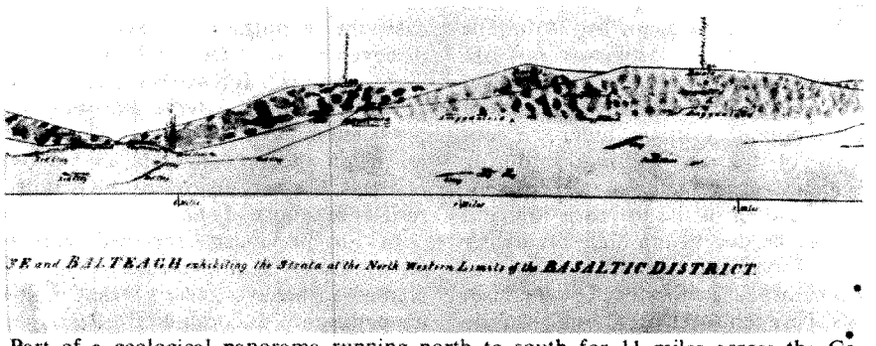
IN March 1832 Henry Thomas De La Beche approached the Master General and Board of Ordnance offering to plot geological lines upon the ordnance maps of south-western England, and the outcome was the establishment in 1835 of the Geological Survey of Great Britain as a branch of the Ordnance Survey. A fact which is much less well known is that as early as 1824 Lieutenant-Colonel Thomas Frederick Colby (1784–1852), the Superintendent of the Ordnance Survey, had himself inaugurated an Ordnance Geological Survey of Ireland, and although it proved abortive, it was a pioneering venture that deserves to be remembered in this the year of its hundred and fiftieth anniversary.

Colby was keenly interested in geology (he had been a member of the Geological Society of London since 1814) and he resolved to bring Irish geology within his purview when in 1824 he was directed to extend the Ordnance Survey's operations into Ireland. The Irish rocks were to receive this particular attention because the survey was under instruction to publish maps of Ireland on the lavish scale of 6 inches to the mile instead of the 1-inch scale that was standard in Britain. Colby hoped that as his men worked their way across Ireland they would also be able to collect geological information as a byproduct of their normal topographical duties. Indeed, writing in February 1826 he looked forward to the completion in Ireland of "the most minute and accurate Geological Survey ever published".

Initially Colby's plan was that the surveyors would collect geological samples, mark the collection sites on maps, and then despatch both samples and maps to the survey's Dublin headquarters where a geologist would identify the specimens and then interpolate geological lines on the maps. This seems to have been the procedure used when the survey began operations in counties Antrim and Londonderry in 1825, but the inadequacy of such random methods soon became apparent. In November 1826, therefore, Captain John Watson Pringle (who lived about 1793–1861), a Waterloo veteran and a former pupil of Friedrich Mohs at the Freiberg School of Mines, was appointed to reshape the survey's geological activities. Under his direction the field parties were themselves instructed in the construction of geological maps and sections, and for their guidance he prepared a pamphlet entitled *Geological and Mineralogical Observations* fifty copies of which were lithographed at the Tower of London in March 1827. From the surveyors Pringle expected a geological map of each parish accompanied by two geological sections drawn from north to south and east to west, and he estimated that using his methods the geological survey of Ireland would be completed by 1834 at the trifling cost of £5,500.

But Pringle's methods were never accorded a fair trial because Colby's superiors decided that geology was interfering with the survey's more legitimate topographical duties and in September 1828 Colby was directed to cease all geological investigations forthwith. He did manage to revive the Irish Survey's geological activities in January 1830 when Captain Joseph Ellison Portlock (1794–1864) took over the duties formerly discharged by Pringle, and the fruit of this resurrected geological survey was Portlock's classical map and memoir of the geology of Co. Londonderry and adjacent areas published in 1843. By then, however, the Geological Survey of Ireland was merely a poor cousin of the vigorous body that De La Beche had created in England, but this should not be allowed to obscure the significance of Colby's achievment back in the 1820s. He had in 1824 inaugurated the earliest official geological survey to operate within the United Kingdom and that only 3 years after the Corps Royal des Mines had planned in France the earliest of all national geological surveys. The French surveyors, however, did not start work in the field until 1825, the very same year as Colby's men 'broke ground' in Northern Ireland.

Sadly, Colby's abortive geological survey of 1824 never published any of its work, but a few geological maps and sections produced under Pringle's direction have recently come to light in the Dublin offices of the Irish Ordnance and Geological surveys. Some of these items have found a place in the exhibition being staged in Trinity College Dublin to mark the anniversary of the foundation of the Ordnance Survey of Ireland. The exhibition opens on June 7 and will run for 2 months.



...E and BALTEAGH exhibiting the Strata at the North Western Limits of the BASALTIC DISTRICT

Part of a geological panorama running north to south for 11 miles across the Co. Londonderry parishes of Drumachose and Balteagh. The original possesses a horizontal scale of 6 inches to 1 mile and a vertical scale of about 1 inch to 430 feet. The panorama which shows the Tertiary plateau basalts (blotched) resting upon Cretaceous chalk and Triassic sandstone, is the work of two of Pringle's officers, Lieutenants Lancey and Fenwick, and it is dated November 28, 1827. The topographical detail and geological inscriptions have been lithographed and geological colours then added by hand, and the panorama was evidently used as a specimen designed to show survey parties the type of work expected of them.

Infrared superhet for detecting pollutants

from our Chemical Physics Correspondent

ANYBODY who, since the 1930s, has built his own radio receiver is aware of the advantages of superheterodyne detection. Not merely can the main amplifier be chosen for a convenient frequency, but more importantly the signal strength is proportional to the product of the electric field strengths of the weak incoming signal and the powerful local oscillator in contrast to the less favourable square of the signal field for direct detection.

Hitherto these advantages have been denied to those who need to detect weak infrared signals, because of the lack of suitable local oscillators whose stability must be good compared with the band width of the amplifier. Stable infrared lasers have altered this position so that the whole problem of the detection of weak infrared emission is being considered. These comments are prompted by an article by Menzies and Shumate (*Science*, **184**, 570; 1974) who use the superhet principle for the detection of small amounts of pollutant gases. But as these authors point out, a wider range of applications is possible including the gathering of meterological information by sensing the atmospheric emission at selected frequencies. And it may not be long before such detection systems are used in conjunction with astronomical telescopes. Furthermore, when tunable infrared lasers are more readily available the scheme can be used in conjunction with frequency scanning.

The heart of the instrument is the mixer detector, in the present case a copper-doped germanium photoconductor. This receives the signal,

chopped with what is effectively a cold chopper, and it also receives 40 mW of power from the c.w. laser at the frequency of interest. The detector is followed by a high radiofrequency amplifier broad banded from 10 to 600 MHz, which amplifies the beat signal between the infrared sources. This device is then sensitive to radiation ± 600 MHz (± 0.02 cm^{-1}) from the laser with the exception of a narrow central gap. Providing in the absence of the emitter gas the source seems to be appropriately cold, a condition ensured in the laboratory by a cold surface beyond the back window of the sample cell, appropriate reference levels may be set and calibration provided.

The gas under study must, of course, emit at the frequency of the laser. Using a CO_2 laser at 1,109 cm^{-1} (9.02 μm) for SO_2 samples at room temperature, the emission was as little as 10^{-2} atm cm for the product of the partial pressure of SO_2 and the cell length. For ethylene at 950 cm^{-1} (10.53 μm), the figure was 5×10^{-5} atm cm in the presence of an atmosphere of nitrogen in each case. There is little doubt that these figures could be appreciably improved with tunable lasers to ensure operation at the peak of the gas line and also if the gas to be detected formed a major component of the sample so that lower pressures could be used.

Oestrogen and progesterone binding

from our Steroid Biochemistry Correspondent

IT is well established that the steroid hormones oestrogen and androgen bind to specific receptor proteins in mammalian reproductive tissues. Binding of progesterone and receptors for it, however, have been more difficult to demonstrate. In rat uterus the progesterone binding protein seems to be the same as the corticosteroid-binding globulin of rat plasma. In guinea pig uterus, however, a progesterone-binding protein (not corticosterone-binding globulin which has only a low affinity for progesterone in this species) is easier to demonstrate.

Two types of binding for oestrogens and androgens exist in the mammalian reproductive tract; one has a high affinity and a limited number of binding sites and, conversely, the other has a low affinity and an unlimited number of binding sites. Progesterone binding in the guinea pig uterus seems to follow the same pattern.

The presence of progesterone receptors has been revealed mainly by sucrose gradient centrifugation. As in the case of uterine oestrogen receptors, two components seem to exist; one has a sedimentation coefficient of 6.7S and the other has one of 4–5S. In addition to sucrose gradient centrifugation which measures directly the concentration of binding protein, binding of progesterone can be shown by simpler methods involving *in vitro* measurements of bound progesterone after removal of free hormone with adsorbents (Freifield *et al.*, *Steroids*, **23**, 93–103; 1974; Atger *et al.*, *Enclocrinology*, **94**, 161–167; 1974).

In both rats and guinea pigs these authors found that treatment with oestrogens increased the uterine binding of progesterone. In the guinea pig the increase began within 4 to 12 h and after 4 days the concentration of high-affinity binding sites per cell showed a ten-fold increase; no further increase occurred if treatment was continued for up to 2 weeks. On stopping oestrogen treatment, binding activity declined with a half life of about 3 days which suggests that the progesterone receptor is a relatively stable protein but that it is dependent on oestrogen stimulation. Further evidence for oestrogenic control of the receptor is the change in the binding protein during the oestrous cycle; the concentration of binding sites is highest at pro-oestrus when oestrogen production is high and the receptor is present mainly in the 6.7S form and lowest at dioestrus when more of the 4–5S receptor is present.

The vagina and uterine cervix of guinea pigs also contains a receptor protein which can bind progesterone. The properties of the receptor in these tissues are similar to those of the uterus although the vaginal receptor seems to be much more dependent on oestrogen than the uterine one.

This effect of oestrogen seems to be antagonised by progesterone. Administration of progesterone to oestrogen-treated castrated guinea pigs caused a rapid decline in uterine binding which reached a minimum by 6 h and which had not increased again by 24 h. The loss of binding activity was not apparently caused by a failure of labelled hormone to exchange with the endogenous bound hormone under the conditions of the test. Similarly, during pregnancy in the guinea pig, progesterone binding in the uterus is low and this may be due to the high circulating levels of progesterone.

In most circumstances synergism exists between oestrogen and progesterone and progesterone only produces a biological response in tissues which have been exposed to oestrogen. This is explainable if oestrogen stimulation is required for the synthesis of progesterone binding protein. The decreased binding activity on progesterone administration, even when oestrogen is present, might suggest that the action of progesterone is self-limiting—once a sufficiently high level of progesterone in plasma is attained, binding of the hormone in the uterus is decreased. More work is, however, required to determine these relationships.

Anomalous Kapitza resistance

from Peter V. E. McClintock Condensed Matter Correspondent

THE Kapitza resistance between liquid He II and a solid exhibits anomalous behaviour near 2 K according to a report in *Physical Review Letters* (**32**, 658; 1974) by Cheeke, Hebral, Richard and Turkington of the Centre National de la Recherche Scientifique (CNRS) at Grenoble.

When heat is passing from one solid to another one made of a different material there is always a jump in temperature across the interface which separates them. The phenomenon can be accounted for reasonably well in terms of an acoustic mismatch theory: by analogy with the partial reflection of light which occurs at an interface between two materials with different refractive indices, there is a partial reflection of the lattice waves (phonons), which constitute the flux of heat, as they try to pass between two materials in which the velocity of sound is different. The temperature discontinuity caused by this reflection of phonons is proportional to the heat flux, the constant of proportionality being known as the thermal, boundary resistance.

The velocity of sound in liquid helium is a factor of one twentieth smaller than in most solids, so that the thermal boundary resistance, between liquid helium and a solid would be expected to be particularly large. In fact, the boundary resistance, called in this case the Kapitza resistance after its discoverer, is indeed relatively very large, although, for temperatures above 1 K not nearly as large as would be predicted on the basis of the acoustic mismatch theory. There is, as yet, no really satisfactory theory of the Kapitza resistance above 1 K.

In all previously reported experiments, however, the phenomenon has seemed to behave linearly: that is, the temperature discontinuity ΔT has always been directly proportional to the heat flux Q, within experimental error. The CNRS group has measured, to a high precision, ΔT between copper or lead and liquid helium. They find that, although a plot of ΔT against Q yields an accurate straight line at small values

of Q there is, for any given temperature, a definite heat flux at which the line changes slope by up to 25%. The effect is at its largest near 2 K, gradually diminishing as the temperature is reduced.

It is now more than thirty years since the existence of the thermal boundary resistance was discovered by Kapitza (*J. Phys. U.S.S.R.*, **4**, 181; 1941) and it seems at first sight very surprising that in the course of the large body of experimental work on the phenomenon since that time, no one has reported observation of the anomalous features now being described. The effect would clearly be easily missed, however, except in cases where a large number of closely spaced data points were taken at small values of Q. Ironically, there is perhaps some evidence for the existence of these anomalies in the original graphs published by Kapitza.

As for the origin of the anomalies, the authors discuss a number of possibilities the most plausible of which concerns the onset of turbulence in the helium very close to the metal surface. Liquid helium at these temperatures, He II, can be regarded as an intimate mixture of two interpenetrating but extremely dissimilar fluids: a normal fluid component which behaves much like any other liquid and which carries all the thermal energy of the helium and a superfluid component which carries no thermal energy, has zero viscosity and is thus responsible for many of the more bizarre properties of the liquid. When heat enters the liquid, a counterflow of the two components occurs, with superfluid approaching the heated metal, being converted into normal fluid and then flowing away again. The authors tentatively conclude that the observed anomalous behaviour arises from the onset of turbulence in the normal fluid component within the very thin layer of helium at the surface of the metal, but further work will be necessary before this interpretation can be confirmed.

The authors have taken considerable pains to try and ensure that they have not been observing a mere artefact arising from their measuring equipment or experimental techniques. If the phenomena which they report are genuine, however, any theory of Kapitza resistance under these conditions will need to take them properly into account, so it seems highly desirable that the experiment be repeated in another laboratory as soon as possible.

Protein folding made simple

from Barry Robson

IT is hardly surprising that nobody has yet predicted how a given protein molecule folds up, considering that there are very much simpler molecules whose conformations defy theoretical analysis. Theoreticians with insatiable appetites for protein molecules, however, can restore at least a modicum of common sense by first examining protein systems in which prediction of the folding process is probably a simpler task.

Ptitsyn and Rashin (*Dokl. Acad. Nauk SSSR*, **213**, 473; 1974 and *Biophys. Chem.*, in the press) have made the first attempt to predict the folding and final overall conformation of a globular protein from its amino acid sequence and known secondary (locally organised) structure. The relatively simple protein system is in this case the myoglobin molecule which along with its haemoglobin relatives, is exceptional in being composed of α helix and very little else. Ptitsyn and Rashin justify starting with the known helical regions in terms of the popular model in which such regions are among the first features to appear during the folding up of the polypeptide chain, a model which is consistent with experimental evidence (Jardetzky *et al.*, *Cold Spring Harb. Symp. quant. Biol.*, **36**, 257; 1971; Robson and Pain, *Proc. Jerusalem Symp. quant. Chem. Biochem.*, **5**, 279; 1973; Sachs *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3790; 1972). Furthermore, recent publications confirm that α-helical regions in proteins can be predicted with reasonable accuracy (see, for example, Chou and Fasman, *Biochemistry*, **13**, 222; 1974).

The method of Ptitsyn and Rashin is to try and predict the folding pathway and resulting most stable configuration of stacked α helices on the basis of hydrophobic interactions between the helices. They find that the most stable configuration "coincides in a rough resolution" with the native conformation. It is surprising, however, that they obtain even such an approximate structure, and it is necessary to ask whether such an agreement could be fortuitous. Helices were treated simply as cylinders with a radius of 5 Å, and dispersion, electrostatic and hydrogen bonding forces between helices are neglected except to the extent that they are included in the parameters of the hydrophobic interactions. Furthermore, a possible contribution of the helix backbone to the hydrophobic interactions was not investigated. Finally, the study was apparently carried out manually and because of the still very large number of possible configurations it would be difficult to exclude an accidental subjective element from entering such calculations where the answer is known in advance.

It is therefore possible that in retrospect the simplifications of Ptitsyn and Rashin will be seen to be too simple, and their work is likely to be best remembered for the more critical reassessments of the problem which it will undoubtedly stimulate. Nevertheless, the concept of interactions between secondary structure features such as α helices leading to the formation of single compact globules is a potent one and extends well beyond the simple case of myoglobin. The recent work of Rossman and Liljas (*J. molec. Biol.*, **85**, 177; 1974) confirms and quantifies the idea that sections of the backbone of more complicated proteins are bunched up locally to form subglobules within the overall globular structure of the molecules, so dividing many protein molecules into two or more distinct lobes. Within such subglobules, interactions between α-helical regions and other secondary structure features are probably very important.

As the first protein to have its conformation determined by X-ray crystallography, myoglobin once surprised biochemists with its unexpected complexity and the lack of any obvious relation between amino acid sequence and conformation. Now the pendulum is beginning to swing from a state of general pessimism to a state of optimism which is probably not unjustifiable.

All excited in Lisbon

from a Correspondent

THE opulent ambience of the Calouste Gulbenkian Foundation Centre in Lisbon on April 18–24 was the choice for an international conference on excited states of biological molecules under the sponsorship of the European Physical Society and European Photochemical Association, with J. B. Birks (University of Manchester) as papers secretary and editor.

As both Sir George Porter (Royal Institution, London) and G. Eisinger (Bell Laboratories, New Jersey) noted in their lectures, 1974 is about 25 years since the seminal Faraday Society Discussion of 1950, when M. Kasha discussed the phophorescence of organic molecules in terms of the Jablonski diagram and singlet–triplet conversion, and about 50 years since Perrin postulated singlet–singlet energy transfer to explain the depolarisation of fluorescence. Excited states in biological molecules were last discussed at an international meeting at Arden House, New York, in October 1969 (see *Nature*, **226**, 113–118; 1970). In the light of that report, the programme of this year's meeting had inevitably a slight sense of *déja vu*, as the main themes have not changed greatly in 5 years.

Porter reviewed the methods avail-

able for the direct observation of excited states, with particular reference to nanosecond and picosecond laser flash photolysis. He emphasised the importance of substituent effects on the reactivity of radical species formed from excited-state ketones, and the biological roles of plastoquinone and ubiquinone, which are the subject of current work in his laboratory.

C. Hélène (Centre National de la Reserche Scientifique, Orléans) gave a lucid review of excited state interactions and energy transfer processes in oligopeptide–nucleotide complexes. He described energy transfer between stacked nucleotides and aromatic amino acid chromophores in frozen aggregates at both singlet and triplet levels. It is necessary to intercalate the bound aromatic residue between the bases of single-stranded nucleic acid for quenching of fluorescence. The ability of small peptides containing an aromatic residue to bind to a small, single-stranded region of DNA containing, for example, a thymidine dimer, followed by photosensitised splitting of the dimer by energy transfer from the excited aromatic residue, provides an elegant example of a specific photoprocess in a model protein-DNA complex.

In a lecture entitled "What We have learnt about Proteins from a Study of their Excited States", G. Weber (University of Illinois) cited the demonstration of processes in the nanosecond or shorter time range, such as fast degradation of excess vibrational energy of the excited state, energy transfer between aromatic chromophores, solvent relaxation effects, molecular or segmental relaxation times and the correlation between free residue rotation and protein unfolding. He considered that with the advent of time-resolved spectroscopy on a nanosecond time scale, future work would be largely concerned with the dynamics of excited state processes. The use of differential phase fluorimetry to study solvent relaxation was described, and also the use of dissolved oxygen (under high pressure in a special cell) as an uncharged fluorescence-quenching agent. From comparisons between plots of fractional fluorescence and fractional lifetimes it is possible to determine if the quenching is homogeneously dynamic, or static and involving complexes. Of the proteins examined, some show deviations from pure dynamic quenching.

The lecture by H. T. Witt (Max-Volmer Institute, Berlin) was an impressive example of the value of a conceptual model in planning definitive experiments in such a complex field as photosynthesis. Witt demonstrated how the specific structural arrangements of the functional membrane (thylakoid) of the photosynthetic system mediates extraordinary reaction patterns not seen *in vitro*. He presented evidence in suport of the following sequence of primary acts of energy conservation and utilisation: (1) the channelling of light absorbed by bulk pigment (carotenoids and chlorophylls) by singlet–singlet transfer to photoactive chlorophyll, with a triplet–triplet transfer path from chlorophyll back to carotene to protect the former from irreversible photo-oxidation at high light intensity; (2) a specific arrangement in which always two photoactive chlorophylls are coupled in series in the membrane and are able to pump electrons from H_2O to $NADP^+$ to form NADPH; (3) the creation of a charge across the membrane; (4) the discharge of the membrane potential by H^+ fluxes through specifically organised channels (ATPase) coupled with the generation of ATP from ADP+P. It is the NADPH and ATP formed in the system which carry out the reduction of CO_2 into sugar and "everything else". The membrane potential is sufficiently high to give rise to a Stark shift in the absorption spectra of adjacent chromophores, and the shift can be regarded as a "molecular voltmeter". Some ingenious experiments to demonstrate this engaging concept were described. It seems that at least four key features of Witt's model of vectorially directed processes in the photosynthetic membrane have been confirmed experimentally.

Eisinger described a more critical approach to the interpretation of singlet energy transfer by the Förster mechanism. Since the transfer efficiency between two luminophoric groups on a macromolecule depends not only on the distance (R) between them, but also on the relative orientation of their transition dipoles, the value used for the orientation factor (κ^2) is crucial for the calculation of R. The widely-used value of 2/3 can be derived for the dynamic random averaging case, which, however, is unlikely to be relevant to real macromolecule situations. Between the extremes of fixed relative orientation, κ^2 varies from zero to 4. Eisinger and Dale have calculated contour maps showing the variation of κ^2 as the transition dipoles are given orientational freedom within cones of specified half-angles for a variety of donor-acceptor group geometries. Their analysis shows it is fallacious to assume that in practice κ^2 values near the extreme values are unlikely. They estimate the extent of orientational freedom from the limiting emission anisotropies of the separate luminophores (from time-dependent polarised emission data), and hence get reasonable upper and lower bounds for κ^2. Since information on the relative orientation of the donor-acceptor pair can be obtained from the depolarisation of transferred energy, a realistic estimate of κ^2 is possible. This procedure was used to obtain an R value for energy transfer from the fluorescent Y-base of the anticodon loop of a t-RNA to a bound dye at the remote end of the macromolecule.

S. A. Latt (Harvard Medical School) used fluorescent probes to study chromosome structure. The fluorescence of bound quinacrin is quenched by guanine–cytosine base pairs of the DNA, so that the fluorescence reveals regions with high adenine–thymine content. Furthermore, the base analogue 5-bromodeoxyuridine biosynthetically incorporated into DNA quenches the fluorescence of the dye Hoechst 33258, and can therefore be used to study the time course of DNA replication in chromosomes, and also some aspects of chromosome structure and stability.

L. G. Christophorou (Oak Ridge National Laboratory and University of Tennessee) described how threshold electron excitation spectroscopy, based on the resonant interaction of slow electrons with vapours, can be used to detect optically-forbidden transitions in aromatic compounds, including triplet states and transient negative ions. The stabilities of the double negative ion resonant states can be simply correlated with established substitution parameters of the benzene ring.

In an interesting application of luminescence spectroscopy, J. W. Longworth (Oak Ridge National Laboratory) reported fluorescence and phosphorescence data for more than 50 Bence-Jones proteins — 'light chain' dimers of γ-immunoglobulins which are secreted as individual variants in myeloma. The light chain is believed to fold in a homologous conformation, the immunoglobulin fold, and comprises two domains of approximately equal size, the variable N-terminal domain, and the C-terminal domain which is constant in all light chains of a given type. The observed diversity of fluorescence, with respect to amplitude, spectral distribution and lifetime, can be ascribed to perturbation of a single tryptophan residue in the variable domain. The phosphorescence spectra are equally diverse, often showing two decay components. Bence-Jones proteins therefore provide an excellent opportunity for studying the effects of limited variations in residue composition on the luminescence properties of a specific protein structure. There is some evidence that interaction between tryptophan and a contiguous disulphide bond is responsible for the short-lived phosphorescence, and some other contributors also considered the possible role of such an interaction in protein luminescence spectroscopy.

I. Tatischeff (Institut du Radium-

Laboratoire Curie, Paris) reported variations of the fluorescence quantum yield of the three aromatic amino acids with excitation wavelength. For all three, the yields, based on both quinine sulphate and sodium salicylate as wavelength-independent quantum counters, were constant in the longwave absorption bands, then fell abruptly to lower constant values in the shortwave absorption bands. The discussion provoked by her findings was about equally divided between explaining it away as due to experimental artefacts or inadequacy of the quantum yield standards, and in accepting them as correct and proposing possible explanations.

From the rather small number of communications concerned with small peptides and polypeptides, that by Ph. Wahl (Centre de Biophysique Moléculaire, Orléans) on the pulse fluorometry of tryptophyldiketopiperazine may be noted. He concluded that quenching of tryptophan fluorescence by the peptide bond only occurs in a folded conformer. The equilibrium constant relating the folded and unfolded conformations seems to be different in the excited and ground states; the ground state value is available from nuclear magnetic resonance studies.

An indication of new spectroscopic techniques was the inclusion of contributions on magnetic circular fluorescence and fluorescence polarisation and on magnetic circular dichroism in the near infrared. The shorter papers on visual pigments were concerned with problems of singlet-triplet intersystem transfer and *cis–trans* isomerisation of polyenes, and in the large number of communications on photochemical reactors, the roles of singlet and triplet oxygen were frequently discussed.

Management of aquatic weeds

from Peter D. Moore
Plant Ecology Correspondent

AT the close of the International Hydrological Decade it is only natural that the world of waterweeds and the problems associated with their control should be receiving some attention in the scientific literature. It is natural too that the problems of the developing countries should receive greatest prominence, for here many essential programmes of irrigation are being set back by the luxuriant and rapid growth of aquatic vegetation in tropical climates. India, most especially, has been at the fore in a series of recent publications on this subject.

Gupta has summarised many of the Indian problems and some of their solutions in his booklet *Aquatic Weed Control* (Rajasthan College of Agriculture, Udaipur, 1973). In Rajasthan the development of a canal system is relieving a serious problem of water distribution; many women were forced to walk several kilometres to fetch pitchers of water from surface ponds. The canal projects, however, have suffered severely because of the growth of aquatic weeds, particularly water hyacinth, *Eichornia*, and the water ferns *Azola*, *Wolfia* and *Salvinia*. From April to June the canals are closed so that clearance by hand can take place, but this control method is ineffective in the long term. Only one mechanical weed cutter operates in Rajasthan but biological control using Chinese carp is being attempted; the use of chemical herbicides is prohibitively expensive. The same sad picture of India's plight emerges from the recent UNESCO symposium held in New Delhi and reported by Tinker (*New Scientist*, **61**, 747; 1974).



Weed Research Organisation

Water channel in India infested with the water hyacinth *Eichornia*.

A very full review of the entire problem has now been published by UNESCO (*Aquatic Vegetation and its Use and Control*, edit. by D. S. Mitchell, Paris, 1974). Although the theoretical consideration of the mathematics of waterweed population growth and the technique of mechanical, chemical and biological control of waterweeds are undoubtedly important as short term approaches to the aquatic vegetation problem, data presented by Boyd (University of Auburn, Alabama) in this collection of papers provide hope for a more permanent solution. The explosive growth of aquatics is itself an indication of eutrophication and nutrient wastage which can ill be afforded, especially in countries such as India. Boyd considers ways in which waterweeds could assist in the retrieval of these last elements.

Many waterweeds provide a better feed for livestock than dried alfalfa hay. Generally they contain rather less crude protein and fibre, but more ash and fat than this commodity. Inorganic ion concentration in aquatics falls within the range generally accepted for crop plants; ash values of more than 25% of the dry weight are recorded, the noxious weed of many Indian waterways, *Hydrilla verticillata*, being responsible for some of the higher figures.

Before these nutrients can be re-used by crops they must be released from the organic tissues of the water plants, and decomposition is not always rapid. *Eichornia* compost takes about 3 months of fermentation before it is suitable for use as a fertiliser. Nevertheless, when it is considered that eutrophicated waters can support a standing crop of 29 t ha^{-1} (dry weight) of *Eichornia* containing 157 kg ha^{-1} of phosphorous and 693 kg ha^{-1} of nitrogen, this process must be considered an important means of cutting nutrient losses.

In the developed countries the cost of retrieving lost nutrients from solution usually exceeds the cost of supplying new ones. In countries such as India, where manual harvesting is cheap and where the foreign exchange needed for chemical fertilisers is in short supply, the use of aquatic vegetation for nutrient recovery may prove to be economically feasible.



Weed Research Organisation

Pistia stratiotes.

What is the nuclear optical potential?

from Peter E. Hodgson, Nuclear Physics Laboratory, Oxford

THE interaction of a nucleon of moderate energy with a nucleus is an exceedingly complicated process. Very many reactions can occur and they depend on the energy levels of the compound and residual nuclei. The conservation of particle flux links all the reaction channels together, so that any abnormal behaviour in one channel, a resonance or threshold for example, produces corresponding changes in all the others. It might well be thought that each interaction would have to be treated as a special case and that the detailed structure of each nucleus would have to be taken into account before the cross sections could be calculated.

One-body potential

In spite of this somewhat pessimistic expectation, the striking regularities in the neutron total and reaction cross sections as a function of energy and from nucleus to nucleus suggested that they could be described by a one-body potential varying smoothly with energy and nuclear size. (Feshbach, Porter and Weisskopf, *Phys. Rev.*, **96,** 448; 1954). The success of this description led to the model being extended to nucleon elastic scattering and the range of other nuclear interactions.

Subsequently it was realised that this success was partly due to the relatively poor energy resolution of the early measurements. Later improvements in experimental techniques showed that the cross sections of reactions passing through the compound nucleus do fluctuate as the incident energy varies (Von Brentano *et al.*, *Phys. Lett.*, **9,** 48; 1964) but if the energy spread of the incident beam is greater than the mean width of the fluctuations an averaged cross section is obtained that generally varies quite smoothly with energy. Detailed analysis showed that this averaged cross section is the sum of compound nucleus and direct reaction components; the former may be calculated using the theory of Hauser and Feshbach (*Phys. Rev.*, **87,** 366; 1952) and subtracted from the measured cross sections. As the energy increases the contribution of the compound nucleus processes falls rapidly and soon becomes negligible.

In precision analyses it is necessary to remove the compound nucleus contribution to the data by a preliminary Hauser–Feshbach calculation or preferably to confine the analysis to energies for which the compound nucleus contribution is negligible. For most nuclei, energies of about 15 MeV or more are adequate for this purpose. The direct cross section is then analysed by a simple optical potential.

Many such analyses were made and soon it was possible to specify potentials defined by rather few parameters that give strikingly good overall fits to a wide range of neutron and proton elastic scattering and reaction data (Perey, *Phys. Rev.*, **131,** 745; 1963; Perey and Buck, *Nucl. Phys.*, **32,** 353; 1962). One of the most successful of these potentials was obtained by Perey from an analysis of the elastic scattering of protons of 9–22 MeV by a range of medium and heavy nuclei. He used a potential of the form

$$V(r) = U_0 f(r_u,a_u) + ia_D W_D(d/dr)[f(r_D,a_D)] + (\hbar/m_\pi c)^2 U_s(1/r)(d/dr)[f(r_s,a_s)]\mathbf{L}.\sigma$$

where

$$f(r_i,a_i) = [1+\exp\{(r-r_i A^{1/3})/a_i\}]^{-1}$$

In the first analyses, the parameters of this potential were adjusted to optimise the fit to each set of data for scattering from a particular nucleus at a particular energy. The most accurate data were the differential cross sections, supplemented by less accurate polarisations and reaction cross sections.

The optimum parameters for different nuclei at different energies were found to be very similar, so the data were reanalysed fixing the form factor parameters to the values

$$r_u = 1.25;\quad r_D = 1.25;\quad r_s = 1.25$$
$$a_u = 0.65;\quad a_D = 0.47;\quad a_s = 0.65.$$

The quality of the fits was still very high, though naturally not quite as good as previously.

The depth of the real potential was found to vary systematically with energy and with the numbers of neutrons and protons in the nucleus, and the variation could be well represented by

$$U = 53.3 - 0.55E + 27\alpha + 0.4Z/A^{1/3}$$

where $\alpha = (N-Z)/A$

The optimum imaginary potential was not so well determined, but a good average value is

$$W_D = 12A^{1/3}$$

The polarisations were best fitted with

$$U_s = 7.5 \quad (E < 17);\quad U_s = 8.5 \quad (E > 17)$$

The fits obtained with this overall potential are very little worse than those obtained when several parameters were adjusted in each case.

Global potential

The striking success of these analyses led to the hope that it would be possible to find an overall or global potential for all nuclei and one has tended to forget that the individual structure of each nucleus must affect the interaction to some extent. This raises difficulties concerning the concept of the optical potential, in particular whether it should give a good overall fit to many sets of data or whether it should be adjusted to fit each set of data as accurately as possible.

Optical potentials of the first type are the same for all nuclei, with perhaps a smooth and easily parameterised dependence on the incident energy and on Z and A. To achieve this one has inevitably to make some sacrifice in the quality of the fits to the data.

Optical potentials of the second type have parameters that are unique to each nucleus at each energy, so that accuracy has been purchased at the expense of loss of generality.

Both types of potentials have their advantages and disadvantages. In some interactions there are quite substantial deviations from the behaviour given by the overall potential, whereas potentials fitted accurately to particular sets of data are sometimes recognisably non-physical. These anomalies have a physical basis, so that one might hope that detailed studies with both types of potential could teach us more about the physical process taking place.

It is important to see what can be done to eliminate the disadvantages of these potentials while preserving their good points. The first step is to identify the physical processes responsible for the deviation from 'normality' and make a physical model that allows them to be calculated explicitly. If this can be done within the framework of the optical model, then the process and its effect on the cross section can be represented by an extra term in the optical potential. In this way, for example, the polarisations of the elastically scattered nucleons were included in the model by the addition of the spin–orbit term to the potential and the dependence on the asymmetry parameter by the term proportional to $(N-Z)/A$.

Small terms

At one stage it was hoped that all new physical effects and deviations from the simpler potential could be brought within the general optical potential by the addition of a series of small terms, giving a potential expressible as a simple analytical function of A and E and capable of fitting the elastic scattering data to high accuracy.

Unfortunately in recent years several deviations from normality have been found that can be understood physically but which cannot be treated in this way. These are of two types; the first can still be treated within the optical model formalism whereas the second requires more sophisticated theoretical treatment.

An example of the first is the exceptionally low neutron reaction cross sections found for magic nuclei at low energies, especially for ^{208}Pb (Perey and Buck, 1962). These are much less than those calculated from the highly successful Perey–Buck potential and can easily be understood as a consequence of the low level density at small excitation energies of such nuclei. The effect can be treated phenomenologically by allowing the absorbing part of the optical potential to depend on the shell structure but the absorbing potential cannot conveniently be expressed as an analytical function of Z and A. This phenomenon only occurs in a limited energy region and is not of general importance.

The effects in the second category may be calculated by appropriate physical models but it is not possible to represent them by an easily-parameterised additional term in the optical potential. It is of course often possible to fit the data through the region of the anomaly with a particular optical potential, so that one can formally define the potential responsible for the effect as the difference between the general optical potential and the particular potential, but this is often highly singular and not conveniently parameterisable.

The extent to which this occurs implies a breakdown of the simple optical model, so that one is now faced with the alternatives of either using an ill-behaved effective potential in the framework of the simple optical model or of continuing to use a general optical potential in a more sophisticated formal framework. The second alternative is clearly the more desirable, even from the purely phenomenological point of view, as it often unifies a range of phenomena in a simple way, whereas the effective potentials are much more difficult to handle.

This may be illustrated by the analysis of elastic scattering from strongly deformed nuclei. These have low lying collective states that are readily excited by inelastic scattering and the cross sections are so large that the elastic scattering cross section is significantly affected.

The inelastic cross sections vary in magnitude with β, but hardly at all in shape, and the elastic cross section departs more and more from its unperturbed value as β increases. If the elastic cross section is analysed for several deformed nuclei, the parameters of the resulting potential vary from nucleus to nucleus depending on the strength of the coupling to the collective states.

In particular, the imaginary potential is found to increase with increasing deformation. A coupled-channels analysis can, however, fit the elastic cross section with the same optical potential for each nucleus provided each has its characteristic deformation parameter. This was first shown by Perey (in *Nuclear Spectroscopy with Direct Reactions* (edit. by Throw) ANL-6848, 114; 1964) in an analysis of the elastic scattering of protons of 17 MeV off some heavy spherical and deformed nuclei.

Another example is provided by the scattering of α particles of 50 MeV by the even isotopes of samarium (Glendenning, Hendrie and Jarvis, *Phys. Lett.*, **26B**, 131; 1968). The elastic scattering changes qualitatively from one isotope to the next; the diffraction oscillations become less and the envelope of the maxima steeper as the mass (and the nuclear deformation) increases. Simple optical model analyses of these data give quite different potentials, but the cross sections are all well fitted by the same optical potential when the coupling to the collective states is taken into account and each nucleus is characterised by its deformation parameter. The deviations from simple normality can thus be brought within the scope of the general optical model, although the deformation parameter cannot be expressed as an analytical function of Z and A.

Coupled-channel analyses

Further evidence for the reduction of the absorbing potential in the region of closed shells is provided by measurements of the s and p-wave strength functions. These show characteristic maxima and minima as a function of A and the overall behaviour is quite well given by the optical model with standard potentials. Closer examination shows that although the maxima are quite accurately fitted, the experimental values are much less than the calculated ones in the regions of the minima. A coupled-channels analysis by Newstead (in *European Conference on Intermediate Reactions*, Plitvice; 1972) found that this behaviour can be obtained by reducing the imaginary potential in a way that varies from nucleus to nucleus. Since the imaginary potential corresponds to absorption it might be expected to be correlated with the number of three-quasiparticle states that can be formed in the initial stages of the nuclear excitation. Newstead estimated the numbers of such states in various nuclei and found that they correlate very well with the imaginary potentials W obtained by requiring a fit to the s-wave strength function data. Thus once again the deviations from normality can be explained by a physical model but not in a way that can easily be parameterised in terms of A and Z.

Another effect that can easily be treated by the coupled-channels formalism is the marked resonances that occur when low energy nucleons are scattered by some light nuclei (Mikoshiba, Terasawa and Tanifugi, *Nucl. Phys.*, **A168**, 417; 1971). Some of these are single-particle resonances in the optical potential; others can be understood as core-excitation resonances. The coupled-channels formalism with a simple model of the nuclear excitation gives a good overall account of these resonances. The data also show higher order resonances that could presumably be treated by including the coupling to more complicated configurations.

The scattering from light nuclei at higher energies frequently shows deviations from normality and in some cases this is due to higher order processes. In the case of medium-energy proton scattering by ^{12}C and ^{16}O the core-polarisation exchange mechanism with intermediate excitation of a giant resonance is responsible for the marked peaks in the backward cross section for inelastic scattering to non-normal parity states that cannot be accounted for by the simple optical model (Von Geramb, *Nucl. Phys.*, **A199**, 545; 1973; *Nuovo Cim. Lett.*, **5**, 333, 1972). This effect reflects back on the elastic channel and so should be taken into account explicitly at those energies where giant resonances are important.

As a final example, the (p, n) quasi-elastic reaction to the isobaric analogue state has significant contributions from the two-step (p, d, n) intermediate particle transfer process (Rickertsen and Kunz, *Phys. Lett.*, **47B**, 11; 1973). If it is analysed with a simple optical potential the fits are not strikingly good and the parameters of the isospin term which is responsible for the transition vary erratically from nucleus to nucleus. If the two-step process is included explicitly, making use of the properties of the known intermediate states, then the fits are significantly improved and the isospin potential is essentially the same for all nuclei. Thus once again by treating explicitly the process responsible for the deviations it is possible to recover a general optical potential.

Polarisations

The polarisations of the elastically scattered particles are much more sensitive to the interaction potentials than are the differential cross sections, as they depend on the differences of scattering amplitudes. This sensitivity

is to a large extent offset by the lower accuracy of the polarisation data, but nevertheless careful analyses of polarisations have shown anomalies that are not yet understood.

An example of this is provided by the work of Boschitz (*Phys. Rev. Lett.*, **17**, 97; 1966), who analysed a series of differential cross sections and polarisations. He was able to obtain good overall fits to the polarisation data but the optimum values of the parameters of the spin–orbit term in the optical potential showed marked variations from one nucleus to another and as a function of proton energy. So far there is no convincing explanation of this behaviour.

From these examples it will be seen that the processes that must be considered explicitly are of several types. The polarisation and the dependence on the nuclear asymmetry can easily be incorporated in the optical model. The others require quite sophisticated calculations before the effects due to the general optical potential and the special process can be disentangled. The calculations with both processes included give directly the elastic cross section and there is no effective optical model potential as an intermediate stage. It is thus not possible to think of a general optical potential in which the effects of coupling to excited states, two-step reactions, core-polarisation exchange processes and so on are included by appropriate analytical terms in a general expression of the optical potential. In this sense the optical potential ceases to be a concept able to give a detailed physical account of all the data; it has been superseded in some regions by more sophisticated calculations that give an accurate account of the data that cannot be obtained in any other way.

One has thus arrived at a third concept of the optical potential as one that includes as many effects as possible in the parameterisation of its parameters as functions of Z and A but which has to be used in a more sophisticated formalism if certain other effects are to be treated adequately. Thus some of the simplicity of the model has been lost, but further insight into more complicated phenomena has been attained.

I thank Dr R. S. Mackintosh for discussions and suggestions.

Correction

In the News and Views article "Vegetational change in an aboriginal environment" (*Nature*, **249**, 11; 1974), the date in line 7 of the first paragraph should be 5,500 radiocarbon years.

Biogenesis of surface membranes

from R. Colin Hughes

ACCORDING to the membrane flow theory, membrane biogenesis involves the physical transfer of membranes from one subcellular compartment to another. The endoplasmic reticulum is the precursor of Golgi membrane which is in turn processed into plasma membrane. Surface membrane proteins and glycoproteins, therefore, are considered as a special class of secreted substances.

The basic assumption of the theory, that membrane proteins are synthesised on the rough endoplasmic reticulum, must obviously be re-interpreted, however, since Lodish (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 1526–1530; 1973) and Lowe and Hallinan (*Biochem. J.*, **136**, 825–828; 1973) have described synthesis of some membrane proteins on free polysomes. The converse proposal that soluble cytoplasmic proteins are made only on free polysomes is similarly an oversimplification. At least one soluble enzyme, serine dehydrase, is synthesised on membrane-bound ribosomes and a similar role seems likely in non-secretory tissues such as brain or muscle. So far as membrane proteins are concerned, it is possible that integral membrane components are made in a way different to extrinsically bound proteins. Synthesis of glyceraldehyde-3-phosphate dehydrogenase on cytoplasmic polysomes is expected, for example, yet this protein forms stable interactions with high affinity for a limited number of sites on the cytoplasmic face of the erythrocyte membrane (Kant and Steck, *J. biol. Chem.*, **248**, 8457–8464; 1973). The enzyme seems therefore to be a specific membrane component, made on free polysomes.

An indication that integral proteins, by contrast, may follow an intracellular route analogous to the secretory process comes from an extensive radioautographic study by Leblond and his colleagues (*J. Cell Biol.*, **60**, 258–284; 1974). This suggests a final step in surface membrane biogenesis in which fusion takes place between the cell surface membrane and Golgi vesicles, the membranes of which carry proteins and glycoproteins destined ultimately for the surface membrane. The chief difficulty in accepting completely this proposal, is that the membrane composition of highly purified rat liver Golgi membranes, as described recently by Palade *et al.* (*J. Cell Biol.*, **59**, 45–72; 73–88; 1973) is clearly different to the homologous plasma membrane. The role of the Golgi vesicles therefore, may be to ferry a few proteins from intracellular smooth membranes to the cell surface as follows: fusion of the vesicles with the cell surface membrane, lateral diffusion of selected proteins into the area of plasma membrane surrounding the fusion site and finally re-entry of the Golgi vesicular membrane into the cell by endocytosis. In this case, the membranes of Golgi vesicles before fusion would contain a relatively small proportion of plasma membrane components superimposed on a background of purely Golgi membrane proteins and after fusion only Golgi specific proteins.

The membrane flow theory also predicts that at earlier stages of membrane biogenesis, the proteins and glycoproteins are restricted to membrane-bounded intracellular vesicles. Presumably, the membrane precursors made on membrane-bound ribosomes are either discharged into the lumen of the endoplasmic reticulum to be inserted into smooth membranes elsewhere or remain inserted directly into membranes at their sites of biosynthesis. Hirano *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **69**, 2945–2949; 1973 found earlier that the membranes of rough microsomal vesicles are assymetrically substituted with carbohydrate groups, capable of reacting with certain lectins coupled with ferritin. The membrane facing inwards carried carbohydrate whereas the cytoplasmic face did not. Since the intralumenal face of the endoplasmic reticulum is equivalent to the external face of the plasma membrane, at which carbohydrates are also localised, this finding is interpreted as a flow of membrane with retention of configuration from intracellular biosynthetic sites to the cell surface. It does not, of course, prove that this is the case. Kreibach *et al.* (*Fed. Proc.*, **32**, 2133-2138; 1973; *J. Cell Biol.*, **60**, 616–627; 1974) now confirm these observations by testing the accessibility of rat liver microsomal proteins to lactoperoxidase labelling. The glycoproteins are labelled only when the closed microsomal vesicles are made permeable by treatment with low concentrations of detergents. Two classes of glycoproteins are labelled; one class is released by mild detergent treatment whereas the other requires higher detergent concentrations and disassembly of the microsomal membranes. It is not clear, however, whether these last components are stable membrane constituents or precursors of glycoproteins.

The glycoproteins detected by Hirano *et al.* and Kreibach *et al.* are not known to be precursors of surface membrane glycoproteins and could equally well be glycoproteins destined for secretion. The next step is application of the techniques for the selective solubilisation of microsomal glycoprotein precursors to defining the kinetics of flow of these substances.

Gangliosides as membrane receptors for tetanus toxin, cholera toxin and serotonin

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Tetanus toxin is bound by a sialidase-labile disialosyl ganglioside, serotonin by another, and cholera toxin by a sialidase-stable monosialosyl ganglioside. There is good evidence that the monosialosyl ganglioside may be the receptor for cholera toxin in cell membranes; what of the disialosyl ganglioside and tetanus toxin?

THERE has been a sharp rise in interest in the possible biological roles of the gangliosides, discovered by Klenk in 1935–42[1,2]. These versatile substances have been virtually ignored in recent authoritative reviews on the structure of cell membranes[3,4], in spite of a number of characteristics which indicate that they might be considered as possible functional components of cell membranes. First, they have an amphiphilic nature, as they are composed of lipophilic ceramide moieties and hydrophilic oligosaccharide moieties; second, in nervous tissue, which is the richest source of gangliosides, they have found to be concentrated in fractions containing synaptic membranes[5,6]; third, they are being found in an increasing number of biological preparations[7]; fourth, although they are water soluble they are almost invariably not extractable with water from biological material, which indicates that they are bound to hydrophobic components[8,9].

(Bretscher[4] proposes that the outer layer of the membrane lipid bilayer is largely composed of ceramidyl phospholipids, and that the glycolipids are located solely in this layer. In such a scheme it would be reasonable to speculate that the ceramidyl moieties of the gangliosides may be buried, leaving the hydrophilic oligosaccharide moieties exposed on the outsides of the membranes.)

No particular function for the gangliosides has, up to now, been clearly demonstrated, but recently there has been an accumulation of evidence that cholera toxin may recognise a particular ganglioside on the surfaces of cell membranes. These observations revive interest in earlier observations of a specific interaction between tetanus toxin and two other gangliosides, and between serotonin and another ganglioside.

Tetanus toxin

Tetanus toxin is a neurotoxin, thought at first to act only on the central nervous system (CNS), producing spastic paralysis by blocking synaptic inhibition in the spinal cord, but now known to act peripherally as well, producing flaccid paralysis by blocking neuromuscular transmission[10,11]. In its action on nervous tissue, it produces no morphologically distinguishable changes in the tissue, yet it binds with the tissue. This was first shown in 1898 by Wassermann and Takaki who found that when a homogenate of nervous tissue was mixed with a solution of tetanus toxin and the suspension filtered, the concentration of toxin in the filtrate was diminished[12]. This phenomenon was investigated by many workers, including Landsteiner, who believed that the substance in nervous tissue responsible for the binding was a cerebroside, but was puzzled by the fact that grey matter had a greater capacity to bind the toxin than white matter, but had a lower cerebroside content[13]. I reinvestigated the Wassermann-Takaki phenomenon and showed that it was apparently tissue specific and toxin specific (that is, of all the tissues tested only nervous tissue bound the toxin, and of all the known bacterial toxins tested only tetanus toxin was bound[14]). When these tests were reported in 1959 cholera toxin was not yet known, its existence being reported by De in the same year[15].

It was then shown that the binding of tetanus toxin was due to the then comparatively unknown substance, ganglioside. It was also shown that there were at least three gangliosides in nervous tissue, differing in their sialic acid contents and their

tetanus toxin-binding capacities, which increased with increasing sialic acid content[16]. Later, when the multiplicity and nature of gangliosides was better understood, it was shown that tetanus toxin had an affinity for two particular gangliosides containing two sialic acid residues attached to the lactose component[17] (Table 1).

Tetanus toxin does not seem to lose its toxicity as a result of being bound by ganglioside; nor does the ganglioside seem to be altered. If tetanus toxin (50 ng ml^{-1}) is incubated in the presence of gelatin for 1 h at 37° C with the gangliosides GGnSSLC and SGGnSSLC (300 μg ml^{-1}, molar ratio of ganglioside to toxin of 4×10^6:1) there is no reduction in toxicity[18]. If 5 mg of mixed gangliosides (containing about 1 mg of GGnSSLC+SGGnSSLC) are injected intramuscularly into mice, however, followed 5 h later by an injection of 0.5 LD_{50} of toxin in the same site, the mice are protected against the more severe degrees of tetanus, but in this case the molar ratio of ganglioside to toxin is much greater (3×10^9:1) (ref. 19).

The interaction between tetanus toxin and ganglioside can be observed in two ways. First, a solution of tetanus toxin (5mg ml^{-1}) and ganglioside (0.1 to 0.5 mg ml^{-1}) is centrifuged in an analytical ultracentrifuge. The complex of ganglioside and toxin will move rapidly down the centrifugal field, leaving some unbound toxin, the amount of which by difference from the original gives the amount bound[16] (Table 1). Second, ganglioside is rendered insoluble by complexing it (as a mixed micelle?) with insoluble (and unreactive) cerebroside, and varying amounts of the complex are suspended in equal volumes of a solution containing about 1.5 ng toxin ml^{-1} (about 45 LD_{50} ml^{-1}). The insoluble ganglioside–cerebroside–toxin complex is spun down and the supernatant tested for toxicity in mice. In this way the least amount of ganglioside that will bind (say) 10 LD_{50} of toxin can be determined. Not surprisingly, at these low concentrations of toxin far less toxin is bound per unit weight ganglioside than at concentrations 3 million times greater, but the differences between the toxin-binding capacities of different gangliosides are still evident (Table 1). The proportion of ganglioside in the insoluble complex is critical—thus complexes containing 25% ganglioside have a much greater capacity to bind tetanus toxin (and to protect mice against the toxin) than have complexes containing either 5 or 50% ganglioside[9].

If relatively high concentrations of almost any ganglioside in solution are incubated for long periods with almost any toxin in the absence of a protective protein such as gelatin, the toxin will be inactivated. This nonspecific effect is of little interest, but has led to some confusion[18].

So far, the specific interaction between tetanus toxin and the sialidase-labile gangliosides GGnSSLC and SGGnSSLC has not thrown any light on the mode of action of the toxin at the molecular level.

Serotonin

I have shown that mixed gangliosides in solution bound serotonin (5-hydroxytryptamine), tryptamine, lysergic acid diethyl amide, and ergometrine, but not adrenaline, noradrenaline, dopamine, histamine, or γ-aminobutyric acid; they also bound the convulsant drug strychnine and the related drugs brucine and thebaine[17]. I later found, however, that serotonin and strychnine were not bound by the ganglioside–cerebroside complexes that have such a great affinity for tetanus toxin[9]. The effect of ganglioside on serotonin has since been shown in other ways. Strips from rat stomach respond to serotonin, and Woolley showed that this response was diminished if the stomach strips were treated with sialidase, and restored if ganglioside was added to the enzyme-treated strips. Similar results were obtained with strips from ganglioside-deficient stomachs from rats reared on a galactose-rich diet[20]. Gielen identified the serotonin-reactive ganglioside as the sialidase-labile SSLC (which, lacking the terminal galactose and the N-acetylgalactosamine residues, is unlikely to interact with tetanus toxin)[21]. But the intact cat erythrocyte contains SSLC, yet is incapable of binding serotonin. This is reminiscent of the observation mentioned above that mixed gangliosides in free solution bound serotonin, but not when complexed with cerebroside.

Cholera toxin

The actions of cholera and tetanus toxins could be considered similar in the sense that they both affect the passage of substances across cell membranes (those of intestinal epithelial cells and nerve cells respectively) without producing any morphological changes in their susceptible tissues. Cholera toxin has been isolated by Finkelstein as a protein with a molecular weight of 84,000 (ref. 22). In the final stages of purification of the toxin, it is possible to isolate an inert protein, choleragenoid (molecular weight 58,000), that is nearly, but not quite, immunologically identical with the toxin, and possibly derived from it[22]. When cholera toxin is introduced into the lumen of the small intestine, it causes an accumulation of fluid, leading to diarrhoea[15,22]. When introduced intracutaneously it causes a local increase in capillary permeability[23]. Indeed, the toxin acts on a wide variety of tissues and cells[22], in which the effect usually appears to be mediated through the activation of adenylate cyclase. The subsequent increased levels of cyclic AMP in turn promote a wide variety of actions, including in the case of the intestinal epithelium, the secretion of chloride ions[22,24].

My collaborators and I have shown that homogenates of intestinal epithelium have a marked capacity to bind cholera toxin, whereas homogenates of liver, kidney, lung and heart seem to have no such capacity. Brain homogenate, however, proved even more effective than intestinal cells and it was then a simple step to show that mixed gangliosides were capable of binding the toxin, and were likely to account for the ability of these tissues to do so (brain contains about ten times as much ganglioside as intestinal cells)[25]. Ganglioside not only binds cholera toxin, but, unlike the case with tetanus toxin, it also blocks its action in the small intestine, in the skin, and elsewhere. I then showed that of all the brain gangliosides, only the sialidase-stable monosialosyl ganglioside, GGnSLC (G_{M1}) had this effect[26,27] (Table 1). The presence of a single sialic acid residue attached by a sialidase-labile bond, even to the terminal galactose residue, completely abolishes the capacity of the ganglioside to interact with cholera toxin. Also, treatment of intestinal epithelial cells with sialidase, an enzyme itself produced by the cholera bacillus, greatly increases their capacity to bind cholera toxin[26].

The blocking of the action of cholera toxin by added ganglioside indicates that when the toxin is bound to the extraneous ganglioside it is unable to attach itself to the receptor on the cell membrane, and therefore that the ganglioside GGnSLC may be very similar to, and even identical with, the membrane receptor of the toxin. GGnSLC also binds the biologically inert choleragenoid, and Pierce has shown that choleragenoid will block the action of cholera toxin in the small intestine, if placed in the intestine in advance of the toxin, one molecule of choleragenoid blocking one molecule of cholera toxin[28]. King and I (unpublished data) have shown that one molecule of choleragenoid will block the capacity of ganglioside (in the form of a ganglioside–cerebroside complex) to block the activity of nearly one molecule of cholera toxin. These data suggest the possibility that choleragenoid may be the part of the cholera toxin molecule that is responsible for the binding of the toxin to the receptor on the cell membrane. This has been supported and amplified by Simon van Heyningen[29] who has shown that when cholera toxin is bound to an insoluble GGnSLC–cerebroside complex, a component, A (molecular weight about 22,000), may be eluted with neutral 8 M urea. In spite of

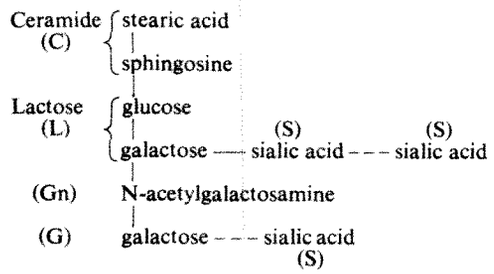
treatment with 8 M urea, this component has about 1% of the activity of the original toxin as tested in the rabbit skin. This activity is distinguished from that of the whole toxin by being unaffected by GGnSLC. The remaining portion of the toxin, in subunits B (molecular weight about 15,000), may be eluted from the ganglioside–cerebroside complex with neutral 6 M guanidine hydrochloride. Subunits B migrated with the same rate as subunits of choleragenoid on electrophoresis in polyacrylamide gel containing 6.25 M acid urea; after removal of the guanidine hydrochloride they were bound again by

Table 1 Interactions of the best-known gangliosides with tetanus and cholera toxins

Toxin	Molecules toxin bound per molecule ganglioside: Tetanus		Cholera
Test	Ultracentrifuge	Toxicity	Toxicity
Toxin concentration	5 mg ml^{-1}	1.5 ng ml^{-1}	10 ng ml^{-1}
Ganglioside*			
GnSLC (G_{M2})	< 0.005		< 0.000,5
GGnSLC (G_{M1})	0.04	0.000,05	0.35
SGGnSLC (G_{D1A})	0.04		< 0.000,5
GGnSSLC (G_{D1B})	0.28	0.000,6	< 0.000,5
SGGnSSLC (G_{T1})	0.28		< 0.000,5

The two sialic acid residues attached to the lactose component are necessary for optimal interaction with tetanus toxin, as is the terminal galactose residue; but the sialic acid residue attached to it seems to play no part. Only one ganglioside, which is sialidase-stable, interacts with cholera toxin, and the terminal galactose residue is again necessary for the interaction.

* It is convenient to use McCluer's abbreviated nomenclature for gangliosides[37] as it is self explanatory, and no less scientific than the widely used nomenclature of Svennerholm given in the brackets[38]; thus, the trisialosyl ganglioside, sialosylgalactosyl-N-acetylgalactosaminylsialosylsialosyllactosylceramide (G_{T1}), becomes SGGnSSLC:



(Broken lines signify sialidase-labile bonds)

GGnSLC, and showed immunological identity with choleragenoid. These data indicate that cholera toxin consists of two parts: a larger portion corresponding to choleragenoid (composed of subunits B) which combines with the GGnSLC receptor on the cell membrane, and thus facilitates the entry of the toxin into the cell; and a smaller portion, component A, which does not combine with GGnSLC and is, somehow or another, responsible for the activity of the toxin within the cell. This is reminiscent of aspects of the composition and activity of diphtheria toxin[30,31], and of the toxic plant proteins, abrin[32] and ricin[33].

The specific blocking action of cholera toxin by the ganglioside GGnSLC (G_{M1}) has subsequently been confirmed by Holmgren[34,35] and by Cuatrecasas[36]. Cuatrecasas has made the interesting observation that if exogenous GGnSLC is added to cells (lipocytes), and the excess ganglioside is washed from the cells, the capacity of these cells to bind and to react to subsequently added cholera toxin is increased. This observation is reminiscent of the effect, discussed in the previous section, of added exogenous ganglioside SSLC in restoring the reactivity to serotonin of ganglioside-deficient stomach strips. Cuatrecasas suggests that the added exogenous ganglioside is actually incorporated in the cell membrane, thus providing more receptor sites for the toxin. This is an interesting suggestion, but there is as yet no direct evidence of incorporation of ganglioside, only increased binding of, and reactivity to, the toxin, and there may be other explanations. For example, added ganglioside might remove some substance blocking preexisting receptor sites (Gill, private communication).

Gill and King (unpublished data) have confirmed that added exogenous GGnSLC increases the response (measured in terms of adenylate cyclase activity) of intact pigeon red blood cells to cholera toxin, provided always that the amount of ganglioside is not in excess (otherwise the toxin would be blocked). If the red cells are first lysed with a bacterial haemolysin, the resulting ghost cells are still reactive to cholera toxin, but the reactivity is not increased by the addition of ganglioside. This indicates that if the toxin can enter the cell by some other means it does not need the entry by binding to the receptor on the outer surface of the cell membrane.

The accumulated evidence that the ganglioside GGnSLC is the receptor for cholera toxin is persuasive, and rekindles interest in the specific reaction between tetanus toxin and GGnSSLC (and SGGnSSLC). Are these the receptors for tetanus toxin on the membranes of nerve cells? Or, as tetanus toxin has both a central and a peripheral action, and as botulinum toxin (which does not react specifically with ganglioside) has only a peripheral action (indistinguishable from that of tetanus toxin), is GGnSSLC responsible only for the central action of tetanus toxin?

1 Klenk, E., *Chem. phys. Lipids*, **5**, 193 (1970).
2 Svennerholm, L., *Methods in Carbohydrate Chemistry* (Academic Press, New York, 1972), **6**, 464.
3 Singer, S. J., and Nicolson, G. L., *Science*, **175**, 720 (1972).
4 Bretscher, M. S., *Science*, **181**, 622 (1973).
5 Whittaker, V. P., *Ann. N.Y. Acad. Sci.*, **137**, 982 (1966).
6 Morgan, I. G., Wolfe, L. S., Mandel, P., and Gombos, G., *Biochim. biophys. Acta*, **241**, 737 (1971).
7 Wiegandt, H., *Hoppe-Seyler's Z. physiol. Chem.*, **354**, 1049 (1973).
8 van Heyningen, W. E., and Woodman, R. J., *J. gen. Microbiol.*, **31**, 389 (1963).
9 van Heyningen, W. E., and Mellanby, J., *J. gen Microbiol.*, **52**, 447 (1968).
10 van Heyningen, W. E., and Mellanby, J., *Microbial Toxins* **2A**, 69 (Academic Press, New York 1971).
11 Mellanby, J., and Thompson, P. A., *J. Physiol. Lond.*, **224**, 407 (1971).
12 Wassermann, A., and Takaki, I., *Klin. Wschr.*, **35**, 4 (1898).
13 Landsteiner, K., and Botteri, A., *Zentbl. Bakt. Parasit. Kde*, **42**, 562 (1906).
14 van Heyningen, W. E., *J. gen. Microbiol.*, **20**, 301 (1959).
15 De, S. N., *Nature*, **183**, 1533 (1959).
16 van Heyningen, W. E., and Miller, P., *J. gen. Microbiol.*, **24**, 107 (1961).
17 van Heyningen, W. E., *J. gen. Microbiol.*, **31**, 375 (1963).
18 van Heyningen, W. E., and Mellanby, J., *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **276**, 297 (1973).
19 Mellanby, J., Mellanby, H., Pope, D., and van Heyningen, W. E., *J. gen. Microbiol.*, **54**, 161 (1968).
20 Woolley, D. W., and Gommi, B. W., *Proc. natn. Acad. Sci. U.S.A.*, **53**, 959 (1965).
21 Gielen, W., *Z. Naturf.*, **23B**, 117 (1968).
22 Finkelstein, R. A., *CRC Critical Reviews in Microbiology* **2**, 553, (Chemical Rubber Company, New York, 1973).
23 Craig, J. P., *Nature*, **207**, 614 (1965).
24 Carpenter, C. C. J., *J. infect. Dis.*, **126**, 551 (1972).
25 van Heyningen, W. E., Carpenter, C. C. J., Pierce, N. F. and Greenough III, W. B., *J. infect. Dis.*, **124**, 415 (1971).
26 van Heyningen, W. E., *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **276**, 289 (1973).
27 King, C. A., and van Heyningen, W. E., *J. infect. Dis.*, **127**, 639 (1973).
28 Pierce, N. F., *J. exp. Med.*, **137**, 1009 (1973).
29 van Heyningen, S., *Science*, **183**, 656 (1974).
30 Gill, D. M., and Dinius, L. L., *J. biol. Chem.*, **246**, 1485 (1971).
31 Collier, R. J., and Kandel, J., *J. biol. Chem.*, **246**, 1496 (1971).
32 Olsnes, S., and Pihl, A., *Eur. J. Biochem.*, **35**, 179 (1973).
33 Olsnes, S., and Pihl, A., *Biochemistry*, **12**, 3121 (1973).
34 Holmgren, J., Lönnroth, I., and Svennerholm, L., *Scand. J. infect. Dis.*, **5**, 77 (1973).
35 Holmgren, J., Lönnroth, I., and Svennerholm, L., *Infect. Immun.*, **8**, 208 (1973).
36 Cuatrecasas, P., *Biochemistry*, **12**, 3547, 3558, 3567, 3577 (1973).
37 McCluer, R. H., *Chem. Phys. Lipids*, **5**, 220 (1970).
38 Svennerholm, L., *J. Neurochem.*, **10**, 613 (1963).

Joint transcription of two $tRNA_1^{Tyr}$ genes from *Escherichia coli*

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Biochemical evidence suggests that the two $tRNA_1^{Tyr}$ genes of E. coli *are transcribed together as a single precursor. A nuclease activity which cleaves incorrectly but specifically the $tRNA^{Tyr}$ precursor has been revealed.*

THE $tRNA_1^{Tyr}$ species of *Escherichia coli* is coded for by two closely linked genes[1]. In *sup*3 mutants, one of these genes has a mutation in the anticodon which allows the tRNA to read UAG[2]. *sup*3-Transducing phages have been constructed. One of them, referred to here as φ80*psup*3 (Kyoto), carries both the suppressor gene and the adjacent wild-type gene[3]. The other will be referred to as φ80*psup*3 (Cambridge), it carries only the suppressor gene as a result of an unequal recombination deleting the wild-type gene[1]. Using φ80*psup*3 (Cambridge), Altman and Smith have shown that the single $tRNA_1^{Tyr}$ gene is transcribed as a precursor containing 41 extra nucleotides at the 5′ end and a few nucleotides at the 3′ end of the mature tRNA sequence[4]. This precursor is then cleaved at the 5′ end of the tRNA sequence by a specific endonuclease, RNase P[5]. The extra 3′-end nucleotides are subsequently removed by a different enzyme. The transcription and maturation scheme is not known in the normal situation where the two genes are present.

We recently reported the characterisation of mutations affecting the *sup*3 gene carried by φ80*psup*3 (Kyoto)[6]; some of these result in greatly diminished synthesis of tRNA because of the low efficiency of correct processing of the tRNA precursor. Similar mutants in φ80*psup*3 (Cambridge) have previously been shown to reduce the yield of suppressor tRNA[7]. Two such mutants are A1 and A2. Other mutants such as U80, U81 and G82 do not appreciably alter biosynthesis of the mutant tRNA. Here we asked the question whether mutants A1 and A2 in the suppressor gene of φ80*psup*3 (Kyoto) also lower the amounts of tRNA synthesised by the neighbouring wild-type $tRNA_1^{Tyr}$ gene. If the two genes are transcribed independently as separate precursor tRNAs, mutations in the suppressor gene would not be expected to alter the synthesis of the wild-type gene.

Our results suggest that the two tRNA genes are transcribed together as a common large precursor. We also show that in a RNaseP⁻ strain[8] the $tRNA^{Tyr}$ precursor is cleaved incorrectly by an specific nuclease.

Mutations in the *sup*3 gene

The phage φ80*psup*3 (Kyoto) carries both the suppressor and the wild-type $tRNA_1^{Tyr}$ genes. We have studied the expression of the two genes in cells infected with the original phage and with five mutants: A1, A2, U80, U81 and G82. These mutants have been previously described[6], each contains a single base change in the amino acid acceptor stem of the suppressor tRNA. The A1 mutation results in a G→A base substitution at the 5′ end of the suppressor tRNA. Three independently isolated φ80*psup*3 A1 mutants (A1·16, A1·56 and A1·79) were compared with φ80*psup*3 (Kyoto) for the expression of the suppressor and the wild-type $tRNA^{Tyr}$ genes. The total RNA synthesised after infection with the transducing phages was labelled with ^{32}P-orthophosphate and subjected to polyacrylamide gel electrophoresis[9] which separates the total $tRNA^{Tyr}$ from other tRNAs, most of which migrate as a broad band running ahead of the $tRNA^{Tyr}$ band ('4S' tRNA).

The total tyrosine tRNA corresponds to a mixture of suppressor $tRNA_1^{Tyr}$, wild-type $tRNA_1^{Tyr}$ and host $tRNA_2^{Tyr}$. The ratio (total $tRNA^{Tyr}$)/('4S' tRNA) was calculated by direct counting of the two bands. The quantitative estimation of the various types of $tRNA^{Tyr}$ was carried out as described in the legend of Table 1. The results are presented in Table 1, lines 1 to 4. Columns I to V show the ratio of total $tRNA^{Tyr}$

Table 1 $tRNA^{Tyr}$ production in A1 infected cells

Mutant	Total $tRNA^{Tyr}$ / '4S' tRNA (%)					Molar yield of the T_1 fragments			
	I	II	III	IV	V	Hexanucleotide UCACAG	pAG	3′ end* C81	3′ end* U81
Single mutants									
A1·16†	29	30	30	31	37	0·12	0·09‡	–	–
A1·56†	28	27	29	ND	ND	0·11	0·10‡	–	–
A1·79†	25	24	37	ND	ND	0·12	0·08‡	–	–
*sup*3	107	ND	118	118	112	ND	–	–	–
Double mutants									
A1·16 U81	ND	ND	ND	103	118	0·017	0·40	0·35	0·32‡
A1·16 P2	ND	ND	ND	63	62	0·031	0·37	–	–
A1·56 P2	ND	ND	ND	60	65	0·036	0·33	–	–
Uninfected§	4	3	ND	ND	ND	0·55	–	–	–

$tRNA^{Tyr}$ production in A1 infected cells. *E. coli* MB94 grown in low phosphate medium[19] at 30° C was infected at a multiplicity of 30 and labelled for 60 min with ^{32}P-orthophosphate[19,20]. The total RNA was subjected to polyacrylamide gel electrophoresis[9]. The ratio (total $tRNA^{Tyr}$)/('4S' tRNA) was determined by direct counting of the two bands. The total $tRNA^{Tyr}$ was extracted from the gels, digested with T_1 RNase and subjected to two-dimensional electrophoresis[11,12]. Since all the mutants give characteristic T_1 fragments[6], it is possible to determine the proportion of mutant and wild-type tRNA among the total $tRNA^{Tyr}$. Similarly, the proportion of host $tRNA_2^{Tyr}$ can be determined since it contains the hexanucleotide UCACAG which is not present in $tRNA_1^{Tyr}$ (ref. 10). Columns I to V show the ratio of the radioactivity present in the total $tRNA^{Tyr}$ to that in the 4S tRNA in five experiments. The radioactivity of the various T_1 fragments was measured by liquid scintillation counting.

* The mutant 3′ end has the C→ U81 base substitution.
† A1.16, A1.56 and A1.79 are three independent isolated A1 mutants.
‡ Average result of two independent determinations.
§ The same results are obtained in uninfected cells or Φ80 infected cells.
– Absence of the fragment.
ND Not determined.

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Table 2 Expression of the $tRNA^{Tyr}$ genes

Mutant	*Phage-coded $tRNA^{Tyr}$ (%) 4S tRNA	Coefficient of expression of the $tRNA^{Tyr}$ genes Total†	Wild-type gene‡	Suppressor gene‡
Single mutants				
A1·16, A1·56, A1·79	25	0·23	0·20	0·02
*sup*3	109	1·0	0·5	0·5
Double mutants				
A1·16 U81	107	0·98	0·55	0·45
A1·16 P2, A1·56 P2	60	0·55	0·325	0·225

* The average ratio tyr tRNA/4S tRNA was calculated from the data in Table 1 and the background of host tyrosine tRNA has been subtracted.
† The full expression of both genes has been given value a of 1 (*sup*3).
‡ The values correspond to the ratio wild-type: suppressor found as result of the T_1 RNase digestion. The efficiency of transfer of the pAG fragment to the DEAE-cellulose paper is 90%; therefore a factor of 1.1 was applied to convert the molar yields of pAG into the ratios A1 tRNA: total $tRNA^{Tyr}$. The efficiency of transfer of the normal and modified 3′ ends were assumed to be identical.

to the bulk of other tRNAs ('4S' tRNA) in five experiments. The last column shows the molar yield of the T_1 RNase fragments pAG (characteristic of the A1 tRNA) and UCACAG (characteristic of $tRNA_2^{Tyr}$ (ref. 10). The results of all three A1 mutants have been pooled in Table 2, column 1. In the second column the average ratio (total $tRNA^{Tyr}$)/('4S' tRNA) is expressed as a function of the average ratio for φ80*psup*3 (Kyoto). This fraction represents the 'coefficient of expression' of the transduced $tRNA^{Tyr}$ genes, equal to 1 in the case of *sup*3 (Kyoto) were both genes are normally expressed. In the last two columns of Table 2 the 'coefficient of expression' is distributed between the wild-type and the suppressor genes according to the relative proportions of the two tRNAs revealed by T_1 RNase digestion. The results show that the A1 mutation reduces the expression of the adjacent wild-type gene to 40% of its normal value. This effect is *cis* specific since the amount of $tRNA_2^{Tyr}$ is not reduced after infection by any of the A1 mutants. The A2 mutation (G→A change at the second position from the 5′ end) reduces the amount of suppressor tRNA to 25-30% and the amount of wild-type tRNA to 70-75% of their normal value (data not shown). The U80, U81 and G82 mutants synthesise both tRNAs at normal levels (data not shown).

Reversion of the effect of A1 and A2 by secondary mutations

Double mutants showing an increased suppressor tRNA synthesis have been isolated from φ80*psup*3 A1 (Kyoto)[6]. One of them, A1U81, has a second base change at position 81, opposite to position 1 in the clover-leaf structure, while the second (A1P2) has no other change in the nucleotide sequence of the mature tRNA. One A1U81 and two independent A1P2 mutants were studied. The $tRNA^{Tyr}$ production in infected cells are reported in Table 1, lines 5-7. The data has been treated as described above and summarised in Table 2. The U81 mutation raises the amount of the suppressor tRNA to almost normal levels. In addition U81 fully reverses the effect of the A1 mutation on the production of the wild-type tRNA. This indicates that the effects of the A1 mutation on the amounts of the suppressor and of the wild-type tRNAs are due to the same structural defect which is corrected by the second C→U81 base change. The P2 mutation also reverses the effect of A1 on both suppressor and wild-type tRNA production, however this reversion is only partial. P2 does not seem to be a promoter mutation since it induces different increases in the coefficient of expression of the two tRNAs. Therefore P2 is most likely a precursor mutation which somehow compensates for the structural defect caused by the A1 mutation. A double mutant, A2U80, obtained from φ80*psup*3 A2(Kyoto)[6] has also been studied. The secondary C→U mutation at position 80 completely reverses the effect of A2 on the synthesis of the suppressor and the wild-type tRNAs (data not shown).

$tRNA^{Tyr}$ precursors in RNase P⁻ cells

To characterise the transcription products of the phage-transduced $tRNA^{Tyr}$ genes we used the temperature-sensitive RNase P⁻ mutant described by Schedl and Primakoff.[8] At 42° C the tRNA-processing enzyme RNase P is inactive, thus allowing the isolation of tRNA precursors in which the 5′ termini has not been removed. We analysed the RNAs

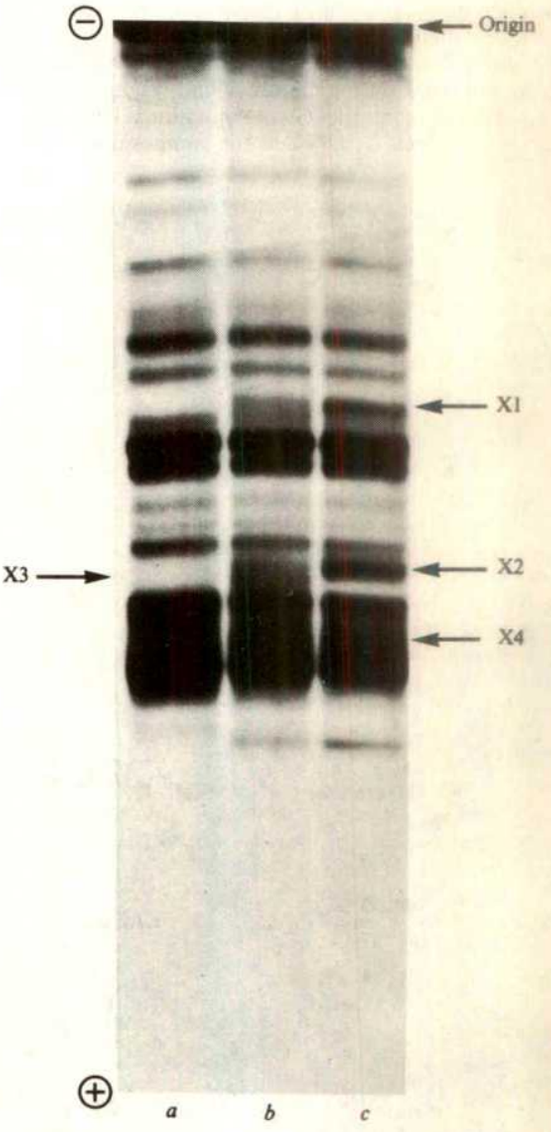


Fig. 1 Polyacrylamide gel electrophoresis of ^{32}P–RNAs from A49 infected cells. A49 cells (RNase P⁻, kindly provided by P. Schedl) grown at 30° C in TPGA medium[21] (5×10^{-4} M phosphate) to 4×10^{8} cells ml^{-1} were collected by centrifugation and re-suspended in one-fifth volume of TPGA medium (5×10^{-5} M phosphate) prewarmed at 42° C. After 10 min of aeration at 42° C, 0.02 vol of M $MgSO_4$ was added and 0.5 ml of cells were infected at a multiplicity of 30. After 10 min adsorption without aeration the cells were diluted 10-fold into TPGA medium (5×10^{-5} M phosphate) prewarmed at 42° C ($t=0$). 5 mCi of ^{32}P–orthophosphate were added at $t=27$ min and the incorporation stopped 10 min later by adding and equal volume of buffer saturated phenol prewarmed at 42° C. This procedure is similar to that described by Schedl and Primakoff[8]. Radioactive samples were applied to 12% polyacrylamide gels as described by Peacock and Dingman.[9] a, φ80; b, φ80 *psup* 3 A1U81 (Kyoto) and c, φ80 *psup* 3 (Cambridge).

synthesised in RNase P deficient cells infected with φ80*psup*3 (Kyoto), φ80*psup*3 (Cambridge) and various mutants. Figure 1 shows an autoradiograph of a polyacrylamide gel electrophoresis of ^{32}P-RNAs from RNase P$^-$ cells infected with (*a*) φ80, (*b*) φ80*p sup*3 A1U81 (Kyoto) and (*c*) φ80*psup*3 (Cambridge) and labelled with ^{32}P-orthophosphate at 42° C. Bands X1, X2, X3 and X4 are absent from cells infected with φ80 and all contain tRNA^{Tyr} sequences. The pattern of RNAs synthesised after infection with φ80*psup*3 (Kyoto) is identical to that from φ80*psup*3 A1U81 (Kyoto). All the bands were extracted from the gels, digested with RNase T_1 and fingerprinted[11,12]. The results for A1U81 are shown in Fig. 2. X1 corresponds to the precursor tRNA described by Altman and Smith[4]. It contains the sequence of precursor A1U81 *sup*3 tRNA, however, and not that of the wild-type tRNA$_1^{Tyr}$ precursor (Fig. 1*a*). The 3′-end fragment of this precursor was shown by pancreatic RNAse digestion to contain the C→U81 base change. It also contains a few additional 3′ nucleotides (including the dinucleotide AU) which are not found in the mature 3′-end fragment. The UAG fragment results from the G→A mutation. The absence of C81-modified 3′ end, ACUG and the composition of the anticodon indicate that no wild-type tRNA sequence is present in this RNA band.

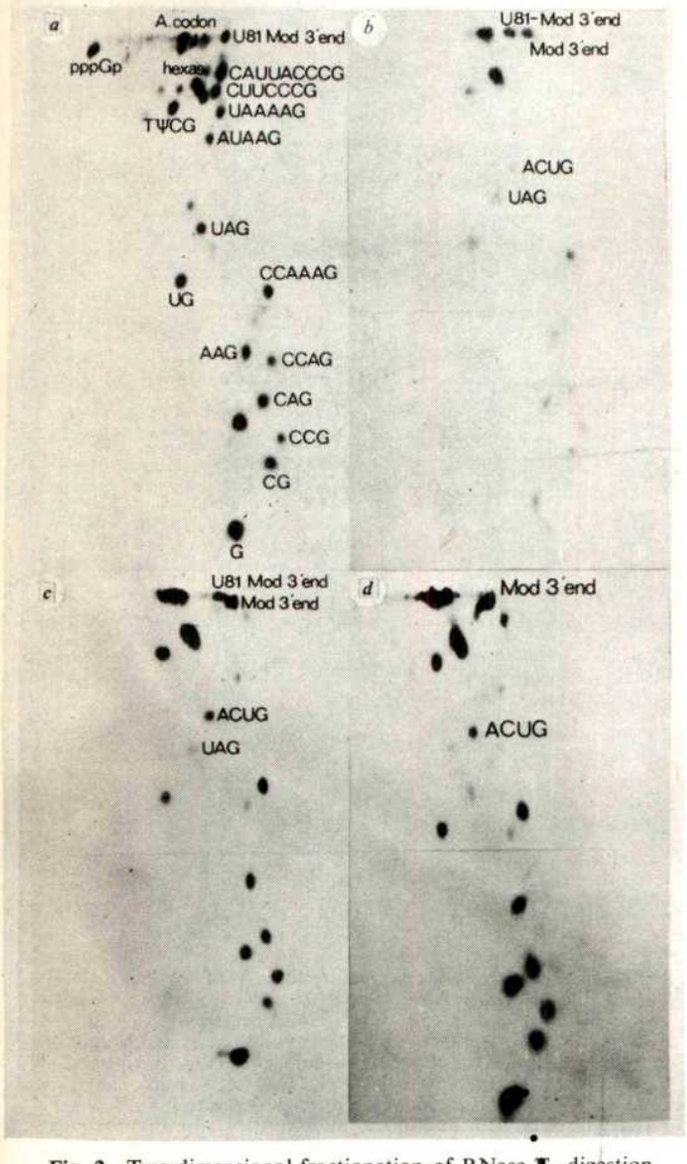


Fig. 2 Two-dimensional fractionation of RNase T_1 digestion products. *a*, A1U81 band XI; *b*, A1U81 band X2; *c*, A1U81 band X3 and *d*, A155P2 band X3. Electrophoresis on cellulose acetate in pyridine acetate, 7 M urea, *p*H 3.5 right to left; and on DEAE paper in 7% formic acid (v/v) from top to bottom. U81–Mod 3′ end=3′-end fragment containing the U81 mutation and the extra 3′ end nucleotides. Mod 3′ end=3′ end fragment containing the 3′-end extra nucleotides.

Band X2 (Fig. 2*b*) corresponds to a 1:1 mixture of A1U81 and wild-type sequences as judged by the quantitation of the two 3′-end fragments (containing U81 and C81 respectively), ACUG and the anticodon fragment. None of the fragments of the precursor 5′ end can be detected, yet these tRNAs differ from the mature tRNAs in the absence of the normal T_1 5′-end fragments (pAG and pGp respectively). In the case of the suppressor tRNA pAG is replaced by a new 5′-end fragment, UAG. This fragment results from the incorrect cleavage of the precursor between residues 40 and 41 (ref. 4) as shown in Fig. 3. Also, pancreatic RNase digestion of band X2 tRNA gives AGU and no pAGU can be detected. The situation however is not clear for the wild-type tRNA^{Tyr} since we do not find any new fragment that replaces pGp. The 3′ end of both tRNAs contain some or all of the 3′-end extra nucleotides present at the 3′ end of band X1 RNA.

Band X3 (Fig. 2*c*) corresponds to a 1:2 mixture of A1U81 and wild-type sequences; no other difference could be detected between the fingerprints of bands X2 and X3. These bands do not always separate; the occasional separation could conceivably be due to a partial loss of some of the 3′-end extra nucleotides.

Band X4 RNA (fingerprint not shown) differs from band X2 and X3 by having mature 3′-end fragments. Since this band is heavily contaiminated and the fingerprints contain pGp it is difficult to assess whether the wild-type tRNA has an abnormal 5′ end. In the case of the suppressor gene we can detect small amounts of pAG, however this fragment is not in molar yields with the U81 3′-end fragment. It is likely then that at least the suppressor tRNA contains an abnormal 5′ end.

Although we analysed all the other bands in the gel in Fig. 1*b*, we have been unable to find a precursor for the wild-type tRNA or a doublet precursor. Further evidence that a singlet precursor for the wild-type tRNA gene cannot be detected has been obtained by analysing the RNAs synthesised in RNase P$^-$ cells infected with A1 mutants. Infection with φ80*psup*3 A1 (Cambridge), which carries only the A1 tRNA gene, yields none of the 1 to 4 bands as a result of the thermosensitivity of the A1 mutation. When the infecting phage is φ80*psup*3 A1P2 (Kyoto), which carries both the A1 and the wild-type genes, only bands X3 and X4 can be detected (results not shown). T_1 RNase digests of band X3 (Fig. 2*d*) and X4 (not shown) confirm that they contain the wild type but not the A1 sequence. Similar analysis of the φ80*psup*3$^-$ phage (Cambridge) which carries only the wild-type gene yields bands X1, a diffuse band in the position of X2 and X3 and band X4 (results not shown).

Common tRNA precursor

Some mutations, which break base pairs in the amino acid acceptor stem of the suppressor tRNA$_1^{Tyr}$ decrease the yield of tRNA coded by the adjacent wild-type gene. This result suggests that the two tRNAs are synthesised as a single transcription product ('doublet' precursor), so that a structural deformation close to the RNase P cleavage point affects the processing, and therefore the yield of both tRNAs. This explanation is further supported by the observation that no 'singlet' precursor can be detected for the wild-type tRNA^{Tyr}. We feel that the origin of the tyrosine tRNAs sequence found in RNase P$^-$ cells is best explained as pictured in Fig. 3. The two genes are transcribed as a long precursor containing both sequences, the suppressor sequence being first from the 5′ end. The length of the spacer between the two tRNAs cannot be derived from our data, it could be 35-70 nucleotides[13]. As a result of endonuclease cleavage(s), this unstable precursor yields a singlet precursor containing the suppressor sequence (band X1), and a wild-type tRNA with inmature 5′ and 3′ ends (bands X2 and X3). The 'singlet' precursor is further cleaved incorrectly but specifically between residues 40 and 41 of the

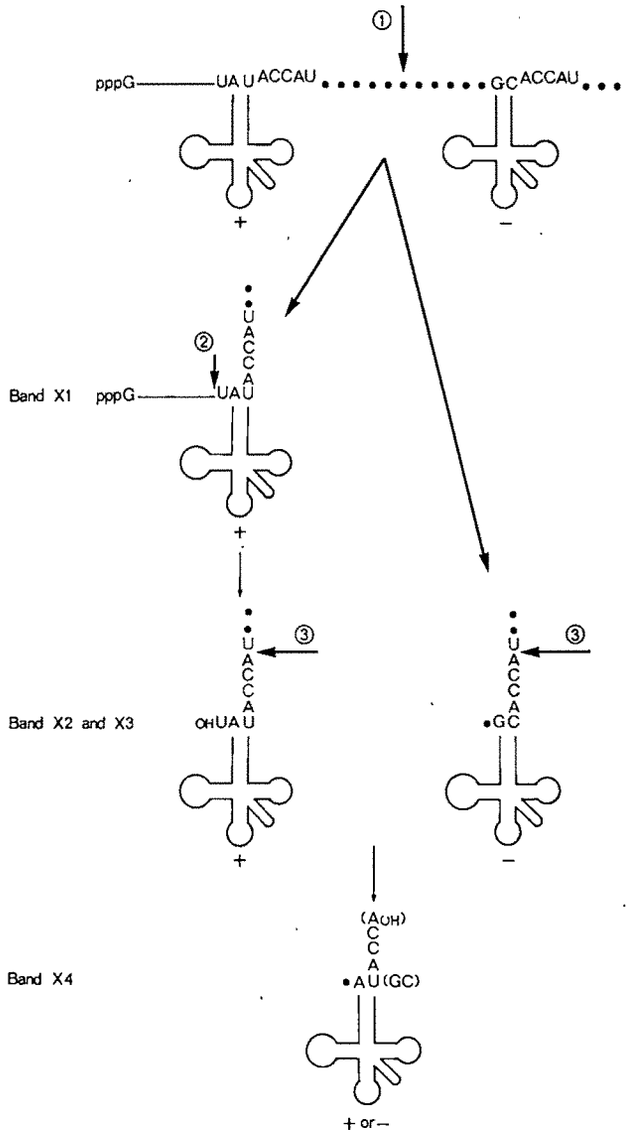


Fig. 3 Possible origin of the precursor cleavage products. The first tRNA sequence of the doublet precursor corresponds to the A1U81 suppressor, the second is the wild-type sequence. The black dots indicate that neither the length nor the sequence of these fragments is known. The arrows indicate the proposed nuclease cleavage. The last residue of the 'mature' tRNA has been written as (A_{OH}) since we have not established its identity. + and − correspond to the suppressor and wild-type anticodon respectively.

precursor fragment[4] (arrow 2 in Fig. 3) to give a suppressor tRNA beginning with HOUAG – – – – at the 5′ end and with immature 3′ end. At present we do not know whether the enzyme that cleaves incorrectly at the 5′ end of the suppressor tRNA is also responsible for the cleavage of the initial doublet precursor at the 5′ end of the wild-type tRNA. Finally, the extra 3′ end nucleotides are cleaved (arrow 3 in Fig. 3). At least in the case of the suppressor tRNA the 5′ end seems to remain abnormal.

Another example of common precursor for closely linked tRNA genes has been reported in the case of the T4 coded tRNAs[14,15]. Furthermore the occurrence of other closely linked tRNA genes in the bacterial genome also raises the possibility of large bacterial tRNA precursors containing two or more tRNA sequences[16–18]. Indeed, Schedl and Primakoff[8] have recently reported that they have found tRNA precursors containing more than one tRNA sequence in RNase P⁻ cells.

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[1] Russell, R. L., Abelson, J. N., Landy, A., Gefter, M. L., Brenner, S., and Smith, J. D., *J. molec. Biol.*, **47**, 1 (1970).
[2] Goodman, H. M., Abelson, J., Landy, A., Brenner, S., and Smith, J. D., *Nature*, **217**, 1019 (1968).
[3] Andoh, T., and Ozeki, H., *Proc. natn. Acad. Sci. U.S.A.*, **59**, 792 (1968).
[4] Altman, S., and Smith, J. D., *Nature new Biol.*, **233**, 35 (1971).
[5] Robertson, H. D., Altman, S., and Smith, J. D., *J. biol. Chem.*, **247**, 5243 (1972).
[6] Ghysen, A., and Celis, J. E., *J. molec. Biol.* **83**, 333 (1974).
[7] Smith, J. D., Barnett, L., Brenner, S., and Russell, R. L., *J. molec. Biol.*, **54**, 1 (1970).
[8] Schedl., P., and Primakoff, P., *Proc. natn. Acad. Sci., U.S.A.*, **70**, 2091 (1973).
[9] Peacock, A. C., and Dingman, C. W., *Biochemistry*, **6**, 1818 (1967).
[10] Goodman, H. M., Abelson, J., Landy, A., Zadrasil, S., and Smith, J. D., *Eur. J. Biochem.*, **13**, 461 (1970).
[11] Sanger, F., Brownlee, G. G., and Barrell, B. G., *J. molec. Biol.*, **13**, 373 (1965).
[12] Brownlee, G. G., and Sanger, F., *J. molec. Biol.*, **23**, 337 (1967).
[13] Wu, M., and Davidson, N., *J. molec. Biol.*, **78**, 1 (1973).
[14] Guthrie, C., Seidman, J. G., Altman, S., Barrell, B. G., Smith, J. D., and McClain, W. H., *Nature new Biol.*, **246**, 6 (1973).
[15] Nierlich, D. P., Lamfrom, H., Sarabhai, A., and Abelson, J. N., *Proc. natn. Acad. Sci., U.S.A.*, **70**, 179 (1973).
[16] Ohlsson, B. M., Strigini, P. F., and Beckwith, J. R., *J. molec. Biol.*, **36**, 209 (1958).
[17] Orias, E., Gartner, T. K., Lannan, S. E., and Betlach, M., *J. Bact.*, **109**, 1125 (1958).
[18] Squires, C., Konrad, B., Kirschbaum, J., and Carbon, J., *Proc. natn. Acad. Sci., U.S.A.*, **70**, 438 (1973).
[19] Abelson, J. N., Gefter, M. L., Barnett, L., Landy, A., Russell, R., and Smith, J. D., *J. molec. Biol.*, **47**, 15 (1970).
[20] Anderson, K. W., and Smith, J. D., *J. molec. Biol.*, **69**, 349 (1972).
[21] Altman, S., *Nature new Biol.*, **229**, 19 (1971).

Simian virus 40 in a human cancer

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Simian virus 40 (SV40) and SV40 T (tumour) antigen have been detected in the malignant melanoma metastases of a patient in which specific viral and T antibodies were demonstrated.

THE patient in our investigation, a plumber born in 1894, had no personal or familial history of chronic illness or congenital anomaly. A pigmented skin growth in his upper back, which he had noticed 2–3 yr before, was removed in 1964 and found to be a malignant melanoma. The diagnosis

was later confirmed in biopsies of axillary and cervical lymph node metastases, in the cells of a pleural exudate that developed shortly before death, and at autopsy, performed in December 1967. No history of immunisation with vaccines likely to contain SV40 was later found. At autopsy there were widespread pigmented or unpigmented metastases in the skin and muscles of the shoulder and chest wall, in the lungs, pleuras, diaphragm, liver, lymph nodes, dura, brain and heart, also hydrothorax and bronchopneumonia.

The medium used for tissue culture consisted of equal parts of McCoy's 5a medium and medium 199, supplemented with foetal bovine serum and antibiotics. Metastatic and normal tissues taken at autopsy were cultured after mincing or trypsinising. The tumour cultures initially contained tumour cells, fibroblasts and some lymphocytes. All cultures were kept for 1–3 months but were gradually lost. Only cultures of normal spleen and normal kidney were frozen for future subculture; none of the others showed adequate growth. Continuous cultures of typical lymphoblastoid cells forming floating colonies eventually emerged from cultures of the spleen and of muscle and lymph node metastases, however. These three exclusively lymphoblastoid cell lines have been cultured for several years.

Virus studies

Several specimens of lung, liver and muscle metastases and of normal spleen and kidney had been frozen since autopsy at −94°C without medium or cryoprotective agents. Beginning 14 months after autopsy, the specimens were thawed, minced, forced through fine sieves while rinsing, centrifuged, and resuspended at 20% concentration in medium containing 10% heated foetal bovine serum. Aliquots were frozen for future use. The suspensions consisted of broken cells and debris. They were centrifuged at 500*g* for 10 min and each of several monolayers grown in 30-ml flasks was inoculated with 0.1 ml of the pellet. The cultures were maintained with medium containing heated foetal bovine serum and examined regularly with an inverted phase contrast microscope.

In the first isolation experiment with various human and animal cell cultures, signs of cytopathic effects characteristic of SV40 were first observed after 30 d in one primary grivet (African green monkey) kidney culture inoculated with a lung metastasis specimen. Ten days later virus was collected (isolate A). All cultures had been observed for more than 1 month before inoculation and were negative for virus and *Mycoplasma*. All negative and control cultures were held for 6 weeks after virus collection; no virus growth occurred.

To avoid the risk of viral contamination in new lots, primary grivet kidney cultures were permanently discontinued after this experiment; primary rhesus kidney cultures were never used. Subsequent isolations were carried out with the grivet kidney-derived BSC-1 cell line; cell stock was obtained from the American Type Culture Collection. From another lung metastasis virus was recovered in three further successive attempts (isolates B, C and D). In two other separate experiments virus was isolated from liver and muscle metastases (isolates E and F). In no case was any incipient cytopathic effect characteristic of SV40 observed sooner than the fourth week of incubation or later than the fifth. In each experiment all negative cultures and controls were kept, without showing cytopathic effects, for at least 8 weeks after virus collection from the positive cultures. Over the years during which these isolations were made no viral or mycoplasmal contamination was detected in several thousand stock and control cultures monitored periodically by electron microscopy or cultural methods.

Several other attempts with the previously positive specimens or with different tumour specimens were unsuccessful. Virus was recovered from about 3% of approximately 500 cultures inoculated with original tumour tissue pellets or with blind passages. In the successful isolations, excluding the other attempts, a total of 11 ml of packed tissues (0.1 ml per culture) yielded 15 positive cultures. It can be estimated that approximately 1 infectious unit was contained in 1 g of the pellets or in no less than 1 g of intact tumour at the time of death, and that virus was detectable only in some parts of the tumours.

In further experiments inoculation of tumour pellets into less sensitive grivet kidney cell lines (CV–1, Vero) yielded no virus and BSC–1 cultures remained negative after inoculation with cell-free tumour extracts. No virus was recovered from BSC–1 cells inoculated with pellets of the normal spleen and normal kidney, nor from human fibroblast and primary embryonic kidney cultures inoculated with pellets of the tumours or of the normal organs. No virus grew, and no viral or T antigen was detected by immunofluorescence, in BSC–1 cultures inoculated with live (cocultivation) or homogenised cells of the three lymphoblastoid lines. Each of these experiments lasted 2–3 months and was repeated at least once.

Electron microscopy of a brain metastasis that had been kept in formalin revealed no virions in several samples of the tumour and of the adjacent white matter. There was good preservation of fine detail.

Mice and Syrian hamsters were inoculated at birth with pellets of the normal and metastatic tissues and of pleural tumour cells obtained before death. No meaningful disease occurred during the natural life span of the survivors.

In cross-neutralisation tests no significant differences were found between the six isolates and two known SV40 strains. Strain J436 and baboon antiserum to J436 were obtained from the National Institutes of Health, strain A2895 from the American Type Culture Collection; rabbit antisera to A2895 and to the six isolates were produced in our laboratory. The two known antisera neutralised the six isolates and two known strains to the same extent; antiserum to each isolate had about the same titre against the particular isolate as against the two known strains. Since each isolate was also proved to be oncogenic for newborn hamsters and no difference from SV40 was found in further work, we have designated isolates A to F collectively as Strain F of SV40, or SF.

Strain F is identical to other strains in its intrinsic properties, growth in cell cultures, cytopathic effects, cell-transforming efficiency, oncogenicity, and electron microscopic features[1–4]. Subcutaneous or intracerebral inoculation of newborn hamsters induced local sarcomas or ependymomas as described for SV40 (refs 5 and 6). Cultures of non-tumour human cells supported its growth and underwent malignant transformation. Inoculation of SF-transformed mouse or hamster cell cultures in the respective hosts resulted in malignant tumours. As within SV40 strains, tumours can be prevented in hamsters inoculated at birth with strain A2895 by injecting SF during the incubation period; conversely, SF tumours can be prevented by a second injection with A2895.

Identity of the cellular T antigen induced by SV40 (ref. 7) with the SF-induced T antigen was demonstrated by indirect immunofluorescence[8]. Coverslip cell cultures fixed in acetone were used immediately or stored at −30° C. Known SV40 T-positive cultures were TT–101, a virus-free SV40 hamster tumour cell line (Flow Laboratories, Rockville, Maryland) and BSC–1 cells inoculated with A2895; SF T-positive cultures were S–419, a virus-free line derived from an SF hamster tumour, and BSC–1 cells inoculated with SF. Infected BSC–1 cells were fixed 3 d after inoculation. Control cultures were normal continuous (BHK–21) or primary hamster kidney cells, and normal BSC–1 cells. Known SV40

T antiserum was obtained from Flow Laboratories and from hamsters bearing transplanted A2895 tumours, SF T antiserum from hamsters with transplanted SF tumours, and control serum from normal hamsters; all T antisera were free of viral antibodies. Fluorescein-labelled γ-globulin against hamster γ-globulin was obtained commercially. The SV40 T antisera stained SV40 T-positive and SF T-positive cells to the same extent, and the same was true for the SF T antiserum; all controls were negative. Each antiserum was absorbed equally well with T antigen extracts from hamster tumours induced by known SV40 or SF.

Antibody and antigen studies

Patient's serum was unavailable, but several specimens of pleural exudate had been stored at −30° C. Early attempts to demonstrate antibodies in exudate obtained shortly before death were unsuccessful. The patient's blood belonged to group O, however, so a search for samples with at least agglutinating antibodies for AB erythrocytes was made. Only the earliest sample, stored 6 d before death, had haemagglutinating activity. It contained IgG, IgA, IgM, and detectable SV40 viral and T antibodies.

Heat-inactivated exudate, diluted 1:2, neutralised 70–100 median tissue culture infectious doses of SF, A2895 or J436 in BSC–1 cells observed over 3 weeks but it did not do so when diluted 1:2 in a concentrated rabbit γ-globulin of a high titre against human γ-globulin, thus indicating activity caused by antibody. The antihuman γ-globulin alone had no effect on the viruses or the cells.

T antibodies were demonstrated as described above by indirect immunofluorescence with known SV40 T-positive cultures (virus-free TT101 cells and A2895-inoculated BSC–1 cells), SF T-positive cultures (virus-free S–419 cells and SF-inoculated BSC–1 cells) and controls. To prevent virus growth, inoculated BSC–1 cultures were maintained during the 3 d of incubation with daily changes of medium containing 20 μg ml^{-1} 5-fluorodeoxyuridine and 20 μg ml^{-1} 1-β-D-arabinofuranosylcytosine. Besides normal BSC–1 and hamster cells, controls included previously T-positive coverslips in which the antigen had been destroyed by storing for several weeks at 4° C and positive controls stained with hamster T antiserum and labelled anti-hamster γ-globulin. The exudate was treated with heparin to inhibit any solublisation of T antigen by RNase it may have contained, and with thimerosal and phenol to inhibit microbial growth. Normal human serum and the labelled globulin applied alone served as controls. The exudate stained typically and exclusively the nuclei of all T-positive cells. Six applications, each followed by 1 d of incubation at 4° C, were needed for best results with the tumour cells. Quicker results were obtained with inoculated BSC–1 cells, which produce more antigen, and with exudate concentrated by ammonium sulphate precipitation. Concentrated exudate absorbed with T antigen extracted from BSC–1 cells infected with SF or A2895 lost its staining activity, but it did not when absorbed with the same extracts previously exposed—after sterilising by filtration—to 'warm' temperatures, that is, to 4° C for several weeks or 22° C for a few days, or when absorbed with normal BSC–1 extracts. Concentrated exudate blocked the binding of fluorescein-labelled hamster T antiserum to T-positive BSC–1 cells, as judged by a conspicuous reduction of the staining intensity. Normal human serum and high titre anti-nuclear sera from patients with systemic lupus erythematosus had no effect.

T antigen was demonstrated in the patient's tumour tissue suspensions. These were sonicated, treated with RNase and centrifuged. The antigen was precipitated from the supernatants with saturated ammonium sulphate (32%) or by acidifying to *p*H 5 with 0.2 M acetic acid[9]. The precipitated antigen was redissolved and dialysed in phosphate-buffered saline, *p*H 7.2. With the small amounts involved little final concentration—but some purification—was achieved. For positive control, an SF hamster tumour that had been kept frozen was extracted in the same way after homogenising. These extracts served also as negative controls after exposure to warm temperatures to destroy the antigen, as described above (inactivated T antigens). Similar extracts from a human placenta and a human myoma were additional control antigens.

A solid-phase microradioimmunoassay[10] was adapted to demonstrate T antigen. Serial two-fold dilutions of antigens in phosphate-buffered saline were placed in wells of microtitre plates, 25 μl per well, and dried at 4° C under vacuum in a desiccator containing calcium sulphate. After methanol fixation the plates were rinsed with buffered saline, and 20 μl per well of each appropriate serum or γ-globulin were successively applied for 1 h at 22° C. The plates were rinsed after each step; the final γ-globulin against hamster or human γ-globulin was labelled with ^{125}I (^{125}I-γ-globulin). The wells were then cut out, placed in tubes and counted for radioactivity in a γ spectrometer. The average c.p.m. bound to triplicate test wells divided by the average c.p.m. bound to control wells gave the binding ratio, ratios of 1.5 or greater

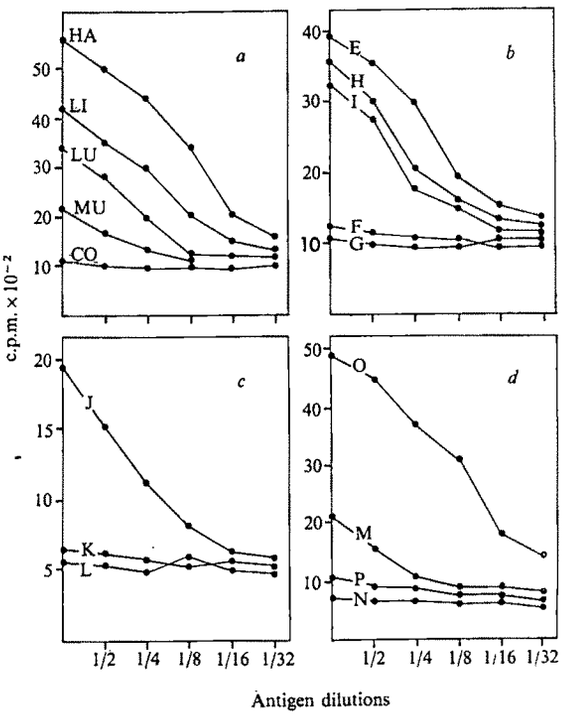


Fig. 1 Radioimmunoassays of the patient's T antigen and antibodies. *a*, Liver (LI), lung (LU) and muscle (MU) tumour antigen extracts; a negative control (CO); an SV40 hamster tumour extract (HA) as a positive control. All reacted with hamster T antiserum and anti-hamster ^{125}I-γ-globulin. The following tests were performed with the patient's liver tumor antigen with a titre of 16. *b*, Absorptions: hamster T antiserum, unabsorbed (E) or absorbed with the patient's T antigen (F), hamster T antigen (G), inactivated patient's T antigen (H) or placenta antigen (I), followed by anti-hamster ^{125}I-γ-globulin. *c*, Antibodies: patient's exudate (J), normal human serum (K) or systemic lupus erythematosus serum (L) followed by anti-human ^{125}I-γ-globulin. *d*, Blocking tests: normal hamster serum (M) or hamster T antiserum (N), both followed by exudate and anti-human ^{125}I-γ-globulin; normal human serum (O) or exudate (P), both followed by hamster T antiserum and antihamster ^{125}I-γ-globulin. (For details see text.)

being taken as significant. The titre was the reciprocal of the highest dilution giving significant binding.

The T antigen titres obtained with hamster T antiserum and anti-hamster ^{125}I-γ-globulin in the extracts of the patient's metastases and of the hamster tumour are shown in Fig. 1*a*. The differences in titre are considered irrelevant. The suspensions of metastases from which the extracts were made had been thawed and rewarmed several times for various periods of time; the hamster tumour had never been used; the muscle specimen contained many normal muscle fibres. The placenta and myoma extracts and the inactivated T antigens gave approximately similar results, no different from the background given by the labelled globulin alone; a representative curve is shown. The following assays were performed with the liver tumour extract with a titre of 16 (patient's T antigen). T antibodies were removed from the hamster T antiserum by absorption with patient's or hamster T antigen but not with inactivated patient's T antigen or placenta extract (Fig. 1*b*). The specificity of the patient's T antigen and antibodies was further demonstrated. The patient's exudate bound significantly to the patient's T antigen but normal human serum or high titre anti-nuclear serum from patients with systemic lupus erythematosus did not (Fig. 1*c*). In blocking tests, hamster T antiserum and exudate inhibited the binding of each other to the patient's T antigen but lupus and normal human serum or normal hamster serum had no effect (Fig. 1*d*).

No viral or T antigen was detected by this method or by immunofluorescence in the three lymphoblastoid cell lines derived from metastatic tissues and from the normal spleen, nor by immunofluorescence in subcultures of the normal renal epithelium or of the splenic mixture of fibroblasts, macrophages and lymphoid cells.

SV40 in humans

The findings in this case correspond to those in hamsters with SV40 tumours. Virus and T antigen were detected in lung, liver and muscle metastases but not in the normal spleen and kidney or in the three lymphoblastoid cell lines; viral and T antibodies could still be detected in the dying patient.

Not much is known about SV40 multiplication in humans. It has rarely been isolated: from faeces after ingestion[11], from throat swabs after respiratory route inoculation[12], and from the brains of two patients with progressive multifocal leukoencephalopathy[13]. Only human cells are truly semipermissive *in vitro*, both undergoing transformation and permitting SV40 growth. Normally, monkey cells are only permissive, and hamster or other animal cells are nonpermissive, that is, can only be transformed. Although humans are apparently inefficient hosts[14], initial *in vivo* growth, sufficient to induce viral (but not anti-T) antibody, is inferred from the titres in persons infected by the respiratory[12] or subcutaneous[15] route, in monkey handlers and in persons (including some cancer patients) with no known SV40 contact[14]; this last group suggests a natural infection from an unknown reservoir and by an unknown route[14].

The minute amounts of SV40 detected exclusively in the patient's tumours could not alone account for a viral antibody response, and do not suggest virus growth but the well known occasional release of minimal residual infectivity by some nonlysing malignant cells. Moreover, since no differences in susceptibility of any human cells for SV40 infection are known, it is very unlikely that this tumorigenic virus could be fortuitously present in three different tumorous organs but not in the normal ones.

The presence of readily detectable T antigen in the tumour extracts cannot be explained by local virus growth. In infected monkey organs SV40 proliferates optimally but individual T-containing cells could not be detected[16]. As well as high anti-viral titres the animals can develop T antibody[16], so the innocuously infected monkey tissues must release immunogenic but *in vivo* probably undetectable amounts of T antigen. By contrast, T antibodies have not been detected in noncancerous persons with high and persistent anti-viral titres[14,15,17]. Some reactivity with SV40 T antigen has been detected in only three cancer-free persons who had insignificant or no SV40 anti-viral activity; it must be considered to be caused by cross-reacting antibodies directed against an antigen produced by BK virus, a different agent[17]. The failure to detect T antibody in many cancer patients[14] may indicate that there are indeed very few SV40 tumours, or that therapy prevented the tumours becoming very large and partly necrotic and releasing the intranuclear antigen[18]. Failure may also be a consequence of the fact that the antibody levels present may be detectable only by more sensitive techniques, or of immunosuppression by anticancer drugs or irradiation.

We have not detected T antigen in other melanomas; the evidence does not imply any obligate association of human malignant melanomas with SV40. Injected intravenously, SV40 can cause leukaemia, lymphosarcoma, reticulum cell sarcoma, osteogenic sarcoma and possibly other malignancies in hamsters[18]. Viral antibodies, and by inference SV40 infection, occur in childhood[14]. Human SV40 malignancies may conceivably[19] develop only occasionally after a very long time or fortuitous triggering. The possibility of SV40 hybridising *in vivo* or *in vitro* with other viruses must be considered, for hybrids could spread the tumour-inducing genes of SV40 more efficiently than the pure virus; the adenovirus–SV40 hybrids that have originated *in vitro* are well known.

SV40 tumour cells, like all other cells permanently transformed by SV40, contain without exception specific T antigen, a nonviral marker of unknown function that indicates integration of the viral into the cellular genome[7,19]. No systematic search for SV40 T antigen in human cancers has been made. Confirmation of the oncogenicity of SV40 for humans will have to come from finding additional cases, especially by T antigen detection.

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1 Melnick, J. L., *Science*, **135**, 1128 (1962).
2 Hull, R. N., in *Virology Monographs*, **2** (Springer-Verlag, New York, 1968).
3 Rapp, F., and Melnick, J. L., *Progr. med. Virol.*, **8**, 349 (Karger, Basel and New York, 1966).
4 Granboulan, N. P., Tournier, P., Wicker, R., and Bernhard, W., *J. Cell Biol.*, **17**, 423 (1963).
5 Eddy, B. E., Borman, G. S., Grubbs, G. E., and Young, R. D., *Virology*, **17**, 67 (1962).
6 Kirchstein, R. L., and Gerber, P., *Nature*, **19**, 229 (1962).
7 Del Villano, B. C., and Defendi, V., *Virology*, **51**, 34 (1973).
8 Pope, J. H., and Rowe, W. P., *J. exp. Med.*, **120**, 121 (1964).
9 Kit, S., Melnick, J. L., Anken, M., Dubbs, D. R., Torres, R. A., and Kitahara, T., *J. Virol.*, **1**, 684 (1967).
10 Rosenthal, J. D., Kozaburo, H., and Notkins, A. L., *Appl. Microbiol.*, **25**, 567 (1973).
11 Melnick, J. L., and Stinebaugh, S., *Proc. Soc. exp. Biol. Med.*, **109**, 965 (1962).
12 Morris, J. A., Johnson, K. M., Aulisio, C. G., Channock, R. M., and Knight, V., *Proc. Soc. exp. Biol. Med.*, **108**, 56 (1961).
13 Weiner, L. P., Herndon, R. M., Narayan, O., Johnson, R. T., Shah, K., Rubinstein, L. J., Preziosi, T. J., and Conley, F. K., *New Engl. J. Med.*, **286**, 385 (1972).
14 Shah, K. V., *Am. J. Epidemiol.*, **95**, 199, (1972).
15 Gerber, P., *Proc. Soc. exp. Biol. Med.*, **125**, 1284 (1967).
16 Shah, K. V., Willard, S., Myers, R. E., Hess, D. M., and DiGiacomo, R., *Proc. Soc. exp. Biol. Med.*, **130**, 196 (1969).
17 Shah, K. V., Daniel, R. W., and Murphy, G., *J. natn. Cancer Inst.*, **51**, 687 (1973).
18 Diamandopoulos, G. T., *J. natn. Cancer Inst.*, **50**, 1347 (1973).
19 Smith, H. S., Gelb, L. D., and Martin, M. A., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 152 (1972).

Outline structure of cytochrome c_3 and consideration of its properties

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An outline structure of cytochrome c_3 from Desulfovibrio vulgaris *has been deducted from sequence and nuclear magnetic resonance data. From this structure conclusions about electron transfer processes have been drawn*

THE cytochromes of the c_3 and c_7 groups form a class of multihaem proteins found in certain bacteria[1,2]. From the reaction chains in which they are involved these proteins seem to serve electron transfer and storage functions much in the same way as do the ferredoxins. As is true of many ferredoxins these cytochromes have more than one redox centre (here haem), are of low molecular weight, and have a low redox potential. Great interest centres in their structures and electron transfer reactions. Here we shall give an outline structure of one such protein, cytochrome c_3 from *Desulfovibrio vulgaris* and go on to describe its electron transfer properties. Information about the outline structure has been deduced from a knowledge of the amino acid sequence, and the nuclear magnetic resonance, the absorption, and the electron spin resonance spectra, studied under a variety of conditions. It should be understood that in principle the structure can be improved so as to produce a full three dimensional map by the methods we have described elsewhere[3–5], but that this procedure is totally different from that used in an X-ray crystallographic series of refinement. In crystallography a rough three-dimensional density map is improved by increasing data input until local molecular configuration can be discerned. The methods which we use start from local knowledge and build up to the full three-dimensional structure using many cross checks. Thus we shall proceed in this paper from a one-dimensional sequence to a trial outline three-dimensional structure. This outline structure is being thoroughly tested. While gathering structural information by NMR much is learnt about the dynamic properties of the protein.

The sequences of five cytochromes of the c_3 and c_7 classes are known. These sequences, including that of *Desulfovibrio vulgaris*, are given in Table 1 (ref. 2). The number of haem residues thought to be bound is four in all cases except that of cytochrome c_7 of *Chloropseudomonas ethylica*. The groupings Cys-*x*-*x*-Cys-His and Cys-*x*-*x*-*x*-*x*-Cys-His where *x* represents an amino acid are obvious haem binding sites, the histidine binding to the iron (compare cytochrome *c*). Haem binding groups in addition to the above cysteine sequences which have been found in other proteins are histidine (cytochrome b_5) methionine (cytochrome *c*) and water (myoglobin). We cannot however exclude a lysine or a carboxylate-containing residue as an iron binding group. The sequences by themselves do not therefore define such groups although some further clues are available from them. There are a further four invariant histidines (33, 36, 47 and 83), apart from those indicated above (46, 64, 97 and 122), in the sequences of the four cytochromes c_3 which have four haems. Close to these histidines lie aromatic amino acids which might be expected to be associated with a haem pocket. (Note these are such small proteins that they have only just enough amino acids to cover the four haem residues, for each haem residue requires about 20 amino acids to cover it.) The sequences also contain invariant lysine residues which could well neutralise the anionic carboxylate groups of the haem residues. It is notable too that certain parts of the sequences are very variable such as between residues 0-25, and 99-108. Much of the region from residues 55 to 90 is missing in the three-haem protein from *Chloropseudomonas ethylica*. This region includes the cysteine sequences 58-64 and also histidine 47. This indicates that these are the binding groups of one haem in the four-haem cytochromes c_3.

Table 1 Amino acid sequences of cytochromes c_3 and c_7.

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                1                   2                   3                   4                   5                   6
    1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3

*                   v d v P A d Ga Ki d f i a g g e Kn l Vv Fn Hs t Hk d v k - Cb b CH Hz p g b k q - y a g Ct t d GC
†                 a p k a P A d Gl Kme - - - - a t Kq p Vv Fn Hs t Hk s v k - Cg d CH Hp v n g k e d y r k Cg t a GC
‡   v d a p a d mv i k a P A - Ga Kv - - - - - - - t Ka p Va Fs Hk g Ha s md - Ck t CH Hk w d g a g a i q p Cq a s GC
§   v d a p g d mv l k a P A - Ga Km - - - - - - - t Ka p Vd Fs Hk g Ha a l d - - Ct k CH Hk w d g k a e v k k Cs a e GC
¶         a d v v t y e - - - - - - - - - - - - - n k Kg n Vt Fd Hk a Ha e k l g Cd a CH - e g t p a k i a i d - - - - - - -

                                                                                1                   1                   1
                    7                   8                   9                   0                   1                   2
    4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6

*   H n i l d k a d Ks - v n s w Yk v v Hd a k g g a Kp t Ci s CHk d k a g d d k e l KKk - - l Tg Ck g s a CHp s
†   H d s md k k d Ks - a k g y Yh v mHd k n t k f K - s Cv g CHv e v a g a d a a k KKd - - l Tg Ck k s k CHe
‡   H a n t e s k - Kg - d d s f Ym a f He r k s e - K - s Cv g CHk - - - - - - - - s mKKg - - p Tk C - - t e CHp k n
1§  H v(b/t s) k(k g Kk s t p k)f Ys a f Hs k s d i - - - s Cv g CHk - - - - - - - - a l KKa t g p Tk C - - g d CHp k k/k
¶   - - - - d k k - - - - - - - - - - s a - Hk k d - - - - - a Ck t CHk s n n - - - - - - - - - - g p Tk C - - g g CHi k
```

* *Desulfovibrio gigas* NCIB 9332 (c_3)
† *Desulfovibrio vulgaris* MCIB 8303 (Hildenborough) (c_3)
‡ *Desulfovibrio desulfuricans* MCIB 8380 (El Agheila Z)(c_3)
§ *Desulfovibrio salexigens* NCIB 8403 (British Guiana) (c_3)
¶ *Chloropseudomonas ethylica* (c_7)
Sequence § (*D. salexigens*) is tentative and incomplete (R. P. Ambler, M. Bruschi and J. Le Gall, unpublished observations). The one-letter notation used is that recommended by the IUPAC-IUB Commission on Biochemical Nomenclature[18]. The haem-binding sites are underlined. Residues common to each sequence are shown in capitals. This choice is somewhat subjective, as deletions can be juggled to improve the apparent match.

Oxidation-reduction states

By adding aliquots of sodium dithionite, the reduction at 25° C of the cytochrome c_3 from *Desulfovibrio vulgaris*, prepared as described previously[6], has been studied by following the paramagnetically shifted NMR resonances[7] which lie to very low field (Fig. 1). The number of methyl resonances in this region indicates the number of oxidised haem groups present. By monitoring selected peaks the disappearance and appearance of three different species can be followed readily during a titration (Fig. 2). The titration shows that there must be four haems per molecule which are reduced in two two-electron steps separated in midpoint potential by about 50 mV.

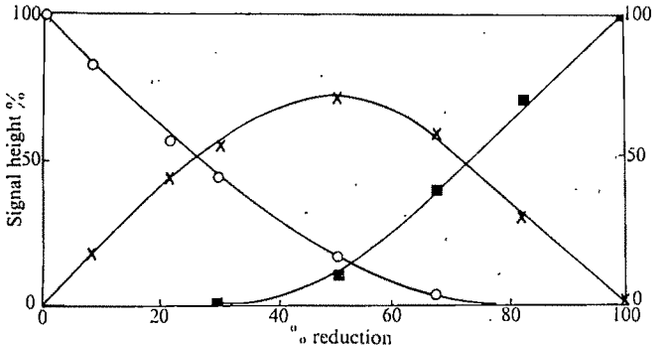


Fig. 1 Appearance and disappearance of NMR lines associated with the three different redox states of cytochrome c_3. O, Concentration of the fully oxidised state which has been followed by measuring the area of several contact shifted methyl resonances at very low field; ×, concentration of the intermediate form, followed by the appearance and disappearance of the new contact shifted methyl resonances on addition of reducing agent, sodium dithionite; ■, the appearance of the diamagnetic form, followed by the appearance of the single proton resonances at very low field attributed to two histidines.

EPR measurements have shown quite independently that the four haems are quite distinct[8]. The NMR reduction-titration (Fig. 2) starts from four distinct and separate paramagnetic haem species, (12-16 low-field methyl resonances, shown more clearly in Fig. 3) has an intermediate of two paramagnetic species (seven or eight low-field methyl resonances) and then gives a fully reduced form of four diamagnetic haem species (no low-field methyl resonances). The reaction is fully reversible and addition of oxygen to the fully reduced species gives an unaltered fully oxidised spectrum. Before discussing the NMR spectra further it is necessary to characterise the spin states of the haems.

The absorption[5,9], the NMR (ref. 10) and the ESR (ref. 8) spectra of cytochrome c_3 from *Desulfovibrio vulgaris* are all characteristic of low-spin Fe(II) and Fe(III) in all oxidation states of the protein. The four cysteine sequences already described as well as the absorption spectra indicate that all the haems are bound through thioether linkages (normal cytochrome *c* links) and thus that one of the iron bound residues of each haem is a histidine of the sequences Cys-*x*-*x*-Cys-His or Cys-*x*-*x*-*x*-*x*-Cys-His. Now as each iron of each haem is low spin it is required to be bound to one other strong ligand which therefore cannot be water or carboxylate, but could be methionine, lysine or histidine. From the *g* values of the EPR spectra of the fully oxidised and half oxidised protein[8], the other ligands are probably nitrogen ligands, that is, either histidine or lysine. The NMR spectrum of the oxidised protein also rules out methionine as a haem ligands, that is either histidine or lysine. The NMR spectrum resonance around +20 p.p.m. (as found in cytochrome *c*)[7] and the absorption spectrum confirms this as there is no 695 nm band typical of methionine binding[9]. McDonald and Phillips[10] have pointed out that the NMR spectrum also excludes lysine as a haem ligand as there are no strongly upfield shifted CH_2 groups. A search of the histidine region of the NMR spectrum revealed only one histidine in its conventional position in all three oxidation states. The four remaining histidines are therefore iron ligands, and in what follows it will be assumed that the four haems are bound to eight of the nine histidines of the *vulgaris* sequence. The sequences of the five proteins in Table 1 strongly confirm this conclusion.

Details of NMR spectra

Using resolution enhancement techniques[11] (compare Fig. 3) the NMR resonance positions of at least one hundred resonances can be followed in each of the three oxidation states. In the fully oxidised state the temperature dependences of the resonances have been studied from 25° C to 55° C. This allows those resonances which are affected by the paramagnetic haem groups (temperature dependent) to be separated from those which are in their normal diamagnetic positions or are shifted from these positions by ring current effects (both temperature independent)[7]. The ring current shifts can then be related to known shifts observed in model compounds using the usual geometric relationships (Fig. 4). The paramagnetic shifted resonances can have been affected either by contact and pseudo-contact shift (this is the case for all groups linked by bonds to the haem iron) or by pseudo-contact shift alone (this is the case for all protein resonances except the groups bound directly to the iron).

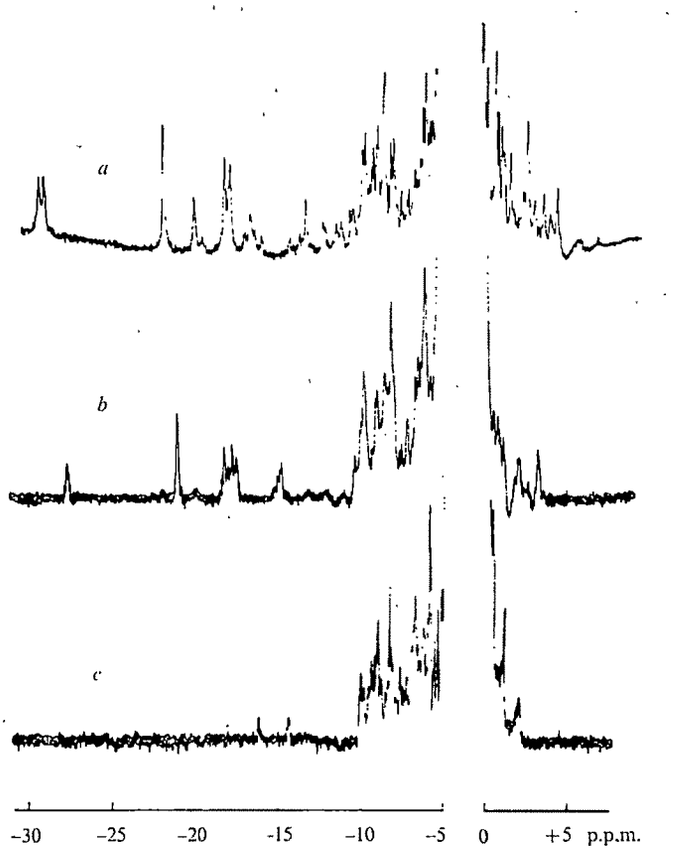


Fig. 2 NMR spectra (measured at 270 MHz) of *D. vulgaris* cytochrome c_3 in the various oxidation-reduction states observed during reduction with sodium dithionite. *a*, fully-oxidised species; *b*, half-reduced species; *c*, fully-reduced species. (Note the vertical scale in *b* and *c* is twice that in *a*.) The titration was carried out with 5 mM protein at 25° C. Dioxan was used as an internal standard but all peaks are referred to TSS.

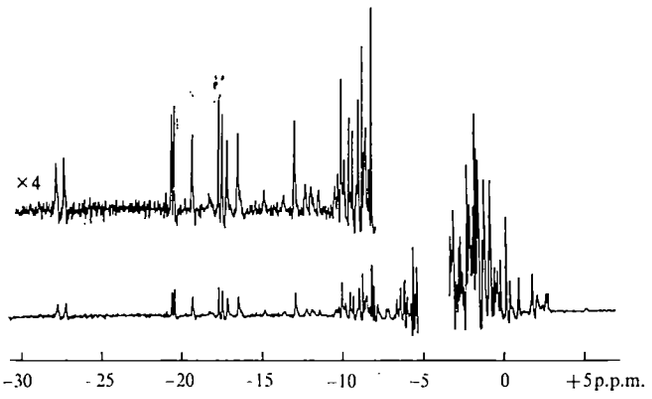


Fig. 3 Resolution enhanced spectrum of oxidised cytochrome c_3 at 50° C.

The pseudo-contact shifts contain much geometric information, which can be used in studies of structure[4,10,12,13]. The greatest problem in using all the shift data, which undoubtedly permit a complete structure determination for this protein, is that of the assigment of the resonances to individual groups. Although we are not yet in a position to carry out such a detailed analysis we can give a qualitative interpretation of our data. We treat each of the three oxidation states in turn.

In the spectrum of fully oxidised cytochrome c_3 there are resonances from +15 to −30 p.p.m. as in cytochrome *c*, but the resonances are spread over the spectrum in a very different way from those in the cytochrome *c* spectrum[7] despite the similarity in EPR *g* values[8]. The most obvious features are shown in Figs 2 and 3. Apart from the fact that there are the expected number of downfield, contact-shifted, haem-ring methyl group resonances (12 to 16 can be seen), there are very many other resonances with large shifts to both high- and low-field. Taking the high-field region first, resonances in this region which show a temperature-dependent shift must either lie in the neighbourhood of and outside the pseudo-contact 'cone' of the haem iron atoms (Fig. 4) or they must experience an appreciable contact shift. From models and studies on other haem proteins, three types of group on the porphyrin are known to give temperature dependent peaks shifted to high field[7]. They are the single proton haem methenes, some of the protons of the propionic acid side chains and at least one of the two methyl groups per haem in the groupings CH_3CH(haem).S-protein, that is the methyl groups of the thioether links. As each of the four iron atoms is bound to two histidines, no other paramagnetically shifted methyl resonances of iron ligands are expected beyond +1.0 p.p.m. Furthermore, in the spectra of all the haem proteins so far examined, no paramagnetically shifted methyl group resonances of non-haem linked residues have been seen at fields higher than +1 p.p.m. In cytochrome *c*, excluding the resonance of the methionine methyl group which is known to be absent in the cytochrome c_3 proteins, the most strongly high-field shifted methyl resonances (due to a thioether methyl group) is seen at +2.2 p.p.m. (ref. 12). By contrast, cytochrome c_3 has some six methyl resonances in the region from +2 to +5 p.p.m. This large number of methyl groups shifted to high field leads immediately to the suggestion that some of the haems are placed end to end producing a region between them of large upfield pseudo-contact shift (see Figs 4, 5). In this region of the spectrum there are also a number of unidentified resonances of one or two protons intensity. Two single-proton peaks are very strongly upfield shifted (+15 p.p.m.) and probably arise from two methene protons.

In the low-field region, apart from the 12–16 contact shifted haem methyl group resonances, there are at least 10 resonances of one proton strength beyond −10 p.p.m. It is likely that these resonances are some of the C_2 and $C_4 \geqslant CH$ groups of histidines which should be strongly downfield shifted by a pseudo-contact mechanism as they are inside the cone (Fig. 4).

The spectrum around −7 p.p.m. where the aromatic protons are generally observed is quite different from that expected, indicating that all or at least most of the five aromatic rings have their resonances displaced by pseudo-contact interactions. Similarly the methyl group resonances, which in a protein normally have strong maxima in the region of −1 to −3 p.p.m. are spread grossly over the whole range from +5 to −5 p.p.m. This is hardly surprising as each of the four haems will interact with some different part of the protein.

The region of the spectrum from −8 to −10 p.p.m. shows only one histidine resonance in roughly the correct position for a possible not bound to iron (see also ref. 10) but it also contains a large number of single proton peaks. We return to these resonances later for they must be very close to the haem group, as is shown by their broadening in intermediate states of oxidation-reduction. It is in this region of the spectrum that >NH resonances are normally observed.

The resonance peaks of the half-oxidised species are readily separated from the fully reduced and fully oxidised

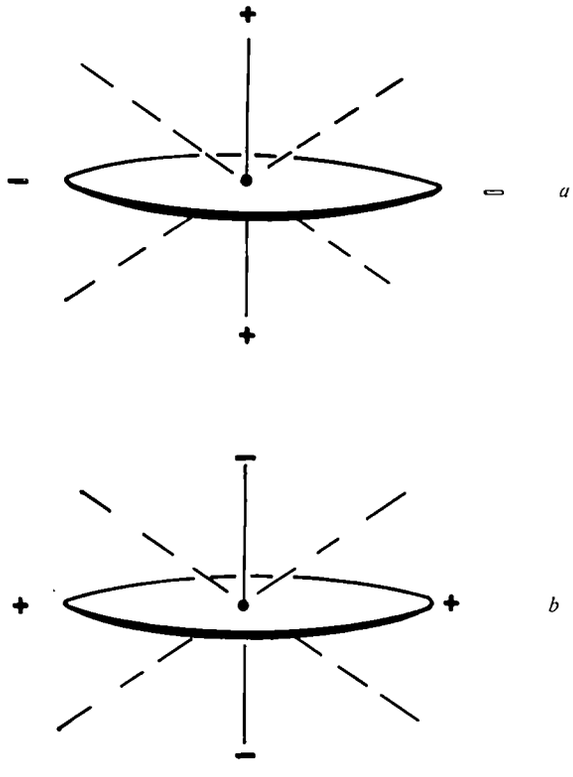


Fig. 4 Schematic representation of the regions of space (cones) which give rise to shifts of NMR resonances. *a*, The case for a ring-current shift which has axial symmetry. Protons lying within the cone experience an upfield shift (+). *b*, Case for axial pseudo-contact shift with the signs appropriate for low spin Fe(III), a proton within the cone experiencing a downfield pseudo-contact shift. As the pseudo-contact term exceeds the ring-current shift by a factor of three, shifts in Fe(III) porphyrin complexes are in the opposed sense to those in diamagnetic Fe(II) porphyrin complexes. In a protein the field around Fe(II) is rhombic, though largely dominated by an axial term, so that care must be taken in the detailed interpretation of pseudo-contact shifts. The cones are derived from a consideration of the angle dependence of shift ($3\cos^2\theta-1$), where θ is the angle between r_i (the vector between the haem iron and the observed proton) and the g_n–axis).

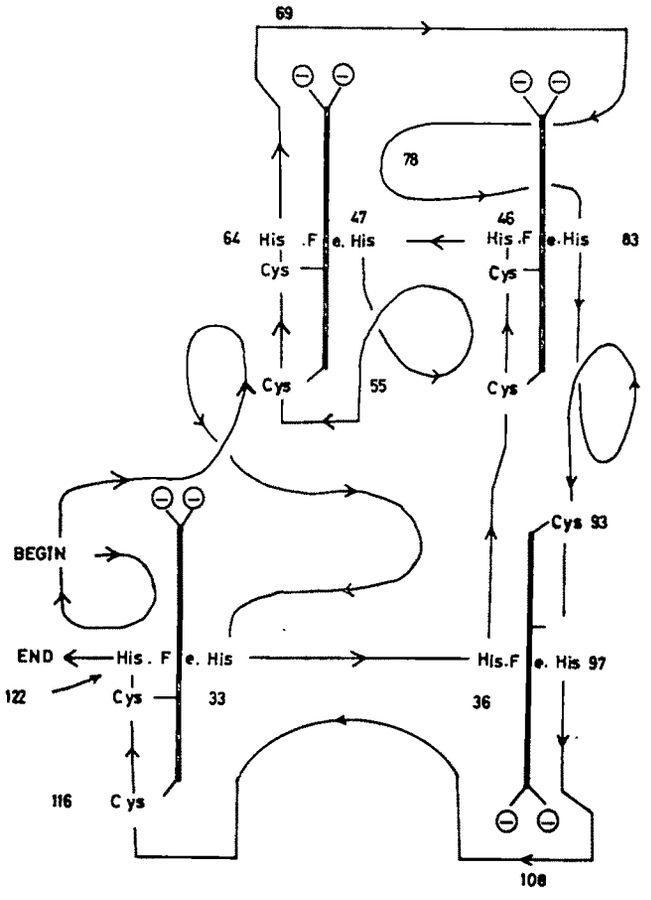


Fig. 5 Proposed outline structure of cytochrome c_3 from *Desulfovibrio vulgaris*.

species (Fig. 2). At very low field, beyond the normal aromatic proton region there are now some six to eight contact-shifted methyl resonances indicating that two haems remain oxidised. There are however only two methyl resonances at very high field, that is above +1.0 p.p.m. although there are also some broad and unidentified one and two proton peaks shifted into this region of the spectrum of oxidised cytochrome *c*, two paramagnetic haem groups replacing the one in cytochrome *c*, showing that the cooperativity required to explain the shifts in the fully oxidised species has disappeared. If two haems in the different oxidation states Fe(II), diamagnetic, and Fe(III), paramagnetic, are packed end to end then the shifts due to one are opposed to the shifts of the other (Fig. 4). The shifts are now anti-cooperative. We emphasise that the downfield contact-shifted methyl resonances move upfield going from fully oxidised to half reduced.

The aromatic protons of the half-oxidised spectrum are again not recognisable in their usual positions but the aliphatic region is more closely that of a conventional haem protein. A single histidine resonance lies in the expected part of the spectrum. The region from −8 to −10 p.p.m. has no structure but as in the oxidised species a rough estimate of the number of >NH protons which do not exchange in D_2O is five to ten.

The fully reduced spectrum has several unusual features. In the upfield region there are more than ten single resonances close to or above 0 p.p.m. We assign many of these single resonances to ring-current shifted protons of histidine bound to iron. They are shifted upfield by the ring current of the haem (Fig. 4). It is known that the α-protons of pyridine bound to a diamagnetic haem are shifted into this region[14]. There is only one possible histidine proton at −8 p.p.m. and thus as in the oxidised and intermediate forms we can state that eight of the nine histidines are bound to the haem. There are no methyl resonances in this upfield region. This shows yet again that unlike cytochrome *c* there are no methionines bound to the central iron. Furthermore, no methyl groups sit centrally inside the cone of the haem rings and close to the haem.

The methyl region, around −2 p.p.m. is now rather similar to that of other proteins. (McDonald and Phillips[10] point out that in fact the methyl region is displaced to low field to some extent.) In the region between −6 and −10 p.p.m. there is a large number of sharp single proton resonances. It is hard to assess the number accurately but it exceeds 10. These are undoubtedly some of the ring methene bridge ⩾CH groups. They are downfield shifted by the ring current (Fig. 4). Again resonances from aromatic protons cannot be recognised in the usual positions. This is expected of course if the aromatic groups are close to the haem so that their resonances experience ring-current shifts. In this region, there is also a number of protons, say five to ten, assigned to nonexchanging >NH from peptide bonds. This region of the spectrum (in D_2O) is not altered after a cycle of oxidation and reduction or after cycling from 25° C to 55° C and holding at the high temperature for some hours, or even after several weeks at 0° C. Some of the >NH protons are very resistant to exchange.

This brings us to the most remarkable feature of the reduced spectrum. At very low field, −14.5 and −16.3 p.p.m., there are two single proton resonances. As this is a diamagnetic system such resonances must originate from >NH groups. It is not possible to conceive of a ring-current shift of minimally 8 p.p.m. downfield of the most downfield ⩾CH resonance. It is known that even the effect on ortho protons of haem-bound pyridine is only about 8 p.p.m. upfield[14], and the chemical shift of imidazole protons of iron-haem ligands is about 0 p.p.m. As the maximum upfield shift is twice the maximum downfield shift, from the angle dependence of shift, $(3\cos^2\theta - 1)$, (Fig. 4), we can eliminate the possibility of downfield shifts of greater than 5 p.p.m. We therefore assign the two resonances to >NH groups of histidine which are known to occur at such a low field in H_2O (ref. 15). Usually such resonances exchange rapidly in D_2O. These two histidines must be in a region of the protein very well protected from water. Since their resonances disappear from this part of the spectrum on oxidation. (Figs 1 and 2), they must be bound to iron.

Introduction of very small amounts of either oxidising or reducing agents to the fully reduced or fully oxidised systems respectively causes a profound change in the line widths of some resonances. In fact all the sharp resonances due to haem protons, for example methene ⩾CH, broadened on altering the redox state of either the fully reduced or fully oxidised protein by less than 10%. These resonances include those assigned to the bound histidines as well as the protons of the haem itself. All the haems seem to be affected. Further oxidation and reduction towards the intermediate form broadens all the resonances with the result that the half-oxidised state gives a relatively poor spectrum in which it is hard to find such resonances as those of the methene bridge protons or those of the histidines. But during oxidation or reduction, well separated resonances of each of the three species titrate without shifting showing that equilibrium between oxidised and reduced species is not fast (on the NMR time scale) for otherwise intermediate resonance positions would be seen.

The broadening of resonances of haem proteins must arise however from relatively fast chemical exchange as the electron relaxation rate of low-spin haem is known to be fast. There are two electron exchange rates to consider: (1) internal exchange between haems of the same protein molecule and (2) exchange between different protein

molecules. A full study of the reaction rates with temperature and concentration will give much knowledge of the electron transfer process in proteins but it is clear that the electron exchange is of intermediate rate rather than very fast or very slow.

Structural features

The sequence and the NMR data on *Desulfovibrio vulgaris* cytochrome c_3 can now be put together. The short lengths of sequence between haem-bound histidines at residues 46–47 and 33–36 force the planes of the haems to be roughly parallel pairs. Each haem is bound to two histidines and to two thioether links. At least some of the haems must also be close together and stacked end to end as the large cooperative upfield shifts of the methyls of the thioether bridges in the fully oxidised state indicates. The anticooperativity of ring-current and pseudo-contact interactions of the haems provided by the arrangement in Fig. 5 also fits observations from the data on the half-oxidised and fully-reduced states. The histidine 80 which is not haem-bound and is found in a normal position in the NMR spectrum is only found in *Desulfovibrio vulgaris*[10]. The aromatic groups must now be placed reasonably close to the haem centres for they do not appear as normal aromatic resonances in the NMR spectra of any of the three forms we have studied. It is also known that in haem proteins it is usual for the two carboxylate residues of the haem to be neutralised by basic groups in their vicinity. In Fig. 5 we have placed invariant basic residues close to the carboxylate groups. McDonald and Phillips[10] point out that the NMR spectra indicate that the lysines are on the outside of the molecule.

The model agrees with the observation on *Chloropseudomonas ethylica* that many (26) amino acids from 55 to 90 and histidine 47 which binds to the haem in the top left hand corner of Fig. 5 are missing. In the three-haem cytochromes this haem must be absent. We have built a working three-dimensional model of the structure to prove that it fills space in a satisfactory way. The distance between haems is about 10 Å, in agreement with assessments of this distance based on one interpretation of the optical spectroscopy[9,16].

Further details of the structure can be unravelled by a thorough assignment of the resonances, and a calculation of the resonance shifts together with model building. Further NMR probes can be introduced as becomes necessary.

The most interesting feature of the protein is that despite the fact that the haems are close together (about 10 Å apart) and are separated by only a single polypeptide chain (Fig. 5) there is relatively slow electron exchange between them. Thus such a protein is not a simple electron conductor but is more likely a storage unit, a capacitor, for electrons. It cannot be the case that normal amino acid residues can give rise to a path for rapid electron exchange. In a model experiment we have shown that collision between low-spin Fe(II) and Fe(III) haems provides fast exchange[17]. Thus the haems in cytochrome c_3 (as in other cytochromes) are not able to come into close contact through intermolecular collisions. The possibility remains that these proteins are devices for multielectron reactions in which electrons are released one at a time but in rapid succession.

C. C. McDonald and W. D. Phillips have measured[10] independently the NMR spectrum of cytochrome c_3. Their data and deductions are very similar to ours. We wish to acknowledges their friendly cooperation. R.J.P.W. is a member of the Oxford enzyme group. C.F.G. acknowledges the receipt of a NATO grant. We thank Dr R. P. Ambler for permission to reproduce the sequences given in Table 1.

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1 DerVartanian, D. V., and LeGall, J., *Biochim. biophys. Acta.* (in the press).
2 Ambler, R. P., *Systematic Zoology*, **22**, 554 (1973).
3 Barry, C. D., North, A. C. T., Glasel, J. A., Williams, R. J. P., and Xavier, A. V., *Nature*, **232**, 236–245 (1971).
4 Barry, C. D., Hill, H. A. O., Mann, B. E., Sadler, P. J., and Williams, R. J. P., *J. Am. Chem. Soc.*, **95**, 4545–4551 (1973).
5 Campbell, I. D., Dobson, C. M., Williams, R. J. P., and Xavier, A. V., *Ann. N.Y. Acad. Sci.*, **222**, 163–174 (1973).
6 LeGall, J., Bruschi-Heriaud, M., and DerVartanian, D. V., *Biochim. biophys. Acta*, **234**, 499–512 (1971).
7 Wüthrich, K., *Struct. Bond.*, **8**, 53–121 (1970).
8 DerVartanian, D. V., *J. Magn. Reson.*, **10**, 170–178 (1973).
9 Drucker, H., Campbell, L. L., and Woody, R. W., *Biochemistry*, **9**, 1519–1527 (1970).
10 McDonald, C. C., and Phillips, W. D., *Biochemistry* (in the press).
11 Campbell, I. D., Dobson, C. M., Williams, R. J. P., and Xavier, A. V., *J. Magn. Reson.*, **11**, 172–181 (1973).
12 McDonald, C. C., and Phillips, W. D., *Biochemistry*, **12**, 3170–3186 (1973).
13 Sheard, B., Yamane, T., and Shulman, R. G., *J. molec. Biol.*, **53**, 35–48 (1970).
Keller, R. M., and Wüthrich, K., *Biochim. biophys. Acta*, **285**, 326–336 (1972).
14 Storm, C. B., *J. Am. chem. Soc.*, **92**, 1423–1425 (1970).
15 Patel, D. J., Woodward, C. K., and Bovey, F. A., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 599–602 (1972).
16 Urry, D. W., *J. Am. chem. Soc.*, **89**, 4190–4196 (1967).
17 Weightman, J. A., Hoyle, N. J., and Williams, R. J. P., *Biochim. biophys. Acta*, **244**, 567–572 (1971).
18 IUPAC-IUB Commission on Biochemical Nomenclature, *Biochemistry*, **113**, 1–4 (1969).

LETTERS TO NATURE

PHYSICAL SCIENCES

Shell flashes in planetary nuclei

THE central stars of planetary nebulae may be the ancestors of some of the white dwarfs[1-3], and the magnetic white dwarfs in particular may have originated in this way[4,5]. There seem to be no mechanisms in the white dwarfs themselves capable of producing fields of the strengths observed, and the magnetic field must therefore be a fossil remnant of an earlier phase of evolution. The hypothesis that a high internal field is exposed by the ejection of the nebular shell has an appealing simplicity.

A satisfactory explanation for the postulated high internal fields in the planetary nuclei has not yet been suggested. Conservation of magnetic flux from the main sequence phase of evolution is adequate only if the main sequence progenitors of the planetaries have average field intensities $B \sim 10^3$

gauss. Such fields do exist in the magnetic A stars, but they seem to be too massive to be the ancestors of the planetaries.

We point out here that a natural mechanism for the production of strong magnetic fields may in fact be present in at least some planetary nuclei. A prominent feature of the evolution of such stars is the occurrence of one or more thermal runaways, 'flashes', in an unstable shell source which produces nuclear energy[6]. Near the peak of a flash a transient convection zone is invariably developed[7-11], and if the star has even a modest amount of differential rotation, the generation of magnetic field in a stellar dynamo can occur. This hypothesis was suggested to us by the recent paper of Ruderman and Sutherland[12], in which it is argued that the transient convection accompanying the explosive burning of carbon in carbon-detonation supernovae, will tangle up any initial 'seed' magnetic field and perhaps cause amplification to the equipartition field strength.

In order to investigate the plausibility of dynamo field generation in the planetaries, it is necessary to have estimates of the rate of differential rotation and of the properties of the transient convection zones in these stars. We are unaware of any determinations of the rotation of planetary nuclei. One of the magnetic white dwarfs is, however, known to rotate with a period, P, of 1.33 d (ref. 13). If this star has indeed evolved from a planetary nucleus, conservation of angular momentum implies a period $P \sim 10^2$ d for the ancestor of the white dwarf. This value seems conservative, and we therefore adopt it for our calculations.

As even less is known about the differential rotation of planetary nuclei, we adopt $\Delta P/P \sim 0.1$, the value appropriate to the sun, where ΔP is the magnitude of the difference in rotation period between the pole and the equator.

Because the precise details of the evolutionary history of the planetaries are uncertain, the exact structure of such stars immediately before the expulsion of the nebular shell is unknown[6]. As a result, a considerable variety of models have been suggested for these objects. Thus, Rose and Smith[7] and Paczynski[8] consider instabilities in the He-shell of double-shell-source models; Wood and Faulkner[9] consider the shell-flash in pure helium stars; Joss *et al.*[10] investigate models in which either He- or C-shell flashes can occur; and Kutter and Savedoff[11] consider shell flashes in pure carbon stars. Unfortunately, except in the latter paper, only very schematic information is given about the structure of the convection zones, and we therefore rely on rather crude estimates for the convective velocities.

The following results are typical of these models. For models that exhibit a He-shell flash[7-10]: the stellar mass is $M \sim 0.8 M_\odot$; the mass of the helium envelope—which is almost entirely convective at the peak of the flash—is $\sim 2 \times 10^{-3} M_\odot$ (the convection zone may, however, cover a substantial fraction ($\sim 1/2$) of the stellar radius; the density at the He-burning shell ρ is $\sim 2 \times 10^3$ g cm^{-3}; the convective velocity v is $\sim 3 \times 10^5$ cm s^{-1}; and the duration of the transient convection is $\sim$10–100 yr. For models that exhibit a C-shell flash[10,11]: the stellar mass is $\sim 1.1 M_\odot$; convection covers $\sim 1/3$ of the stellar mass; the density at the C-burning shell ρ is $\sim 10^5$ g cm^{-3}, the convective velocity v is $\sim 10^5$ cm s^{-1}, and the duration of the transient convection is $\sim 2 \times 10^2$ yr. In either case the stellar radii R are $\sim 0.1 R_\odot$ (as they must before planetary nuclei) and the equipartition magnetic field strengths B_{eq}, are $\sim (8\pi \cdot (1/2)\, \rho v^2)^{\frac{1}{2}} \sim 10^7$–$10^8$ gauss. This is more than adequate to account for the magnetic fields of $\sim 10^7$ gauss detected in the magnetic white dwarfs (which have radii of about $\sim 10^{-2} R_\odot$), provided that the transient convection lasts long enough for the dynamo to generate such fields.

In the conventional picture of a dynamo[14], an initial poloidal 'seed' field of intensity B_0 is distorted by the differential rotation to produce a toroidal field component. This is a continuing process, with the toroidal field doubling in a time $\tau \sim P^2/\Delta P$. At the same time a new poloidal field is generated by the action of the cyclonic convection, which lifts and twists the toroidal field lines[14]. Growth of the field is ultimately halted when the magnetic intensity approaches the equipartition field strength, B_{eq}; not only is the convection then too weak to deform the field lines, but also the stronger field suppresses the convective motions.

That this schematic picture is qualitatively correct can readily be seen by applying it to the Sun. In the solar convection zone, $B_{eq} \sim 4 \times 10^3$ gauss, which is the order of magnitude of the fields seen in sunspots, where magnetic field lines from the interior protrude through the solar photosphere. Furthermore, the doubling time is $\tau \sim 1$ yr, and the time required for an initial 'seed' field of intensity $B_0 \sim 1$ gauss (the general solar field) to reach this limiting intensity is $t \sim \tau \log_{10}(B_{eq}/B_0)/\log_{10} 2 \sim 10$ yr, which is almost exactly half the sunspot cycle.

For the planetary nuclei, our previous estimates of the differential rotation indicate a doubling time $\tau \sim 3$ yr. If the initial 'seed' field is again taken to be $B_0 \sim 1$ gauss (it would be $\sim 10^2$ gauss if flux conservation from the main sequence were assumed), the time required for the field to reach its equipartition strength of $\sim 10^7$–10^8 gauss is $t \sim \tau \log_{10}(B_{eq}/B_0/\log_{10} 2 \sim$ 70–80 yr. This is comparable with the duration of the transient convection zone, and it therefore seems entirely possible that fields of the intensity ($\sim 10^5$ gauss) required in order to explain the magnetic white dwarfs can be generated in at least some planetaries.

It is tempting to suggest that the production of a magnetic field necessarily accompanies the ejection of a planetary nebula or even, perhaps, that nebular ejection is caused by the production of strong magnetic fields. This is certainly premature at present, although if the latter conjecture were true, all planetary nuclei should have strong magnetic fields, and it should be possible to verify this by observation.

If dynamo generation of strong magnetic fields actually occurs during shell flashes in planetary nuclei, as we have suggested, a prime candidate for the observational test of this hypothesis would seem to be the remarkable star FG Sge. This star is known to have ejected a planetary nebula $\sim$6,000 yr ago, and it seems to be undergoing an active shell flash at the present (refs 8, 15, and G. E. Langer, *et al.*, unpublished information). Most remarkable, however, is the recent discovery (G. E. Langer, *et al.*, unpublished information) that the surface layers are currently being enriched in s-process nuclei, which are thought to be produced in the He-shell flash. Along with these nuclei strong magnetic fields would also be expected to be transported to the surface if our hypothesis is correct. Measurements of the magnetic field of this star thus seem to present a crucial test of this idea.

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[1] O'Dell, C. R., *Astrophys. J.*, **138**, 67 (1963).
[2] Harman, R. J., and Seaton, M. J., *Astrophys. J.*, **140**, 824 (1964).
[3] Weideman, V., *A. Rev. Astron. Astrophys.*, **6**, 351 (1968).
[4] Imoto, M., and Kanai, M., *Publ. astr. Soc., Japan*, **23**, 363 (1971).
[5] Fontaine, G., Thomas, J. H., and Van Horn, H. M., *Astrophys. J.*, **184**, 911 (1973).
[6] Salpeter, E. E., *A. Rev. Astron. Astrophys.*, **9**, 127 (1971).
[7] Rose, W. K., and Smith, R. L., *Astrophys. J.*, **173**, 385 (1972).
[8] Paczynski, B., *Acta astr.*, **21**, 417 (1971).
[9] Wood, P. R., and Faulkner, D. J., *Astrophys. J.*, **181**, 147 (1973).
[10] Joss, P. C, Katz, J I., Malone, R. C., and Salpeter, E. E., *Astrophys J.*, **181**, 409 (1973).
[11] Kutter, G. S., and Savedoff, M. P., *Astrophys. J.*, **156**, 1021 (1969).

[12] Ruderman, M. A., and Sutherland, P. G., *Nature phys. Sci.*, **246**, 93 (1973).
[13] Angel, J. R. P., and Landstreet, J. D., *Astrophys. J. Lett.*, **165**, L67 (1971).
[14] Parker, E. N., *Astrophys. J.*, **122**, 293 (1955).
[15] Sparks, W., and Kutter, G., *Astrophys. J. Lett.*, **183**, L121 (1973).

Gravity-induced electric polarisation near the Schwarzschild limit

IT has long been recognised that besides carrying the fundamental unit of charge an electron is also endowed with inertia. Only recently has attention been paid to the possibility that phenomena may exist which depend on the gravitational mass[1]. Here we point out that strong gravitational fields, such as those encountered in collapsed stellar objects, may produce observable electromagnetic effects arising directly from the gravitational mass of electric charges.

Some sixty years ago Tolman and Stewart[2] described an experiment in which the inertia of electrons is revealed. This involved a conductor, such as a circular horizontal wire, which oscillates about a vertical through its centre. Because of their inertia the free electrons will move relative to the crystal structure of the wire and so an oscillating current will be generated. To a first approximation, if **a** is the acceleration of the wire at any instant, then as far as the free electrons are concerned an effective electric field **E** given by

$$\mathbf{E} = \left(\frac{m}{e}\right)\mathbf{a} \qquad (1)$$

is present in the reference frame of the wire (m and e are the electronic mass and charge, respectively). In principle a method for obtaining the specific charge e/m was made available.

From the point of view of relativity theory, we may regard the acceleration of the wire as equivalent to the action of a gravitational field (of oscillatory character in the above case), in accordance with Einstein's principle of equivalence. In fact whenever a conductor is held in a gravitational field **g** we may expect an effective electric field

$$\mathbf{E} = -\left(\frac{m}{e}\right)\mathbf{g} \qquad (2)$$

to be manifested to the free electrons. In this case m is the electron's gravitational mass, indistinguishable from the inertial mass in the light of the equivalence principle.

If this is the effect of gravitation on free electrons (admittedly a very small one in the gravitational field of the Earth) then a corresponding phenomenon must influence bound charges in a dielectric. We may think of this as a gravitational polarisation

$$\mathbf{P} = \chi\mathbf{E} = -\chi\left(\frac{m}{e}\right)\mathbf{g} \qquad (3)$$

where χ is the gravitational susceptibility of the dielectric. If indeed **P** exists then, as in ordinary electric polarisation, an equivalent volume distribution of charge $-$ div. **P** and surface distribution **P.n** would result in accordance with conventional analysis.

For the gravitational field of the Earth such a polarisation no doubt produces extremely small consequences. But in a collapsed star lying near to its Schwarzschild limit the local value of g may be large enough to produce observable effects, especially in conjunction with a rapid rotation of the star[3]. Thus if we apply the usual Schwarzschild metric to a condensed object then the local value of the gravitational intensity on the surface $R = R_S$ is given by

$$g = (GM / R_S^2)\,(1 - [2GM/(c^2 R_S)])^{-1} \qquad (4)$$

M being the total mass and G the Newtonian gravitational constant. Here g is the proper acceleration (invariant) of a freely falling test particle relative to the fixed surface, and is equal to the magnitude of the first curvature vector of the world line of an atom fixed under stress at $R = R_S$. Equivalently g is the acceleration (in the opposite direction) of the fixed atom relative to a freely falling geodesic frame instantaneously at rest at $R = R_S$. Clearly if $R_S = R^*$, where $R^* = 2GM/c^2$ (the Schwarzschild gravitational radius) g may become extremely large, with corresponding intensification of the effects we have described on free and bound electric charges. Even in a neutron star (the limiting consequence of these effects) there will be such charges near the surface.

We now consider in particular what happens to a 'standard clock' S located in such intense gravitational fields. If the standard clock is an atomic emission line then we cannot expect this to be at the standard laboratory frequency in such circumstances. There must necessarily be an effect related to the electric Stark phenomenon. For example although the usual redshift formula will apply to the emitted radiation as received by a remote observer O, it is not sufficiently realised that this formula gives only the ratio ν_S / ν_0 for the frequencies emitted and observed in terms of the change in the gravitational field between S and O. It tells us nothing about the emitted frequency absolutely. The emitted frequency will in fact be standard only for an atom in free fall, or when the gravitational field is negligible. It is usually assumed in the case of the Sun, for example, that these conditions prevail. But clearly the assumption is invalid when we consider an atom fixed on the surface of a highly condensed object.

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[1] Schiff, L. I., and Barnhill, M. V., *Phys. Rev.*, **151**, 1067 (1966).
[2] Tolman, R. C., and Stewart, T. D., *Phys. Rev.*, **8**, 97 (1916).
[3] Kumar, N., and Nandini, R., *Phys. Rev.*, **7**, 3586 (1973).

Meteorological features affecting large aperture synthesis radio telescopes

VARIATIONS of atmospheric radio refractive index can cause phase differences between signals received at spaced aerials and limit the resolution of large radio telescopes using the aperture synthesis technique[1]. Hinder[2] found that anomalous phase differences recorded at the One-Mile telescope at Cambridge[3] were caused by thermal updraughts and fair weather cumulus; the mean size of the irregularities was 700 m. Earlier calculations[4] had shown that the gradients of refractive index across fronts were likely to be important but it was believed that corrections using ground-based measurements of pressure, temperature, and humidity could be applied to reduce errors from such large scale ($\approx$ 500 km) weather systems[5].

The first few months of observing with the 5-km telescope at the Mullard Radio Astronomy Observatory, University of Cambridge, showed that there were frequent significant effects from intermediate systems, with scales of 10 to 100 km or more. An investigation was undertaken to relate these periods of anomalous phase changes—'events'—to meteorological phenomena and the results are summarised here.

Telescope operational periods (excluding those spent observing complex emitting regions) event times to within 1 h and event intensities, for part of May, June to September, and

Table 1 Event distribution by month and time of day

Time GMT	May*		June		July		August		September		December		May - September		
	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(c)
00 – 06	111	242	198	675	128	535	109	607	64	483	35	580	610	2542	24.0
06 – 12	52	173	45	267	13	347	106.5	455	62	363	30.5	621	278.5	1605	17.3
12 – 18	50	134	42	485	174	548	142	733	83	419	117.5	740	491	2319	21.2
18 – 24	20	247	260	873	92	733	164.5	866	85	535	25	699	621.5	3254	19.1
Total	233	796	545	2300	407	2163	522	2661	294	1800	208	2640	2001	9720	20.6
(c)	29.3		23.7		18.8		19.6		16.3		7.9		20.6		
Nos of Events	9		18		13		19		10		10		69		
Intensity															
Max.	0.67		0.89		0.87		0.93		0.58		0.33		0.93		
Min.	0.32		0.24		0.14		0.19		0.17		0.10		0.14		
Mean.	0.44		0.50		0.47		0.46		0.34		0.23		0.45		
Medan	0.41		0.46		0.44		0.41		0.32		0.24		0.42		

(a) Total duration of events in tenths of an hour. (b) Period of operation of telescope in tenths of an hour. (c) % of operational time occupied by events.
* May data commenced on May 21.

December 1972 were considered. The intensity of an event is a normalised measure of the rate of increase in phase shift with aerial separation with first order geometrical differences removed. The observational programme was independent of weather conditions.

Table 1 gives the distribution of events by month and time of day. The data for December show that in winter, when the absolute water content of the atmosphere is at a minimum, events are likely to pose less of a problem, so the analysis was confined to the period May to September.

A classification of vertical variations of temperature, based on radiosonde reports for Hemsby (52° 41′ N, 01° 41′ E) and Crawley (51° 05′ N, 0° 13′ W), showed no relationship with occurrence of events; however, some events appeared to be associated with sharp vertical gradients of humidity marking the lower boundary of very dry mid-tropospheric layers. Such layers are associated with fronts[6] and may persist long after the front becomes inactive; such an association implies a sloping interface and hence a much larger horizontal gradient of radio refractive index than might be expected with more extensive dry layers in anticyclones.

Table 2 Precipitation at Cardington during operational periods at Cambridge (May to September only)

	Moderate or heavy rain	Thunder	Showers	Slight rain	Dry	Total
Event	3.9	1.0	18.6	12.2	164.4	200.1
No event	6.1	2.0	31.9	48.9	683.0	771.9
% of time occupied by events	39.0	33.3	36.8	20.0	19.4	20.6

All values are given in hours and tenths.

Considerations of 12-h changes in air mass characteristics at Hemsby and Crawley, and pressure variations recorded by the microbarogram at Kew Observatory, were inconclusive; in both cases only the few large changes showed any possible association with events. No additional relationships were found from a study of more detailed vertical soundings in the lowest 1,200 m, carried out four times daily at Cardington (52° 06′ N 00° 25′ W).

When hourly weather reports from Cardington were scrutinised, some events were found to coincide with the onset or cessation of rain. Table 2 gives the total times in hours and tenths when various precipitation types and intensities occurred in operational periods with and without events. If slight rain is disregarded, the relationship between events and other precipitation types is significant at the 0.1% level. No event in September coincided with precipitation (at Cardington); indeed a 12 h operational period from 1745 GMT on 8 September marked by continuous, sometimes heavy, rain throughout was free of events. Recent investigations into the structure of frontal systems[7,8] have established the existence of rain bands of approximate scale 100 × 30 km, within which are 'clusters' of order 20 × 30 km marked by higher rainfall rates; these could explain the relationship between moderate and heavy rainfall and events. The prolonged rain of September 8 and 9, mentioned above, did not seem to conform to this model. On that occasion rapid and intense cyclonic development occurred off South-west England, the resulting depression passing near Cambridge. In the early stages of a development of such intensity, the precipitation structure would not be expected to have consisted of organised cluster bands. Events were also noted during periods of convective activity. This is not surprising in view of the large humidity contrast between the upward rising convective columns and the neighbouring drier environment, and typical variations[9] in the depth of the convective boundary layer on distance scales of tens of km.

This investigation was limited by the lack of fine scale meteorological data, particularly upper air observations, from the vicinity of the telescope itself; a detailed synoptic study would require surface and upper air observations at the site. But it has indicated three systems which may have caused many of the phase differences observed: rain 'clusters'—usually associated with fronts, areas of showery activity and occasional very dry mid-tropospheric layers. These systems are all associated with humidity or liquid water content variations in the vertical and the horizontal, which are not limited to the lowest layers of the atmosphere. Therefore the likely frequencies of such systems must be taken into account when considering sites for new radio telescopes.

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[1] Ryle, M., and Hewish, A., *Mon. Not. R. astr. Soc.*, **120**, 220–230 (1960).
[2] Hinder, R. A., *J. atmos. terr. Phys.*, **34**, 1171–1186 (1972).
[3] Elsmore, B., Kenderdine, S., and Ryle, M., *Mon. Not. R. astr. Soc.*, **134**, 87–95 (1966).
[4] Bean, B. R., and Dutton, E. J., *Radio Meteorology*, Monogr. No. 92 (National Bureau of Standards, Washington, 1966).
[5] Hinder, R. A., and Ryle, M., *Mon. Not. R. astr. Soc.*, **154**, 229–253 (1971).
[6] Miles, M. K., *Q. J. R. met. Soc.*, **88**, 286–300 (1962).
[7] Browning, K. A., Hardman, M. E., Harrold, T. W., and Pardoe, C. W., *Q. J. R. met. Soc.*, **99**, 215–231 (1973).
[8] Harrold, T. W., *Q. J. R. met. Soc.*, **99**, 232–251 (1973).
[9] Harrold, T. W., and Browning, K. A., *Q. J. R. met. Soc.*, **97**, 330–339 (1971).

Neutral oxygen in comets

MEASUREMENTS of the strength of permitted spectral lines from neutral oxygen in comets should provide both a better understanding of gas dynamics in comets and an assessment of whether the production ratio of oxygen to hydrogen in comet atmospheres is close to a half. If this ratio is confirmed, it would provide strong support for the growing evidence[1] that the 'parent' molecules which make up the solid comet nucleus are dominantly H_2O.

A better understanding of the role of H_2O in comets can be obtained from the study of both atomic oxygen and hydrogen. Most observations of atomic oxygen in comets seem to be of the forbidden lines at 6,300 Å and 6,363 Å. These lines presumably occur only in cases in which an oxygen atom was left in the $^1D_2$ level at the time of dissociation, and they are thus related to the production rate of atomic oxygen rather than to the oxygen density (ref. 2). Measurement of permitted oxygen lines which result from photoexcitation by solar photons would provide a direct probe of neutral oxygen density.

Photoexcitation of neutral oxygen from the $2p^4\ ^3P_2$ ground state to the $3s\ ^3S_1^0$ level gives rise to three resonance-fluorescence lines at 1,302 Å, 1,305 Å and 1,306Å. These lines have been observed in the upper atmospheres of both Venus and Mars[3,4] and in Comet Kohoutek[5]. The fact that the same OI lines appear in emission in the solar chromosphere can considerably enhance the fluorescence. In a cometary atmosphere, however, the neutral oxygen may not be excited by these lines if the radial velocity of the comet with respect to the Sun is large enough to Doppler shift the wavelength of the exciting lines. In this case the excitation would be expected to be by the solar continuum, which is of a much lower intensity than the chromospheric OI lines. There is, however, another method of exciting OI in a comet. This is possible because of the almost exact coincidence of the oxygen lines at 1,025.77 Å and the Lyman-β hydrogen line at 1,025.72 Å, which appears in emission in the solar chromosphere (see also ref. 6). Lyman-β has a width of approximately 0.4 Å in the Sun, and therefore the radial velocity of the comet with respect to the sun will not be enough to prevent the occurrence of fluorescence.

The neutral oxygen atoms in the $3p^4\ ^3P_2$ ground state are excited to the $3d\ ^3D^0_{3,2,1}$ states by Lyman-β photons. The excited OI atoms can return to the ground state either by direct transitions, or through transitions to the $3p\ ^3P$ and $3s\ ^3S^0$ states, which will give rise to lines at 11,287 Å, 8,446.5 Å and the three lines near 1,304 Å. There is thus the interesting possibility of detecting the presence of neutral atomic oxygen in comets, from allowed transitions in the near infrared spectrum.

The concentration of neutral oxygen in the atmosphere of a comet will depend on the rate of production, the rate of escape from the nuclear region and the rate of removal by ionisation. The rate of production of oxygen can be deduced if it is assumed that the hydrogen cloud around a comet is the result of photodissociation of water vapour, in which case the rate of production of oxygen should be half of the rate of production of hydrogen.

The rate of escape of oxygen from the nuclear region is difficult to estimate accurately, because there is evidence that collisions in the region near the nucleus may be rather frequent[7], resulting in an equipartition of energy between the component particles. In this case the outflow velocity of oxygen atoms into the coma would be a quarter of the hydrogen velocity. For oxygen atoms released by photodissociation of OH at a distance from the nucleus, conservation of momentum imparts a velocity to the oxygen atoms of one sixteenth that of hydrogen.

The ionisation of hydrogen in comets is predominantly a result of charge exchange by protons in the solar wind. This mechanism is about 10 times more important than photoionisation[8]. The lifetime against photoionisation of oxygen by solar photons (found from the cross sections in ref. 9) is very close to that for hydrogen. The cross section to ionise oxygen by charge exchange with solar wind protons is, however, two to three times smaller than the hydrogen cross section[10], so the total lifetime of neutral oxygen atoms is still determined by the lifetime against charge exchange. The smaller charge-exchange cross section of oxygen allows most of the oxygen atoms from a comet 1 AU from the Sun to remain neutral out to at least 10^6 km from the nucleus, in spite of their lower velocities. At a distance of 10^6 km from the nucleus, the oxygen density is about two to five times that of hydrogen, for hydrogen to oxygen velocity ratios of 4 and 16 respectively, assuming that the hydrogen and oxygen atoms both come from H_2O.

From the published transition probabilities[11] for O and H the photon flux of the 8,446.5 Å OI line from a comet can be estimated in terms of the 6,563 Å Hα line, because both are nearly proportional to the Lyman-β flux from the Sun. The calculated photon flux for the 8,446.5 Å OI line is 0.8 to 2.0 of the Hα flux, when the oxygen density is two and five times the hydrogen density respectively. This assumes that the comet is optically thin to the solar Lyman-β photons, but as both the oxygen and hydrogen clouds in the comet are expected to be considerably larger than the radius at which Lyman-β becomes optically thick (about 10^5 km) (ref. 12), it seems justifiable to assume a small optical depth.

The observation of Hα in Comet Kohoutek, using a Fabry–Perot interferometer[13], suggests that it might be possible to observe the OI line at 8,446.5 Å using an instrument with a high enough resolution to discriminate against the continuous background spectrum. Such observations, together with more observations of the far ultraviolet spectra of hydrogen and oxygen, the OH emission at 3080 Å, and the forbidden oxygen lines at 6,300 Å and 6,363 Å, may provide sufficient information on the physical and dynamical processes occurring in comets to allow a critical evaluation of the importance of H_2O in the dirty-ice model.

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[1] Delsemme, A., *Astrophys. Lett.* **14**, 163–167 (1973).
[2] Biermann, L., and Trefftz, E., *Z. Astrophys.*, **59**, 1–28 (1964).
[3] Moos, H. W., and Rottman, G. J., *Astrophys. J.*, **169**, L127–130 (1971).

[4] Barth, C. A., Hord, C. W., Pearce, J. B., Kelly, K. K., Anderson, G. P., and Stewart, A. I., *J. geophys. Res.*, **76**, 2213–2227 (1971).
[5] Opal, C., and Carruthers, G., *IAU Circ. No. 2618* (1974).
[6] Keenan, P. C., and Hynek, J. A., *Astrophys. J.*, **111**, 1–10 (1950).
[7] Biermann, L., *Q. Jl R. astr. Soc.*, **12**, 417–432 (1971).
[8] Keller, H., *Astr. Astrophys.*, **23**, 269–280 (1973).
[9] Comes, F. J., Speier, F., and Elzer, A., *Z. Naturf.*, **23A**, 125–133 (1968).
[10] Fite, W. L., Smith, A. C. H., and Stebbings, R. F., *Proc. R. Soc.*, **A268**, 527–536 (1962).
[11] Wiese, W. L., Smith, M. W., and Glennon, B. M., *Atomic Transition Probabilities*, NSRDS–NBS 4, **1**, (US Government Printing Office, Washington, 1966).
[12] Biermann, L., *Joint Institute for Laboratory Astrophysics, Report No. 93*, (Univ. Colorado, Boulder, 1968).
[13] Donn, B., *IAU Circ. No. 2605* (1973).

PAN over the Atlantic and the smell of clean linen

OZONE, at concentrations in excess of 0.1 parts per million (p.p.m.), has been observed in the summertime air over southern England[1]. The most probable source is photochemical reaction among air pollution products. We therefore sought the presence of another characteristic product of urban atmospheric photochemistry[2], peroxy acetyl nitrate (PAN). Preliminary measurements (S. A. Penkett and F. J. Sandalls, personal communication) show that PAN is present in the air over southern England. During episodes of increased ozone and other atmospheric pollution indicators the concentration increased, but as with ozone there was always a background concentration of PAN even in clean air arriving from the Atlantic. The voyage of the German Research Vessel Meteor from Hamburg to Santo Domingo in October 1973 presented an opoprtunity to discover whether or not there is a natural background of PAN.

Analysis was by gas chromatography using an electron capture detector. Air samples were collected at the bows of the ship by drawing 5 ml of air into a glass syringe; the sample was then quickly injected into the gas chromatograph. The apparent concentration of PAN during the voyage is shown in Fig. 1. Concentrations of CCl_3F are also included because increases in the background level of this compound indicate the presence of air from urban industrial regions[3]. The term 'apparent' is used because it was discovered during the voyage that the unexpectedly high concentrations over the Atlantic were not present in the air, as such, but had formed by reaction on the glass surface of the sampling syringe. No PAN could be detected in air collected in polypropylene syringes, but the addition of a twist of glass wool to such syringes before the air was drawn into them restored the concentration to levels similar to those found with glass syringes. One-litre samples of fresh air were also drawn through plugs containing 1 g of glass or cotton wool, and these were found to be strong sources of PAN and of peroxy proprionyl nitrate (PPN). In addition the wools possessed the characteristic bleach-like odour of linen after it has been dried in the open air. The PAN potentials of Fig. 1 are approximate and probably too low. No corections were made for losses by adsorption or for the extent to which the detector was not coulometric.

The potential for formation of PAN was most marked on days of unobstructed sunshine (see Fig. 1). October 13 and 14, when the ship passed through a polluted air mass (which was indicated by the high concentrations of CCl_3F) were exceptions. PAN formation was greatest at approximately 1400 h and was between 3 and 10 times lower at dawn and in the evening. Simultaneous measurements from the ship, of the aerial concentration of atmospheric hydrocarbons, indicated levels of betwen 0.1 and 2 parts per 10^9 (p.p.b.) for the lower unsaturated hydrocarbons (R. A. Rasmussen, personal communication). The sea was apparently the most probable source. The concentration of

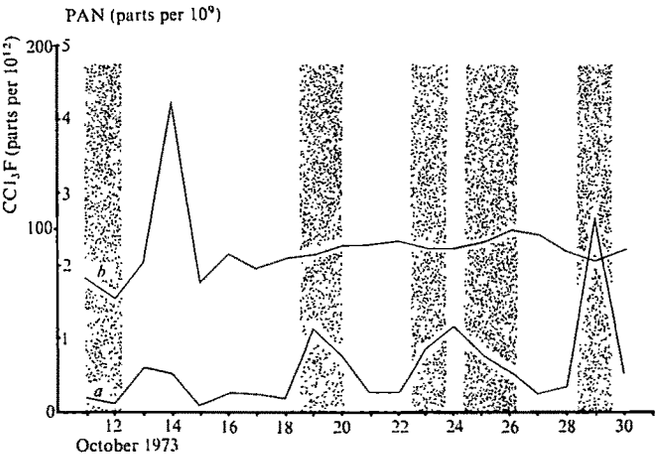


Fig. 1 PAN from the air over the Atlantic. Average daily concentrations of 'apparent' PAN × 10^{-9} (*a*) and $CCl_3F \times 10^{-12}$ (*b*) by volume. Shaded columns indicate days with less than 50% cloud cover.

the other ingredients necessary to form PAN by photochemical reaction were not measured. Ozone is ubiquitous and can be presumed to have been present in at least its background concentration of about 50 p.p.b.

These preliminary observations suggested that PAN and similar compounds can be formed in the clean air of remote maritime regions by heterogeneous reaction if a suitable surface is provided. They raise some interesting and important questions. What are the mid-Atlantic precursors? Is PAN formation in urban photochemical smog also by heterogeneous reaction? If so what is the surface on which it forms? Finally the quantities of PAN observed suggest the presence of a high background concentration of NO_x or of some related molecular species.

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[1] Atkins, D. H. F., Cox, R. A., and Eggleton, A. E. J., *Nature*, **235**, 372 (1972).
[2] Scott, W. E., Stephens, E. R., Hanst, T. L., Doerr, R. C., *Proc. Am. Petrol. Inst.*, Sec. III, **37**, 171 (1957).
[3] Lovelock, J. E., *Atmos. Environ.*, **6**, 917–925 (1972).

Vertical distribution of heat production in the Basement of the Eastern Alps

A LINEAR relationship between heat flow and heat production within plutonic bodies has been observed by a number of researchers[1,2] and can be written:

$$q = q^* + DA(0) \quad (1)$$

where q is the surface heat flow, $A(0)$ is the surface heat production, and q^* and D are constants within a given geographic province.

Many heat production–depth models satisfy equation (1), but Lachenbruch[2,3] suggests that an exponential model (equation (2)) is needed if equation (1) is to be preserved after differential erosion of the source layer.

Thus, the following equation is initially assumed to be valid for the area under discussion:

$$A(z) = A(0)\exp(-z/D) \quad 0 < z \leqslant z^* \quad (2)$$

Data are therefore presented in the form:

$$\log_e A(z) = \log_e A(0) - z/D$$

$$\text{or } z = -D\log_e A(z) + D\log_e A(0) \quad (3)$$

The distribution of heat production with depth in a granitic terrain is discussed here and the results of this direct measurement approach are compared with those already obtained from heat flow data elsewhere. The granitic rocks (the Zentralgneis) from the Pennine Basement of the Tauern Window in Austria were chosen because of the excellent exposure and because a vertical section of 4 km is available for sampling.

The Zentralgneis is a Variscan polyphase intrusive series of tonalites, granodiorites, and granites, which has undergone deformation, and subsequent amphibolite facies metamorphism during the Tertiary. A representative sample of the lithologies within each unit 1 km thick was obtained whenever possible. Particular care was taken to ensure that each unit was represented by a similar number of samples. Measurement of the amounts of uranium, thorium, and potassium was carried out using standard γ-ray spectrometry techniques and heat production was calculated from these data using the formula published by Birch *et al.*[1].

The depth of each sample (m) was calculated from a map published by Cliff *et al.* (ref. 4, page 234) on which the contours of the projected base of the overlying unit, the Peripheral Schieferhülle, are extrapolated over this area. Errors are difficult to estimate but are thought to be no greater than ±250 m over the whole of the sampled area.

Heat production falls off markedly with increasing depth (Fig. 1); but the scatter of data points is such that it is not possible to distinguish an exponential model from, for example, a linear diminution of heat production with depth. The same problem of a high background noise on the fitted curve also led Lachenbruch and Bunker[5] to an identical conclusion in the only other attempt to verify the exponential model by direct measurement.

In both cases, the difference in scale between the thermal equilibrium, which enables a heat flow value to be representative of large volumes of rock, and the petrological and geochemical equilibria, which are commonly confined to a sample the size of a hand specimen in such terrains appears unbridgable. But heat flow values tend to smooth out the small scale chemical inhomogeneities and, it is therefore possible that such a geophysical approach will add a new large scale dimension to the petrogenetic studies of composite intrusive series.

The second important result is that, although heat production does fall off with depth as predicted by Lachenbruch's interpretation of the heat flow data, it falls off considerably faster in the Zentralgneis than in any other province so far investigated.

Lachenbruch has explained the linear relationship described by equation (1) in terms of a steady state one-dimensional model, assuming the lateral gradients in heat production to be negligible compared with the vertical gradients. Consequently, the vertical section of 4 km in the Zentralgneis has been subdivided into four horizontal units 1 km thick and the

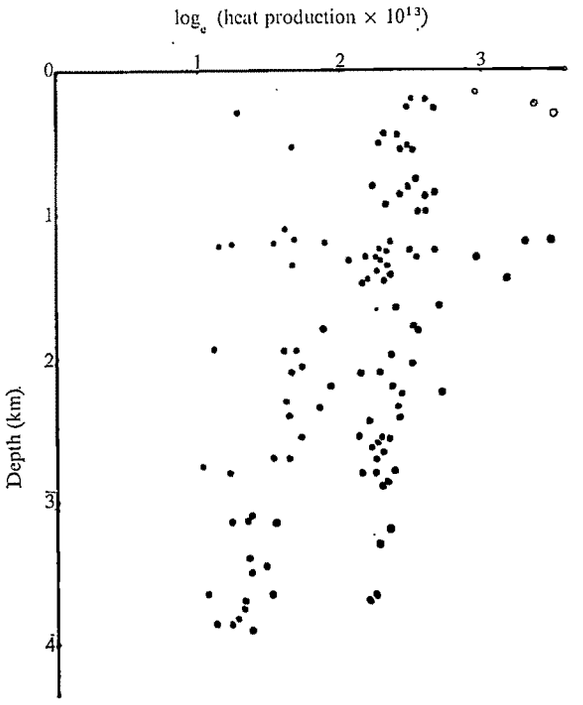


Fig. 1 Distribution of log$_e$ (heat production × 10^{13}) with depth (km) below the top of the basement complex in the Tauern Window. All samples were taken from the metaplutonic rocks of the Zentralgneis.

mean heat production of each unit has been calculated. The results are then presented in the form of equation (3), that is, a straight line graph of log$_e$ (heat production × 10^{13}) against depth (Fig. 2). The slope of the best fit straight line gives $D = 3.5 \pm 1$ km (2σ).

Considering the scatter of the original data, the fit of the mean values of heat production to the best straight line is remarkably good. It is, however, apparent from Fig. 2 that the value of D is to some extent controlled by the surprisingly low value of mean heat production in the fourth unit. This low value is a result of the relative depletion of uranium with respect to thorium in the basal unit, which in turn reflects the fact that it is only at this structural level that tonalite predominates over the other, more fractionated, granitoid lithologies. It is, however, difficult to further evaluate this low value of mean heat production because the rocks exposed in the basal unit crop out in a smaller area than those of the other three units; and it is not known how typical the lithologies in the fourth unit are of this structural level in the Zentralgneis.

Nonetheless, despite these geological ambiguities concerning the value of mean heat production in the basal kilometre, the scatter of the values of log$_e$ (mean heat production × 10^{13}) about the best straight line (Fig. 2) is within the assigned experimental errors. Consequently, the distribution of heat production with depth in the Zentralgneis seems to be consistent with an exponential model (equation (2)) in which $D = 3.5 \pm 1$ km, and $A(0) = 15.7 \times 10^{-13}$ calorie cm^3 s^{-1}.

Other workers have reported results obtained from both heat flow[1,2] and direct measurement techniques[5], which are consistent with $D = 9$–10 km. Roy *et al.*[6], however, suggested that in stable continental areas, D may be approximately 7.5 km. All these values are significantly greater than the value of D reported here.

Although the Zentralgneis has been deformed and metamorphosed to amphibolite facies, recent geochemical work (C. J. Hawkesworth, unpublished) and the preservation of premetamorphic Rb/Sr whole rock ages[7], suggests that on a large scale a primary igneous distribution of elements is being observed. Consequently, the low value of D cannot be explained by late upward concentration of the radioactive elements during metamorphism.

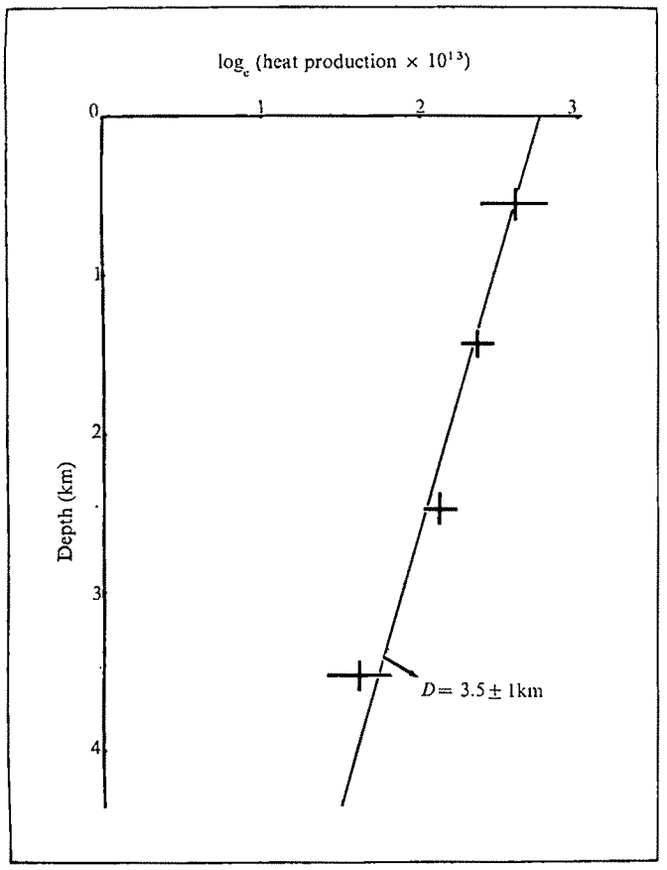


Fig. 2 Distribution of the values of $\log_e$ (mean heat production $\times 10^{13}$) for each of the four horizontal units, with depth in kilometres below the top of the basement complex. The values of mean heat production were calculated from the data in Fig. 1.

Furthermore, this value of D measured at the surface is unlikely to be true for all depths in the present Alpine crust. Recent geophysical work[8,9] suggests not only that the base of the crust below the Tauern Window is molten but also that below a depth of 10 km there is a large granitic body of assumed Alpine age. This will almost certainly have redistributed the radioactive elements in the lower crust, perhaps with another value of D.

Finally, the results presented here illustrate that the rate of decrease of heat production with depth may be more variable than was initially anticipated. The value of D presumably varies with the regional conditions under which fractionation and intrusion of magma took place. At this time, however, it is not possible to decide whether an exponential decrease of heat production with depth is the direct consequence of fractional crystallisation processes, or whether other factors, such as the effect of the gravitational field on the distribution of large ionic complexes[10], also contribute significantly.

The γ-ray spectrometry was carried out at the Mineralogisk-Geologisk Museum, Oslo at the invitation of Professor K. S. Heier. I thank him and his colleagues, P. Killeen and G. Raade in particular, for help and hospitality. I thank my supervisor E. R. Oxburgh, and also K. O'Nions, S. W. Richardson, M. J. Bickle, P. England and D. Waters for discussions throughout the project.

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[1] Birch, F., Roy, R. F., and Decker, E. R., in *Studies of Appalachian Geology: Northern and Maritime* (edits by Zen, E-an., White, W. S., Hadley, J. B., and Thompson, J. B., jun.), 437 (Interscience, New York, 1968).
[2] Lachenbruch, A. H., *J. geophys. Res.*, **73**, 6977 (1968).
[3] Lachenbruch, A. H., *J. geophys. Res.*, **75**, 3291 (1970).
[4] Cliff, R. A., Norris, R. J., Oxburgh, E. R., and Wright, R. C., *Jb. geol. Bundesanst.*, **114**, 121 (1971).
[5] Lachenbruch, A. H., and Bunker, C. M., *J. geophys. Res.*, **76**, 3852 (1971).
[6] Roy, R. F., Blackwell, D. D., and Birch, F., *Earth planet. Sci. Lett.*, **5**, 1 (1968).
[7] Cliff, R. A., *Contr. Miner. Petrol.*, **32**, 274 (1971).
[8] Giese, P., *Schweiz. miner. petrogn. Mitt.*, **50**, 597 (1970).
[9] Angenheister, G., Bögel, H., Gebrande, H., Giese, P., Schmidt-Thomé, and Zeil, W., *Geol. Rdsch..*, **61**, 349 (1972).
[10] Oxburgh, E. R., and Turcotte, D. L., *Science*, **176**, 1021 (1972).

Iron and manganese encrustations in Recent sediments

Cores taken from the north-eastern Mediterranean (Fig. 1) during cruise 4/72 of the RRV *Shackleton*, using a Lehigh 4-inch hydroplastic gravity corer, contain layered organic structures encrusted with either manganese or iron minerals (Table 1, Fig. 2).

To our knowledge these encrustations have not previously been reported from seafloor sediments. The bulk of the manganese deposits, found in layers in the top 5–25 cm of the cores, occur on tubes, which we consider to be the agglutinating benthonic foraminiferan *Rhabdammina* (Fig. 3*a* and *b*). The deposit is also found on small branching rods (Fig. 3*d*) which are believed to be of organic origin but have not yet been identified. Smaller deposits are occasionally found on the shells of some of the pteropods and planktonic foraminifera (Fig. 3*c*); large tubes (Fig. 3*e*) are thought to be the encrusted linings of burrows. The iron deposits, present in layers beneath the manganese deposits, occur as encrustations on what seem to be mineralogically altered *Rhabdammina* tubes and, rarely, on planktonic foraminifera; no iron equivalent of the branching rods or large tubes has been found. When a core is

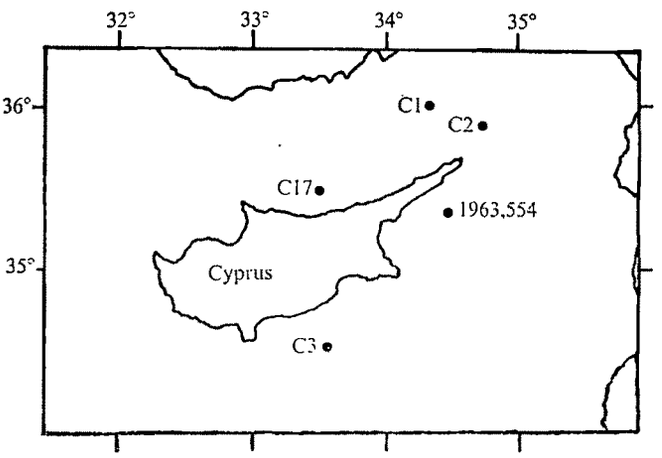


Fig. 1 Location of cores, C. C1, BM1973,0,1; C2, BM1973, 0,2; C3, BM1973,0,3; C17, BM1973,0,17. 1963,544 is a British Museum valve lead sample.

sectioned the manganese deposits appear as black streaks, and the iron-encrusted tubes can be seen as ochre coloured layers. Both types of encrustation were separated from the sediment by washing carefully with hydrogen peroxide (10 vol.), which disaggregates the clay minerals. The manganese encrustation was removed from the smaller tubes with a solution of 1 M hydroxylamine hydrochloride. This exposed a tube similar to the encrusted *Rhabdammina* found in the top 0–5 cm of the cores. The iron encrustation was removed with 1 M hydrochloric acid, leaving a

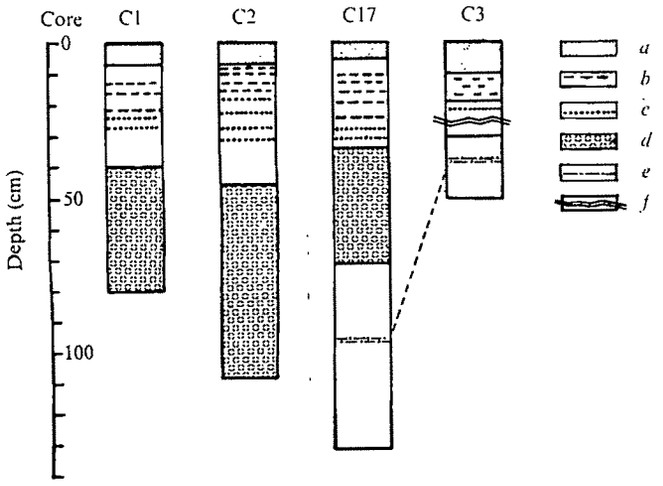


Fig. 2 Sedimentology of the cores. *a*, Orange-yellow ooze; *b*, layer of black manganese-encrusted tubes in light brown ooze; *c*, layer of brown iron-encrusted tubes in light brown ooze; *d*, grey ooze; *e*, light green layer in green-grey ooze (containing no benthonic foraminifera, and possibly representing a sapropel). Cooler water planktonic foraminifera occur below this level. *f*, position of hiatus.

siliceous tube similar to that produced when unencrusted *Rhabdammina* were similarly treated.

Examination by X-ray diffraction of the material rich in manganese showed that it was composed mainly of todorokite, a mineral commonly present in managanese nodules[1]. This analysis was confirmed by X-ray powder photography. The structural spacings which we have measured differ slightly from those previously reported[2], possibly because of the greater amount of magnesium in the structure. The nature of the material rich in iron is not known, but it is amorphous to X rays and is probably a hydroxide.

The faunal content of the cores indicates that they are post glacial. The planktonic foraminiferal population indicates a warm temperature and is dominated by *Globigerinoides ruber* (d'Orbigny), which makes up 60–90% of the samples larger than 250 μm. It is associated with many gastropods and pteropods, which indicate postglacial sediments in this region[3,4]. In core C17 below 100 cm, and in core C3 below 45 cm, the occurrence of the foraminiferal species *Globorotalia inflata* (d'Orbigny) and *Globigerina pachyderma* (Ehrenberg) provide evidence of cooler conditions. The sediments in core C3 seem to have been disturbed. The distinction between the layers of manganese-rich and iron-rich tubes is less apparent in this core, and the grey ooze section is missing (Fig. 2). A light green layer is present in green-grey ooze at 95 cm in core C17 and is also seen at 27 cm in core C3; this suggests that there is a hiatus somewhere between 20 and 30 cm in core C3, although no physical break is visible in the longitudinally sectioned core. The sedimentation rate in core C17, assuming that a date of 10,000 yr BP (ref. 5) represents the commencement of the postglacial period, is at least 13 cm per 1,000 yr. This implies that the manganese-rich horizons have an age range of about 770 to 2,000 yr BP.



Fig. 3 Manganese encrustations: *a*, Smooth surfaced *Rhabdammina* tubes (typically 0.2–1.2 mm in diameter, and greater than 3 mm long); *b*, *Rhabdammina* tubes with globular protuberances (typically 0.25–0.5 mm diameter, and more than 4 mm long); *c*, planktonic foraminiferan *Globigerinoides ruber* (d'Orbigny); *d*, branching rods (typically 0.075 mm diameter, length variable); *e*, large tube, probably a (?worm) burrow lining (typically 1 mm in diameter and 3 mm long).

Table 1 Chemical analyses of *Rhabdammina* tubes

	Unencrusted	Manganese-rich encrustations			Iron-rich encrustations
Core No.	C2	C3	C3*	C1	C2
Depth (cm)	0–4	15–16	15–16	12.5-14.5	20-21
Acid-soluble fraction weight (%)					
Mn	0.1	35.3	26.9	32.5	0.3
Fe	4.2	1.3	1.4	0.7	30.6
Ca	9.0	4.9	5.9	4.2	3.6
Mg	0.6	3.6	3.2	3.3	1.3
Insoluble SiO_2	67.8	14.5	19.7	< 14.9	11.3

*Globular protuberances.

The *Rhabdammina* tubes and other attendant organic debris seem to produce preferential surfaces for the deposition of manganese and iron, as the encrustations only occur in the *Rhabdammina* layers. The presence of the manganese-rich layers above the iron-rich layers may be related to the migratory processes believed to be responsible for the formation of manganese nodules[6–8].

We thank Miss E. Fejer for confirming the identification of todorokite by X-ray powder photography, the Captain and Officers of the Natural Environment Research Council RRV Shackleton, and Drs R. Chester and G. Evans, chief scientists for the cruise.

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1 Cronan, D. S., and Tooms, J. S., *Deep Sea Res.*, **16**, 335 (1969).
2 Straczec, J. A., Horen, A., Ross, M., and Warshaw, C. W., *Am. Miner.*, **45**, 1174 (1960).
3 Parker, F. L., *Rep. Swedish Deep Sea Expedition, 1947–1948*, **8**, 219 (1958).
4 Herman, Y., *Proc. second Planktonic Conf.* (edit. by Farinacci, A.), (Technoscienza, Rome, 1971).
5 Wiseman, J. D. H., *Rep. Swedish Deep Sea Expedition, 1947–48*, **7**, 287 (1965).
6 Bonatti, E., Fisher, D. E., Joensuu, O., and Rydell, H. S., *Geochim. cosmochim. Acta*, **35**, 189 (1971).
7 Bonatti, E., and Nayudu, Y. R., *Am. J. Sci.*, **263**, 17 (1965).
8 Lynn, D. C., and Bonatti, E., *Mar. Geol.*, **3**, 457 (1965).

Photolysis of CO-NH_3 mixtures and the Martian atmosphere

One of the puzzling aspects of the Martian atmosphere is the absence of nitrogen or compounds containing nitrogen[1]. Instead, the atmosphere is found to be CO_2 along with smaller amounts of CO and H_2O. (ref. 1). One suggestion is that nitrogen

atoms gain sufficient energy to escape the gravitational field when N_2 undergoes photodissociation with ultraviolet light[2]. Alternatively, nitrogen may be conserved in compounds in the Martian crust[3].

We have already noted that although neither CO_2 nor H_2O affected the rate of NH_3 photolysis, CO acclerated the photodecomposition of ammonia, with the formation of a solid product[4]. We have investigated the photolysis of NH_3 in the presence of CO in greater detail not only because of the potential significance to atmospheric photochemistry on Mars but also because of the possibility of photocatalytic reactions of NH_3 and CO on the Martian surface[5] and in the interstellar medium[6]. These photochemical reactions may also have occurred on the primitive Earth[7].

Ammonium cyanate has been identified as the major product of the photolysis of gaseous NH_3–CO mixtures at wavelengths of 206.2 and 184.9 nm. This compound was probably not detected by previous workers because it sublimes readily at room temperature and atmospheric pressure (see refs 8-10). Urea and biurea were isolated in yields that were approximately 10% of the yield of ammonium cyanate and formamide, and the yields of semicarbazide, cyanide and biuret were about 10% of the yield of urea and biurea.

Water-soluble ^{14}C–organic compounds are formed on siliceous substrata which are irradiated in the presence of ^{14}CO, NH_3 and either N_2 or $^{12}CO_2$ diluent gas. For example, 119 nmol of ^{14}C–organics were produced when 0.5 g of vycor glass particles containing 0.28 mmol of adsorbed NH_3 were irradiated for 12 d in the presence of 250 mmol of CO, using an ultraviolet source which had spectral quality and intensity of the sunlight which reaches Mars. This synthesis occurs using an ultraviolet source with a wavelength of approximately 210 nm or greater than 250 nm. Little reaction was observed with dark irradiated samples or when the light source contained only wavelengths longer than 280 nm. Silica gel and volcanic ash shale were also used as substrata for the synthesis. Higher yields were obtained when the irradiations were carried out at −110° C. Substrata which were predried at 700° C or 400° C *in vacuo* were more effective than those which were predried at 150° C even though more NH_3 was absorbed by the latter. One factor responsible for these changes may be the level of water adsorbed on the substrata. Addition of water vapour to the gas mixture inhibits, but does not completely prevent, the formation of organic compounds.

Thin layer chromatography (TLC) and autoradiography provided tentative identification of ^{14}C–urea and showed it to be a major product. The identification was substantiated by eluting the suspected ^{14}C–urea spot from the TLC plates, and demonstrating that it was digested by urease. ^{14}C–urea was also detected on substrata which had been predried at 150° C, but TLC and hydrolysis indicated that formamide was the major product. ^{14}C–formaldehyde has been tentatively identified as a minor product on substrata which had been predried at 700° C or 400° C by the dimedon derivative procedure (ref. 11, and J. P. Ferris *et al.* not yet published). Using hot water extraction to remove the products from the substrata would have destroyed any ammonium cyanate.

In the experiments which used light with a wavelength of more than 250 nm the reactions were obviously catalysed by wavelengths longer than those absorbed by the gaseous reactants. The absorption coefficient for NH_3 is less than 10^{-4} cm^{-1} at 240 nm[11] whereas the longest wavelength at which CO shows any ultraviolet absorption is 206 nm (ref. 12). Thus the reactions are initiated by excitation and/or photolysis of the gas(es) which are adsorbed on the surface, by the longer ultraviolet wavelengths.

The findings discussed here indicate an additional possible mechanism of nitrogen conservation. If NH_3 is outgassed from the Martian crust it would be photolysed and would react with CO, yielding cyanic acid. The cyanic acid could well precipitate as ammonium cyanate which in turn would be converted to urea.

Surface reactions involving CO and NH_3 would also be compatible with conditions existing on Mars. The dust storms known to occur on Mars would expose large amounts of the surface to sunlight. Such dust storms could also effectively bury the particles, thus protecting the organic products from photodestruction.

If NH_3 has been outgassed during the recent history of Mars, it seems highly probable that the molecular analysis experiment on the 1976 Viking mission[13] will detect nitrogenous compounds, such as urea, in the Martian soil. If the Viking mission obtains evidence suggesting that life exists on Mars, a test for urease[14] may be useful in characterising metabolic processes in later Mars landings.

Both NH_3 and CO occur in interstellar space. CO is the most abundant compound detected in the Sagittarius and Orion nebulae[6]. Cyanic acid and formamide have also been identified in these nebulae[7] and it is therefore possible that these compounds were produced photochemically from CO and NH_3. The adsorption of these molecules on the surfaces of particles would increase the probability of reaction even though their concentrations in interstellar space are extremely low. Furthermore, the reactions could make use of the more abundant ultraviolet light at wavelengths of up to 250 nm. Our observation of an increased synthetic rate at low temperatures are also compatible with interstellar conditions. Recent experiments have suggested that the irradiation of silica gel in the presence of CO yields a silica ester of formic acid which may undergo further photochemical reactions with NH_3 to produce urea and formamide. It has also been demonstrated that ultraviolet light with long wavelengths (>250 nm) causes the formation of CO_2H and CO_2^- radicals from CO and silica gel[15]. The surface radicals produced were unusually stable at low temperatures. Thus the formation of formate ester and/or radicals on interstellar grains could provide stable conditions for subsequent reactions with NH_3.

There is some disagreement concerning the composition of the atmosphere of the early Earth. A highly reduced CH_4, NH_3 and H_2O atmosphere is preferred by some whereas others believe that a more oxidised CO_2, N_2 and H_2O atmosphere (with some more highly reduced compounds) is more likely[7]. In our view any ammonia in the primitive atmosphere would have been rapidly photolysed to N_2 and H_2. In the presence of CO formed by outgassing or by the photolysis of CO_2, the NH_3 could, however, have been efficiently converted to cyanate. Some of this cyanate would be hydrolysed back to CO_2 and NH_3, and some would be thermally converted to urea[16]. Some of it, however, may also have effected the formation of pyrimidines[17] and high energy phosphate bonds[18]. If these reactions were significant they probably occurred early in the history of the Earth. The rapid rate of the photolysis of ammonia limits the time period in which it would have been present in significant amounts on the early Earth[4]. The surface-dependent syntheses described here would be of questionable importance on the primitive Earth because of their susceptibility to inhibition by water. The reaction yielding formate, formaldehyde and other products[5,11] seems more likely to have occurred on the terrestrial surface if CO was indeed present.

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[1] McElroy, M. B., *Science*, **175**, 443 (1972).
[2] Brinkmann, R. T., *Science*, **174**, 944 (1971).
[3] Mitz, M. A., *Origin of Life* (formerly *Space Life Sciences*) (in the press).
[4] Ferris, J. P., and Nicodem, D. E., *Nature*, **238**, 268 (1972).
[5] Hubbard, J. S., Hardy, J. P., and Horowitz, N. H., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 574 (1971).
[6] Sagan, C., *Nature*, **238**, 77 (1972).
[7] Lemmon, R. M., *Chem. Rev.*, **78**, 95 (1970).
[8] Berthelot, D., and Gaudechon, H., *C.r. hebd. Séanc. Acad. Sci. Paris*, **155**, 207 (1912).
[9] Emeleus, H. J., *Trans. Faraday Soc.*, **28**, 89 (1932).
[10] Ya Dodonova, N., *Vestn. leningr. gos. Univ.*, **17**, No. 16, *Ser. Fiz. Khim.*, No. 3, 144 (1962); *Chem. Abstr.*, **58**, 5511f (1963).
[11] Hubbard, J. S., Hardy, J. P., Voecks, G. E., and Golub, E. E., *J. mol. Evol.*, **2**, 149 (1973).
[12] Thompson, B.A., Harteck, P., and Reeves, R. R., *J. geophys. Res.*, **68**, 6431 (1963).
[13] Anderson, D. M., Biemann, K., Orgel, L. E., Oro, J., Owen, T., Shulman, G. P., Toulmin, P., and Urey, H. C., *Icarus*, **16**, 111 (1972).
[14] MacLaren, A. D., in *Biology and the Exploration of Mars* (edit. by Pittendrigh, C. S., Vishniac, W., and Pearman, J. P. T.), (National Academy of Science, Washington DC, 1966), 147-63.
[15] Tseng, S. S., and Chang, S., *Origin of Life* (formerly *Space Life Sci.*) (in the press).
[16] Frost, A. A., and Pearson, R. G., *Kinetics and Mechanism*, 307-316 (John Wiley, New York, 1961).
[17] Ferris, J. P., Sanchez, R. A., and Orgel, L. E., *J. molec. Biol.*, **33**, 693 (1968).
[18] Miller, S. L., and Parris, M., *Nature*, **204**, 1248 (1964).

Crystalline (xanthotoxin)$_2$.KI$_3$

STUDIES of the crystal structure of many organic and inorganic tri-iodides have shown that they are salts[1]. What happens when a tri-iodide salt forms a complex with a neutral, but polar, organic molecule? Examples, which are formed by related organic molecules, are (coumarin)$_4$.KI$_3$ (ref. 2) and (xanthotoxin)$_2$.KI$_3$ (ref. 3). (Xanthotoxin is 8-methoxy-3′,2′:6,7-furocoumarin). The crystals of the first of these substances show one-dimensional disorder[4] and its structure is not known; we have now determined the crystal structure of (xanthotoxin)$_2$.KI$_3$, which is fully ordered, and find that there are potassium cations, tri-iodide anions and neutral xanthotoxin molecules in the crystal. The structure were obtained: triclinic, $a = 9.59(1)$ Å; $b = 11.09(1)$ Å; $c = 7.96(1)$ Å; $\alpha = 118.1(1)°$; $\beta = 86.7(1)°$; $\gamma = 114.5(1)°$; space group $P\bar{1}$ (according to the structure analysis); $D_m = 2.11$ g cm^{-3}, $D_c = 2.11$ g cm^{-3} for 1 formula unit per cell.

Intensities of about 1,900 reflections were measured using a Stoe semi-automatic Weissenberg diffractometer (Mo Kα, graphite monochromator). Appropriate corrections were made for geometrical and absorption factors. The structure was solved by Patterson and Fourier methods and refined by least squares, using anisotropic temperature factors for all non-hydrogen atoms. The present R factor is 8.2%.

The crystal structure (Fig. 1)[7] can be conveniently described in terms of extended two-dimensional slices, one xanthotoxin molecule thick, which are bound by $(2\bar{2}0)$ and $(\bar{2}20)$ planes (Fig. 2). The bonding between these slices is by dispersion forces and is appreciably weaker than that within the slices, which results from ionic and dipolar interactions. Each slice is composed of stacks of xanthotoxin molecules, interleaved by columns of alternating cations and anions. The axes of both stacks and columns extend in the [001] direction. Within the xanthotoxin stacks the successive molecules are antiparallel, with an interplanar distance of 3.44 Å. In crystalline xanthotoxin[8] the stacks contain parallel molecules, with an interplanar distance of 3.58 Å. The shortening in the present compound is presumably because of dipole–dipole interaction between antiparallel molecules, in addition to the normal dispersion forces. There is also an appreciable cation–anion interaction in the [001] direction; each K^+ ion lies at the centre of a parallelogram formed by central and outer iodine atoms of tri-iodide ions above and below it along [001]. The distance of K^+ to the outer iodine is 3.53 Å, equal to the shortest K^+---I^- distance in crystalline potassium iodide[9], and to the central iodine 3.98 Å (the distance to the second outer iodine is 6.04 Å). The main interaction between stacks and columns is an ion–dipole interaction between K^+ ions and the methoxy and lactonic oxygens of the xanthotoxin molecules, with K^+--O distances of 2.75 and 2.88 Å respectively. These distances are similar to the K^+--O distances of 2.76 and 2.80Å found in potassium hydrogen bis (homophthalate)[10]. On the other hand the C--I distances between iodines of the I_3^- ion and the non-polar part of the xanthotoxin molecule, lie in the range 4.10–4.24 Å, showing that these are normal C–H---I contacts.

The dimensions of the xanthotoxin molecule are similar to those found in crystalline xanthotoxin[8], but the carbon atom of the methoxyl group is less rotated out of the mean plane

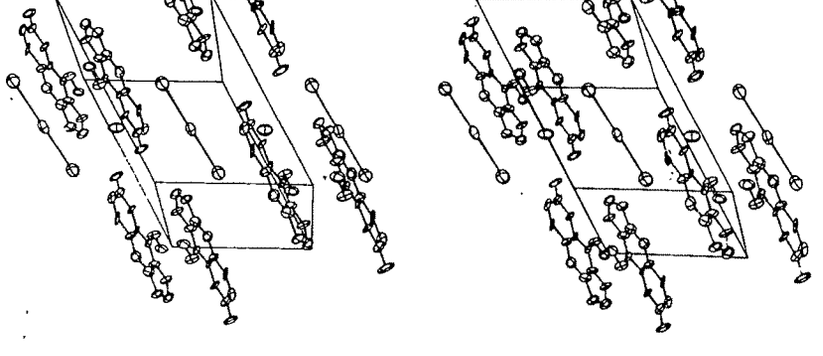


Fig. 1 An ORTEP[7] stereoview of the unit cell. The origin of the unit cell is at the centre of the parallelepiped. The stacks of xanthotoxin molecules run along the eight edges of the parallelepiped; a pair of xanthotoxin molecules in each of these stacks has been omitted to avoid complicating the diagram. The K^+ and I_3^- ions are both centred at centres of symmetry and the pairs of xanthotoxin molecules shown are related by centres of symmetry. Hydrogen atoms have been omitted.

is thus similar to those of other neutral molecule–salt complexes, such as (succinimide)$_2$.tetraethylammonium bromide[5]. Presumably (5,5-diethylbarbituric acid)$_2$.KI$_3$ has a similar structure but only a very brief report has appeared[6].

Strongly dichroic reddish-brown rhombs, somewhat elongated along [001] and showing (100) and (010) faces, were grown from ethanol. The following crystallographic results

of the furocoumarin moiety than in crystalline xanthotoxin (deviations from the mean plane are 0.27 and 0.94 Å respectively). The tri-iodide ion is centrosymmetric, with $d(I–I)=2.939(2)$ Å, a value similar to those found in other structures containing noninteracting I_3^- ions[1].

Thus (xanthotoxin)$_2$.KI$_3$ is a neutral molecule—salt complex, with the individual structural elements clearly discernible

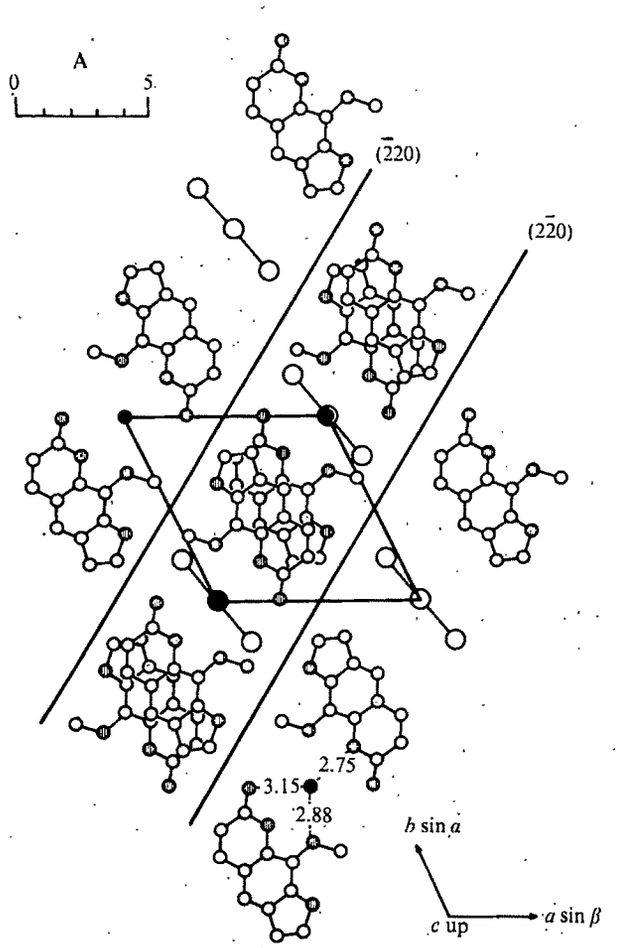


Fig. 2 The crystal structure seen in projection down [001]. An infinite two-dimensional slice, bounded by (22̄0) and (2̄20) planes and extending normal to the page, is shown. The shorter interatomic distances mentioned in the text have been included in the figure. Hydrogen atoms have been omitted. The slices on the edges of the figure each contain only a single layer of structural units so as not to complicate the diagram. Large open circles, iodine atoms; small open circles, carbon atoms; solid circles, potassium atoms; hatched circles, oxygen atoms.

the crystal. The mutual arrangement of these is such as to provide both local neutralisation of charge, the importance of which has been emphasised by Powell and Wait[5], and an antiferroelectric interaction in the stacks of dipolar xanthotoxin molecules.

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1 Runsink, J., Swen-Walstra, S., and Migchelsen, T., *Acta crystallogr.*, **B28**, 1331–1335 (1972).
2 Clover, A. M., *J. Am. chem. Soc.*, **42**, 1248–1260 (1920).
3 Starkowsky, N. A., *Egypt. J. Chem.*, **2**, 111–117 (1959).
4 Cramer, F., and Windel, H., *Chem. Ber.*, **89**, 354–365 (1956).
5 Powell, H. M., and Wait, E., *J. chem. Soc.*, 1866–1872 (1958).
6 Robbins, A. H., and Craven, B. M., *Abstr. Am. crystallogr. Ass. Winter Meeting, Gainsville*, 87 (1973).
7 Johnson, C. K., *ORTEP. Report ORNL-3794* (Oak Ridge National Laboratory, Oak Ridge, Tennessee, 1965).
8 Stemple, N. R., and Watson, W. H., *Acta crystallogr.*, **B28**, 2485–2489 (1972).
9 Hambling, P. G., *Acta crystallogr.*, **6**, 98 (1953).
10 Gupta, M. P., and Dubey, D. S., *Acta crystallogr.*, **B28**, 2677–2681 (1972).

New vapour deposition technique

THERE are various techniques of vapour deposition for putting down thin films, or thicker coatings and deposits of one material on another[1,2], which are of interest to a growing number of industries. The techniques range from chemical vapour deposition at relatively high gas pressure and temperature to physical vapour deposition based on thermal evaporation in a high vacuum for deposition on to hot or cold substrates. In recent years interest has grown in several intermediate techniques in soft vacuum (10^{-3} to 1 torr) which may be physical and/or chemical in nature[3-5]. Gas discharges are sometimes used to influence both the physical and chemical aspects of the process—to generate vapour by sputtering, to cause chemical reactions among vapour species, or to encourage bonding to the substrate. Here I outline the basic features of a new 'intermediate' technique which depends simply on evaporation into a heated gas. The aim is to cause the vapour species to diffuse to the substrate while avoiding conditions favouring homogeneous nucleation in the gas phase. Chemical reactions may or may not occur depending on the choice of gas and gas discharges can be employed for substrate cleaning, or other purposes if required.

Homogeneous nucleation within an ambient gas occurs readily at high concentrations of a vapour species and indeed has long been exploited for the manufacture of powders[1] (such as carbon black and pigments). It can readily occur in soft vacuum[6] and particularly fine powders can be made this way[7]. But its occurrence under vapour deposition conditions is

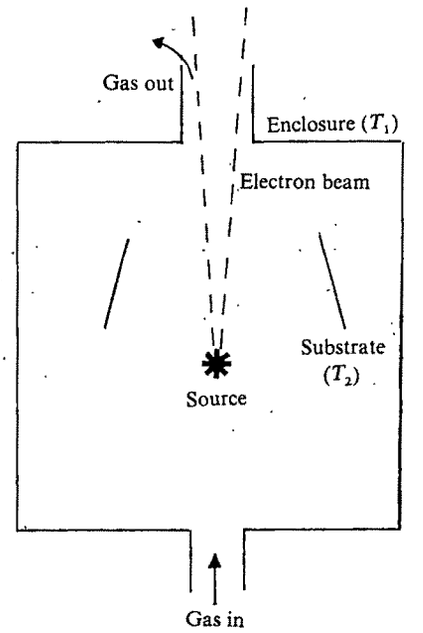


Fig. 1 Schematic diagram of apparatus.

usually undesirable since a poorly bonded granular deposit results. There have been several attempts to construct accurate theoretical models for homogeneous nucleation[8,9], though as yet no entirely satisfactory experimental verifications have been made. At least one investigator[10] thinks that the theoretical problem is beyond precise solution at present. But inspection of the theory reveals that the rate of formation of stable clusters should increase with vapour concentration, but decrease as the ambient temperature increases owing to an increase in the critical size for stable nuclei. Lydtin[11] showed how to raise the soot limit and consequent growth rate of pyrolytically deposited graphite by mechanical means for reducing the vapour concentration.

The vapour concentration C at any specified coordinates in a given geometry of source and sinks is, from the diffusion equation, proportional to the source strength S and inversely proportional to the diffusion coefficient D (which in turn is

proportional to the product of the average molecular speed c and the mean free path λ). Thus

$$C \alpha S/D \alpha S/(c\lambda) \alpha S/T^{3/2}$$

because of the dependence on temperature (T) of c and λ. Raising the gas temperature will reduce the vapour concentration and increase the size of stable nuclei. So it should be possible to find a range of operating conditions of gas temperature, pressure and rate of evaporation which avoids homogeneous nucleation and precipitates material in molecular form onto the substrates (light gases are favoured by virtue of a larger mean free path).

An experimental investigation of a technique based on these principles was made with apparatus the essential features of which are illustrated in Fig. 1. The heated enclosure was mounted within a soft vacuum chamber (10^{-2} to 1 torr). Electron beams generated by a glow discharge gun[12] were conveniently used for heating purposes. Dense deposits of ceramics (silica, alumina, magnesia, spinel, silicon nitride, titanium carbide and carbon), metals (aluminium, titanium, chromium and molybdenum) and a semiconductor (silicon) have been put down on a variety of substrates including glasses, ceramics, metals and, again, silicon. Several gases were investigated including hydrogen, helium, nitrogen, oxygen and argon and rates of deposition were typically in the range 10^{-2} to 1 mm h^{1}. At enclosure temperatures T_1 in the range 300° C to 600° C homogeneous nucleation was insignificant at gas pressures of $400/d$ mtorr, where d(cm) is the dimension of the enclosure (and therefore equivalent to several tens of mean free paths in a heavy gas such as argon). No investigation of the range of appropriate gas pressures has yet been made, except in the case of hydrogen, for which satisfactory deposits were still obtained at $4000/d$ mtorr and this is probably not an upper limit.

The structures of the deposits obtained were essentially similar to those well-known in chemical vapour deposition[1]. Depending on the substrate temperature T_2 and the properties of the source material, structures from amorphous through polycrystalline to single crystal were obtained. The bond to the substrate was dependent on chemistry and could sometimes be influenced by the chemical nature of the gas. The texture of deposits was dependent on the smoothness and cleanliness of the substrate surface. Stresses of well known character were found in the deposits, though in a general way the higher the substrate temperature T_2 the lower the grown-in tensile stress.

The advantages of the technique are several. Only roughing pumps are needed, at least for small-scale operations. The transport by diffusion results in good 'throwing power'[13]. The heating of the enclosure degasses the contents and thereafter purity is maintained by the flowing gas which, because of a relatively long and narrow exit orifice, prevents back diffusion of contaminants from outside. On the other hand the gas readily permits an electron beam (of 15–30 keV) to enter though any suitable method for vapourising the source, including sputtering, can be used. Since many of the parameters can be adjusted independently a high degree of process control is possible. The technique is readily adaptable to the deposition of alloys, complex compounds and multilayers and is compatible with other beam techniques for processing materials (such as welding, machining and ion implantation). In a general way it offers a versatile and reproducible method for fabricating special materials.

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[1] Powell, C. F., Oxley, J. H., and Blocher, J. M., *Vapour Deposition* (Wiley, New York, 1966).
[2] Chopra, K. L., *Thin Film Phenomena* (McGraw Hill, New York, 1969).
[3] *Sputtering and Ion Plating* (edit. by Lundin, B. T.), *Proc. Conf. on, Lewis Research Centre*, 1972 (NASA SP-5111).
[4] Kurtz, R. G., *Vapor-Ion Methods of Film Deposition* (Dow Chemical Co. 1970).
[5] Keller, K., *Härterei-tech. Mitt.*, **26**, 120 (1971).
[6] Reifenschweiler, O., *Rev. Sci. Inst.*, **35**, 456 (1964).
[7] Avery, R. G., and Ramsay, J. D. F., *J. Colloid Interface Sci.*, **42**, 597 (1973).
[8] Lothe, J., snd Pound, G. M. in *Nucleation* (edit. by Zettlemayer, A. C.) p.118 (Dekker, New York, 1969).
[9] McGinty, D. J., *J. chem. Phys.*, **55**, 580 (1971).
[10] Sutugun, A. G., *Phys. Lett.*, **29A**, 233 (1969).
[11] Lydtin, H., *Proc. 2nd Intern. Conf. on Chemical Vapour Deposition* p.71 (The Electrochemical Society, 1970).
[12] Dugdale, R. A., *Nature*, **220**, 1179 (1968) and *Glow Discharge Material Processing* (Mills and Boon, ME/5, 1971).
[13] Burns, R. M., and Bradley, W. W., *Protective Coatings for Metals*, third ed. (Reinhold, 1967).

dsDNA made by RNase-sensitive DNA polymerase from RSV-transformed cells

THE hypothesis that the RNA-directed DNA polymerase (reverse transcriptase) may function in normal cells[1] as part of the machinery for gene amplification is being investigated[2–8]. Cytoplasmic fractions of normal[6,7,9,10], neoplastic[11–14] and virus-transformed cells[3,15], contain RNase-sensitive DNA polymerase activities. Most studies revealed that cellular DNA polymerases differed greatly from the oncorna virus RNA-directed DNA polymerase[7,9] but the nature of the endogenous template of the cellular DNA polymerases was not described. In view of these findings and reports on the participation of RNA in DNA synthesis by DNA polymerases in bacteria[16–20], and mammalian[21–23] cell systems, we investigated the nature of the template and the role of RNA in the synthesis of DNA by the cytoplasmic RNase-sensitive DNA polymerase activity found in rat cells transformed by the B77 strain of Rous sarcoma virus (R(B77) cells). We found that such preparations contained DNA molecules which served as the template for the RNase-sensitive DNA polymerase activity. We also found that RNA has a function in the synthesis of DNA since nascent DNA molecules, covalently linked to RNA, were synthesised at the very beginning of the reaction.

R(B77) cells, a cell line of rat embryo fibroblasts transformed *in vitro* by the B77 strain of Rous sarcoma virus (RSV), were used[24,25]. The cells were maintained in culture by weekly transfers. The cells were seeded at a concentration of 5×10^5 cells per plastic Petri dish (diameter 90 mm; Nunc, Denmark) in 10 ml Eagle's medium containing 1 or 2 μCi ml^{-1} of 5-^{3}H-uridine (13.8 Ci mmol^{-1}; Nuclear Research Center, Negev, Israel) or 0.1—0.2 μCi ml^{-1} of 2-^{14}C-uridine (62 mCi mmol^{-1}; Radiochemical Centre, Amersham, England). The cultures were labelled for 24 h, unless otherwise stated. To label cellular DNA, the medium of R(B77) cells was replaced with fresh Eagle's medium containing 1 μCi ml^{-1} of methyl-^{3}H-thymidine (8.2 mCi mmol^{-1}: Nuclear Research Center, Negev). BUdR was incorporated into cellular DNA by adding 30 μg ml^{-1} of BUdR (Sigma) to the medium of R(B77) cells. The cells were labelled with ^{3}H-deoxycytidine (1 μCi mmol^{-1}; specific activity, 19.4 Ci mmol^{-1} Radiochemical Centre) and were collected after 48 h.

High speed pellets (HSP) of cells were prepared as described by Coffin and Temin[3]. The standard assay for the avian sarcoma RNA-dependent DNA polymerase was according to Temin and Mizutani[26], as described previously for purified B77 virions[15,27]. The DNA polymerase activity in HSP was studied under the same conditions as for the avian sarcoma virus enzyme.

High speed pellet (HSP) preparations from R(B77) cells contained a DNA polymerase activity which was markedly inhibited by RNase treatment before and after initiation of DNA synthesis (Table 1). This indicates that the synthe-

sis of DNA by the HSP DNA polymerase activity is RNA dependent. To rule out the possibility that HSP preparations contain RNase and DNase activities, ^{3}H-uridine and ^{3}H-thymidine labelled HSP preparations were incubated with or without exogenous nuclease. It was found that the labelled nucleic acids were not degraded after incubation for 60 min at 37° C (not shown).

Table 1 Effect of RNase on DNA synthesis by HSP DNA polymerase activity

Treatment of enzymatic reaction with RNase*	Time (min) 5	15	30
	^{3}H-TMP incorporated (c.p.m. per 25 μl)		
Untreated	1,310	2,050	2,215
Pretreated for 10 min	160	175	215
Treated at 0 time	390	500	660
Treated at 5 min	1,320	1,420	1,590

* The HSP preparation was divided in four portions:one was left untreated, the second was preincubated with RNase A (50 μg ml^{-1}) at 37° C, to the third RNase was added immediately before the initiation of the reaction, and to the fourth, RNase was added 5 min after initiation of DNA synthesis *in vitro*. The standard assay of Temin and Mizutani[26] was used. Duplicate samples were removed and the TCA-precipitable radioactivity was determined.

The polymerase activity was also characterised by studying the effect of addition of synthetic polynucleotides (Table 2), which can help to distinguish between cellular DNA polymerase and the RNA-directed DNA polymerase of oncorna viruses[28]. Addition of poly(rA)·oligo(dT) to HSP preparations only slightly stimulated incorporation of ^{3}H-TMP into DNA whereas it markedly stimulated the RNA-directed DNA polymerase of RSV and poly(dA)·poly(dT) stimulated ^{3}H-TMP incorporation by both enzymatic activities to the same level. In this respect, the HSP RNase-sensitive DNA polymerase resembles other DNA polymerases present in eukaryotic cells[7,8].

Analysis by zonal centrifugation of the DNA molecules synthesised *in vitro* by the HSP RNase-sensitive DNA polymerase showed that the product banded in the sucrose gradient (15–65% (w/v) for 3 h at 45,000 r.p.m. using rotor SW50.1 of the Beckman ultracentrifuge) with a sedimentation coefficient of about 10S. Analysis on a sucrose gradient of the HSP DNA synthesised *in vivo* showed DNA molecules sedimenting to the bottom of the sucrose gradient and two bands of labelled DNA, of which the major peak had a sedimentation constant of about 10S (not shown).

The DNA synthesised by HSP DNA polymerase activity during 1 min and 20 min incubation was analysed by chromatography on hydroxylapatite columns (Fig. 1*a*). The DNA synthesised *in vitro* was eluted from the hydroxylapatite columns by 0.25 M phosphate. Labelled DNA prepared from R(B77) cells had the same elution profile as the DNA synthesised *in vitro*. Cellular DNA was eluted before the ^{14}C-labelled HSV DNA (0.27 M phosphate) at the position of nuclear DNA. This demonstrated that the DNA molecules synthesised *in vitro* are double-stranded and resemble nuclear DNA in their elution properties. There was no difference between the DNA molecules synthesised during a short (1 min) or a long (20 min) period.

To study the effect of actinomycin D on DNA synthesis by the HSP DNA polymerase, actinomycin D (250 μg ml^{-1}) was added to the standard reaction mixture which was then incubated for 20 min at 37° C. After extraction the DNA product was compared with DNA synthesised by HSP in the absence of inhibitor. Actinomycin D inhibited DNA synthesis by 80% compared with the untreated control. The DNA product synthesised by the HSP treated with actinomycin D was analysed by chromatography on a hydroxylapatite column. It was found (Fig. 1*b*) that this DNA was eluted by 0.25 M phosphate, similarly to the cellular double-stranded DNA (dsDNA) molecules. The HSP DNA polymerase activity differs from Rous sarcoma virus reverse transcriptase, in respect of the effect of actinomycin D (Fig. 1*c*). In the presence of actinomycin D, the viral enzymatic activity synthesises single-stranded DNA (ssDNA) molecules which are eluted from hydroxylapatite by 0.1 M phosphate.

High speed pellets were prepared from R(B77) cells, labelled or unlabelled for 48 h with ^{3}H-deoxycytidine and incubated in the presence or absence of BUdR. The DNA was extracted with phenol and banded in CsCl gradients (Fig. 2*a*). Untreated DNA banded at a density of 1.70 g ml^{-3} whereas the BUdR-containing DNA banded at the density of 1.74 g cm^{-3} (Fig. 2*d*). Samples from two unlabelled HSP preparations (prepared from cells grown in the absence or presence of BUdR) were incubated *in vitro* for 60 s under conditions of DNA synthesis with the four deoxyribonucleoside triphosphates (including ^{3}H-TTP). The DNA was extracted and banded in CsCl gradients. The DNA synthesised *in vitro* in the HSP from untreated cells banded at a density of 1.70 g cm^{-3} (Fig. 2*b*), the density of cellular DNA. The radioactive DNA synthesised *in vitro* in the presence of BUdR-containing DNA (HSP from BUdR-treated cells) partly banded at the density of the BUdR-labelled DNA and part was shifted to a smaller density (Fig. 2*e*). Longer incubation of the BUdR-containing DNA from HSP (5 min) resulted in a further shift to a lower density (Fig. 2*f*), similar to untreated DNA (Fig. 2*c*). These results demonstrate that the DNA polymerase activity present in the HSP utilised the DNA molecules present in the HSP as the template. The complete inhibition of this reaction by RNase suggests the involvement of RNA as a primer or an intermediate during DNA synthesis by the HSP DNA polymerase.

High speed pellet preparations were incubated under conditions of DNA synthesis and the processes were stopped at 1 min and 5 min after the initiation of the enzymatic reaction. The ^{3}H-TMP labelled DNA synthesised *in vitro* was extracted with phenol, precipitated by ethanol, resuspended in Tris-EDTA buffer, and divided into three: one portion was left as native DNA, the second was heat denatured, and the third was denatured by alkali, and each sample was banded in a Cs_2SO_4 gradient. Most of the native DNA product banded at the density of dsDNA (1.42 g cm^{-3}) but some radioactive DNA appeared at higher densities (1.58–1.69 g cm^{-3}) which are those of DNA molecules complexed or associated with RNA. The amount of DNA in this region, slightly increased after heat denaturation, but the radioactivity disappeared after treatment with alkali. Similar results were found with the DNA synthesised during a reaction period of 5 min. We then investigated the nature of the DNA molecules synthesised during the shorter reaction times, 7, 15, and 30 s (Fig. 3). Analysis of the 7-s DNA product (Fig 3*a*) revealed DNA molecules banding throughout the gradient, mostly at the densities of dsDNA (1.42 g cm^{-3}) and RNA (1.69 g cm^{-3}). The DNA molecules which initially banded at the density of RNA (1.69 g cm^{-3}), most probably DNA:RNA hybrid molecules, were not detected in Cs_2SO_4 after heat denaturation or alkali treatment ((Fig. 3*b* and *c*). Analysis of the DNA molecules synthesised during 15 or 30 s revealed that the total amount of native heavy DNA (which banded at 1.69 g cm^{-3}) was greatly decreased (Fig. 3*d* and *g*). After heat denaturation, however, the amount of radioactive DNA which appeared in the Cs_2SO_4 gradient between the DNA and RNA, most probably DNA–RNA complexes (1.58–1.69 g cm^{-3}) also increased (Fig. 3*e*). The same findings were obtained with the DNA product of a 30-s reaction (Fig. 3*g* and 3*h*). These reaction products disappeared on treatment with alkali after which the labelled molecules accumulated in the position of ssDNA (Fig. 3*f* and *i*). In all nine experiments carried out there was a significant increase of radioactivity in the region of RNA–DNA complexes after heat denaturation which disappeared after alkali treatment. This

indicates that part of the newly synthesised DNA molecules may be linked to RNA molecules. After a short incubation (7 s) the DNA molecules may have been synthesised on RNA molecules, which serve as templates or initiators. The labelled native DNA molecules, band at the density of dsDNA (1.42 g cm^{-3}) although some of it might still be covalently bonded to RNA and can be detected after heat denaturation.

The presence of an RNase-sensitive DNA polymerase activity in normal and transformed cells, which differ from the oncorna virus RNA-directed DNA polymerase, has recently been reported[3,6,7,9,10,15]. Our study confirms the presence of an RNase-sensitive DNA polymerase activity in R(B77) cells. The novel finding of this study is the demonstration that the HSP of R(B77) cells contains 10S DNA molecules of cellular origin which act as the template for this enzymatic activity and that RNA molecules also participate in the synthesis of DNA.

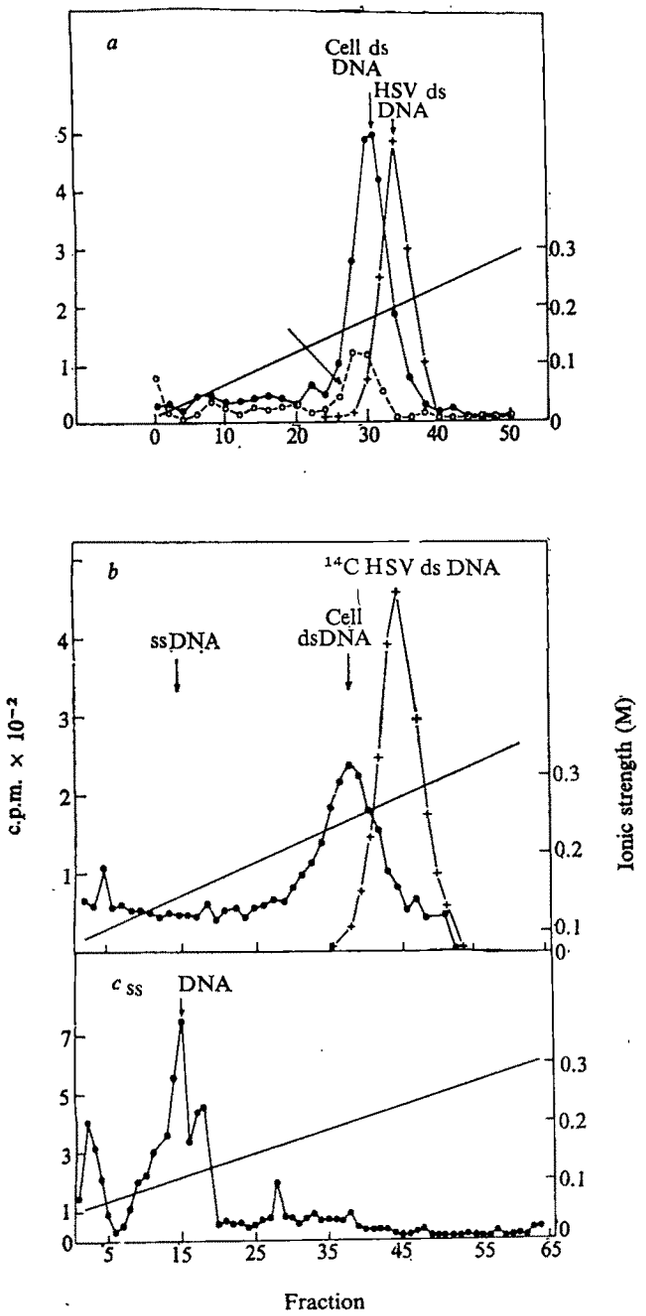


Fig. 1 Hydroxylapatite chromatography of DNA molecules synthesised by HSP DNA polymerase and Rous sarcoma virus reverse transcriptase in the absence and presence of actinomycin D. *a*, HSP was incubated with the standard reaction mixture and samples were removed at 1 and 20 min, treated with 1% SDS, the DNA was extracted with phenol and characterised by chromatography on hydroxylapatite columns (Bio-gel HTP; Bio-Rad Laboratory, Richmond, California), eluted with a linear gradient ranging from 0.05 M to 0.35 M of sodium phosphate (total volume 100 ml) and collected in about 40 fractions. TCA precipitable radioactivity in each fraction was collected on filters and counted in a liquid scintillation counter. Purified herpes simplex virus (HSV) DNA, labelled with ^{14}C-thymidine as well as BSC_1 DNA and HeLa DNA were used as markers. The results of three columns are combined in the graph. O, DNA molecules synthesised by a HSP preparation during 1 min; ●, DNA molecules synthesised by a HSP preparation during 20 min; +, ^{14}C-HSV DNA marker. The position of cell DNA is indicated by the arrow. *b*, An HSP preparation was incubated with actinomycin D (250 μg ml^{-1}) and the ^{3}H-TMP labelled DNA was phenol extracted and chromatographed on a hydroxylapatite column. ^{14}C-HSV DNA and cellular dsDNA served as markers. ●, ^{3}H-DNA synthesised by HSP enzymatic activity in presence of actinomycin D; +, ^{14}C-HSV DNA marker. Cell DNA eluted identically to HSP DNA. *c*, Viral reverse transcriptase activity. Purified B77 virions were disrupted with Nonidet P-40 and incubated *in vitro* under conditions of DNA synthesis by the viral reverse transcriptase, actinomycin D (250 μg ml^{-1}) was added and the ^{3}H-DNA molecules synthesised during 20 min were extracted by phenol and analysed by chromatography on a hydroxylapatite column [27,30] (●).

Table 2 Effect of poly(A)·oligo(dT) and poly(dA)·poly(dT) on DNA synthesis by the RNase-sensitive DNA polymerase activity from transformed R(B77) cells and the B77 Sarcoma virus reverse transcriptase activity

Pretreatment† of HSP	Polymer added	^{3}H-TMP incorporated (c.p.m.)* R(B77) enzyme	Viral‡ enzyme
Exp. 1 Buffer	None	1,887	1,871
RNase	None	365	ND§
Buffer	Poly(A)·oligo(dT)	2,310	126,540
Exp. 2 Buffer	None	1,145	5,551
Buffer	Poly(dA)·poly(dT)	2,193	10,245

* The standard assay for oncorna virus RNA-dependent DNA polymerase and HSP DNA polymerase activity was according to Temin and Mizutani[26]. To each reaction mixture, 0.01 $A_{260\ nm}$ units of poly(dA)·poly(dT) or poly(A)·oligo(dT) (Collaborative Research, Waltham, Massachusetts) were added.

† The enzyme preparations were incubated for 15 min at 37° C with RNase (50 μg ml^{-1}) or buffer before the addition of the standard reaction mixture. ‡ B77 virus treated with NP-40. § Not done.

The DNA polymerase activity present in HSP resembles the oncorna virus-associated reverse transcriptase in its sensitivity to RNase and in its ionic requirements[3,15]. Nevertheless, marked differences were found between the two enzyme activities in agreement with the findings of Bobrow *et al.*[9] and Kang and Temin[7]. We demonstrated that (a) the HSP enzyme was only slightly stimulated by poly(rA)·oligo (dT) which stimulates the viral enzyme, whereas poly(dA)·poly(dT) had the same stimulatory effect on both HSP and the viral enzymes; (b) the main product of the HSP RNase-sensitive DNA polymerase is dsDNA even after a very short reaction; (c) although actinomycin D inhibits both enzymes, only with the viral enzyme is there an accumulation of ssDNA molecules (Fig. 2); and (d) the endogenous template for the cellular RNase-sensitive DNA polymerase is DNA (Fig. 3), whereas for the viral enzyme, it is RNA. These results show that the HSP DNA polymerase activity is an RNA-dependent DNA polymerase, which utilises the cellular dsDNA molecules present in HSP as template.

What is the function of RNA during the synthesis of DNA? Analysis in Cs_2SO_4 density gradients of the DNA

products resulting from very short reactions revealed radioactive DNA banding at the density of RNA (1.69 g cm^{-3}) as well as labelled DNA molecules which banded as dsDNA. Radioactive DNA also banded at densities ranging from that of RNA to DNA (1.69–1.58 g cm^{-3}) suggesting that the DNA molecules are attached to RNA molecules. The amount of label which appeared in these regions, increased after heat denaturation and shifted to the density of ssDNA after alkali treatment, proving that they are DNA molecules linked covalently to RNA chains (Fig. 3) which initially banded together with the dsDNA molecules. It is possible that the RNA–DNA complexes described here resemble the RNA–DNA pieces described

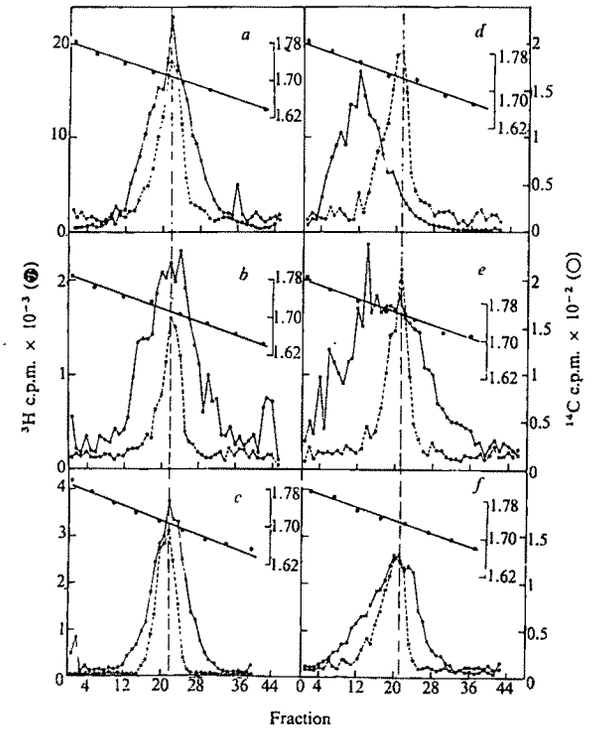


Fig. 2 HSP DNA as template for the endogenous RNase-sensitive DNA polymerase. Twelve Roux bottles containing R(B77) cells were incubated for 48 h in the presence or absence of BUdR (5-bromo-2′-deoxyuridine). One Roux bottle from each group was labelled with 1 μCi ml^{-1} of ^{3}H-deoxycytidine. After incubation, HSP was prepared. Unlabelled BudR-treated and control HSP preparations were incubated for 1 min and 5 min with the reaction mixture, and the reactions were stopped by addition of SDS to final concentrations of 2%, 0.5% Baycovin, and 50 μg of yeast DNA. The nucleic acids from all preparations were extracted according to the Hirt method, modified by Kang and Temin[10]. The alcohol precipitates and marker ^{14}C-thymidine labelled DNA from BSC_1 cells were added to 8 ml of a CsCl solution and the mixtures centrifuged for 60 h at 35,000 r.p.m. in the Beckman 50 Ti rotor at 22° C. The gradients were collected and the radioactive TCA precipitates counted. Densities were determined by weighing 100 μg samples (●). *a*, R(B77) cells were labelled with ^{3}H-deoxycytidine for 24 h after which the DNA was extracted (●). ^{14}C-thymidine labelled R(B77) nuclear DNA was used as a marker (○). The DNA preparations were mixed and analysed by centrifugation in a CsCl gradient. *b* and *c*, HSP preparations were prepared and incubated *in vitro* under conditions of DNA synthesis in the presence of ^{3}H-TMP. The ^{3}H-DNA was extracted with phenol after 1 min (*b*) and 5 min (*c*) reaction periods. The ^{3}H-TMP labelled DNA (●) was mixed with ^{14}C-thymidine labelled BSC_1 dsDNA (○) and centrifuged in CsCl gradients. *d*, R(B77) cells were incubated with BUdR and ^{3}H-deoxycytidine for 24 h. The DNA was extracted (●) and mixed with ^{14}C-thymidine labelled DNA (○) and centrifuged in a CsCl gradient. *e* and *f*, HSP preparations were prepared from R(B77) cells incubated for 24 h with BUdR. The HSP was incubated for 1 min (*e*) and 5 min (*f*) under conditions of DNA synthesis in the presence of ^{3}H-TTP. The DNA was extracted with phenol (●) mixed with ^{14}C-DNA (○) and centrifuged in gradients.

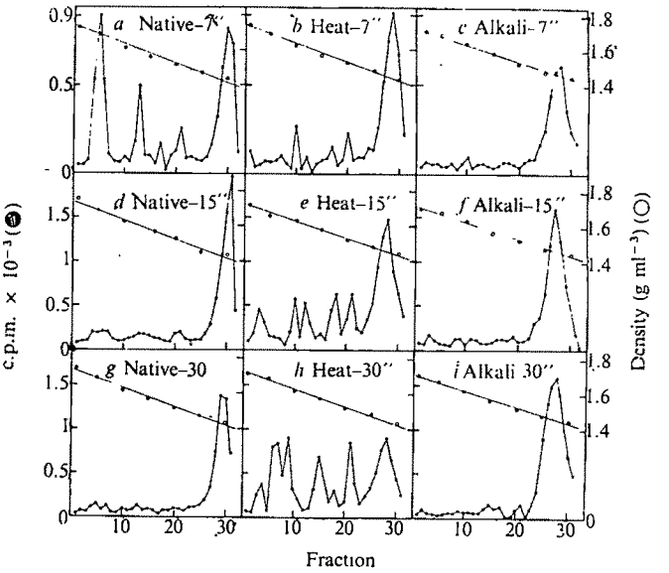


Fig. 3 Charcterisation of the DNA molecules synthesised by HSP DNA polymerase activity. HSP was incubated at 38° C for 7, 15, and 30 s with the standard reaction mixture. The reaction was stopped by adding SDS, and the DNA products were purified as described in the legend for Fig. 2. The DNA samples were resuspended in Tris–EDTA buffer and each DNA preparation was divided into three: one was left untreated (*a*, *d*, and *g*), one sample of each was boiled for 10 min and chilled immediately (*b*, *e*, and *h*), and the third sample was treated with 0.25 M NaOH for 10 min and then neutralised by HCl in ice (*c*, *f*, and *i*). All nine DNA preparations were mixed with Cs_2SO_4 to a final volume of 6 ml and centrifuged at 45,000 r.p.m. for 64 h at 22° C in the 50 Ti rotor of the Beckman centrifuge. The gradients were fractionated dropwise, the densities determined by weighing 100 μl of the indicated samples, and the TCA radioactive precipitates were counted. ●, Radioactive DNA; ○, density.

by Sugino, Hirose, and Okazaki[18]. The participation of RNA in DNA synthesis has also been suggested for mammalian cells[23] and for polyoma virus[29]. The presence of labelled DNA molecules after 7 s of DNA synthesis which band at a density of RNA (1.69 g cm^{-3}) (Fig. 3*a*) and disappear after heat denaturation (Fig. 3*b*) may indicate DNA synthesis on an RNA template, an activity ascribed to oncorna virus reverse transcriptase[7,26]. It is possible that low levels of reverse transcriptase activity may be detected only after a very short reaction period, whereas the major DNA polymerase activity in HSP of R(B77) cells is a DNA polymerase activity which requires RNA for its function, most probably as an initiator and not as a template. The role of this enzyme and its DNA template in the R(B77) transformed cell is still to be studied.

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[1] Temin, H. M., *J. natn. Cancer Inst.*, **46**, III–VII (1971).

2 Brown, R. D., and Tocchini-Valentini, G. P., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1746–1748 (1972).
3 Coffin, J. M., and Temin, H. M., *J. Virol.*, **8**, 630–642 (1971).
4 Crippa, M., and Tocchini-Valentini, G. P., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2769–2773 (1971).
5 Ficq, A., and Brachet, J., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2774–2776 (1971).
6 Kang, C., and Temin, H. M., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1550–1554 (1972).
7 Kang, C. Y., and Temin, H. M., *Nature new Biol.*, **242**, 206–208 (1973).
8 Mahdavi, V., and Crippa, M., *Proc. natn. Acad. Sci U.S.A.*, **69**, 1749–1752 (1972).
9 Bobrow, S. N., Graham Smith, R., Reitz, M. S., and Gallo, R. C., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3228–3232 (1972).
10 Penner, P. E., Cohen, L. H., and Loeb, L. A., *Nature new Biol.*, **232**, 58–60 (1971).
11 Gultai S. C., Axel, R., and Spiegelman, S., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2020–2024 (1972).
12 Sarngadharan, M. G., Sarin, P. S., Reitz, M. S., and Gallo, R. C., *Nature new Biol.*, **240**, 67–72 (1972).
13 Scolnick, E. M., Aaronson, S. A., Todaro, G. J., and Parks, W. P., *Nature*, **229**, 318–321 (1971).
14 Fridlander, B., Fry, M., Bolden, A., and Weissbach, A., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 452–455 (1972).
15 Kotler, M., Weinberg, E., Haspel, O., and Becker, Y., *J. Virol.*, **10**, 439–446 (1972).
16 Keller, W., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1560–1564 (1974).
17 Lark, K. G. *J. molec. Biol.*, **64**, 47–60 (1972).
18 Sugino, A., Hirose, S., and Okazaki, R., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1863–1867 (1972).
19 Sugino, A., and Oazaki, R., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 88–92 (1973).
20 Wickner, W., Brutlag, D., Schekman, R., and Kornberg, A., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 965–969 (1972).
21 Schekman, R., Wickner, W., Westergaard, O., Brutlag, D., Geider, K., Bertsch, L. L., and Kornberg, A., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2691–2695 (1972).
22 Stavrianopoulos, J. G., Karkas, J. D., and Chargaff, E., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1781–1785 (1972).
23 Stavrianopoulos, J. G., Karkas, J. D., and Chargaff, E., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2609–2613 (1972).
24 Altaner, C., and Temin, H. M., *Virology*, **40**, 118–134 (1970).
25 Kotler, M., *J. gen. Virol.*, **12**, 199–206 (1971).
26 Temin, H. M., and Mizutani, S., *Nature*, **226**, 1211–1213 (1970).
27 Kotler, M., and Becker, Y., *J. Israel Med. Assoc.*, **81**, 412–416 (1971).
28 Goodman, M. C., and Spiegelman, S., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2203–2206 (1971).
29 Magnusson, G., Pigiet, V., Winnacker, E. L., Abrams, R., and Reichard, P. *Proc. natn. Acad. Sci. U.S.A.* **70**, 412–415 (1973).
30 McDonnell, J. P., Garapin, A. C., Levinson, W. E., Quintrell, N., Fanshier, L., and Bishop, J. M,. *Nature*, **228**, 433–435 (1970).

RNA-dependent RNA synthesis in rat brain

RIBONUCLEIC acid-dependent RNA polymerase (replicase) was first isolated from *Escherichia coli* infected with RNA bacteriophage Qβ which has specific template requirements[1]. Such an enzyme has been described in tumour cells and several types of mammalian cells infected with RNA viruses[2–5].

It has been reported that in the normal mammalian cell polyribonucleotides are synthesised by a nontranscriptive process and enzymes which polymerise ATP to poly(A) have been found[6–8]. Ribonucleoprotein particles isolated from rat liver nuclei polymerise ATP, UTP, GTP and CTP to the corresponding ribohomopolymers[9]. These reactions have been suggested to be post-transcriptional additions to heterogeneous nuclear RNA. The microsome fraction of rat liver also incorporates the four ribonucleotides into polyribonucleotides a reaction which is stimulated by RNA but not DNA[10]. We report here the isolation of an enzyme fraction from rat brain which can synthesise RNA[12], both as a heteropolymer or a homopolymer, only when provided with a suitable RNA template or primer.

The enzyme fraction was isolated from rat brain (see legend to Table 2) and its RNA polymerase activity was measured by the radioactivity incorporated into acid-insoluble product from ^{3}H-UTP after incubation in various modifications of the standard assay medium (see legend to Tables 1 and 4). The reaction product satisfied the standard tests for RNA (Table 2). The reaction clearly depended on the presence of RNA, the amount of incorporation increasing linearly to a maximum as the concentration was increased (Fig. 1). Magnesium ions were also necessary, the optimum concentration of $MgCl_2$ being 12 mM. Only the four ribonucleoside triphosphates (NTPs) and not the corresponding deoxyribonucleoside triphosphates (dNTPs) could act as substrates. With enzyme from adult rats the omission of any one NTP from the medium markedly reduced incorporation (Table 1*a*) indicating that when they were all present the product was mainly heteropolymeric RNA. By contrast, the fraction from newborn rat readily synthesised RNA when provided with only a single NTP substrate (Table 1*b*): presumably this was

Table 1 Characterisation of the reaction in the presence of four ribonucleoside triphosphates

a Assay mixture (μg)			^{3}H-UMP incorporated (pmol)
Experiment 1	Complete		11.0
	+DNase	10	9.3
	+Actinomycin D	2	8.1
	+RNase (+RNA)	10	1.7
	(−RNA)	10	0.5
	+$(NH_4)_2SO_4$ (0.32 M)		3.4
	+$(NH_4)_2SO_4$ (0.41 M)		1.1
	−RNA		2.1
Experiment 2	Complete		8.3
	−ATP		3.8
	−GTP		4.4
	−CTP		5.5
	−$MgCl_2$		0.5
	−RNA		2.0
b			^{3}H-NMP incorporated (pmol)
Experiment 1	Complete (^{3}H-UTP)		23.9
	+DNase	10	22.7
	+Actinomycin D	2	23.6
	+RNase (+RNA)	10	4.7
	(−RNA)	10	0.5
	+$(NH_4)_2SO_4$ (0.41M)		7.4
	−RNA		7.7
Experiment 2	Complete (^{3}H-UTP)		32.8
	−3NTP		25.2
	−RNA		6.0
	Complete (^{3}H-ATP)		22.9
	−3NTP		20.3
	−RNA		6.1
	Complete (^{3}H-GTP)		23.2
	−3NTP		15.3
	−RNA		6.0

Where indicated 10 μg of DNase, 10 μg of RNase or 2 μg of actinomycin D was added, and one of the four ribonucleoside triphosphates was omitted. 3 NTP indicates the three ribonucleoside triphosphates other than the tritiated one. *a*, Enzyme and RNA were from adult brain; *b*, enzyme and RNA were from newborn brain (1-d-old). The standard reaction mixture had a total volume of 0.25 ml and contained 22 μmol Tris-HCl (*p*H 7.4), 3.0 μmol $MgCl_2$, 4.5 nmol 2-mercaptoethanol, 0.2 μmol each of ATP, CTP, GTP and ^{3}H-UTP (4×10^6 c.p.m. per 0.2 μmol): when assaying adult brain there was 80 μg enzyme protein and 10 μg template RNA; for newborn brain the quantities were 34 μg and 15 μg respectively. The reaction mixture was incubated at 37° C for 10 min for adult enzyme and 20 minutes for newborn enzyme, unless otherwise indicated. The reaction was stopped by addition of pyrophosphate and trichloroacetic acid. Precipitates were collected on membrane filters, washed eight times with cold 5% trichloracetic acid, and dried according to standard procedures[1]. In some experiments the reaction was stopped by blowing aliquots of the assay mixture on filter paper treated with pyrophosphate which was then washed for 2 h in 5% cold trichloroacecti acid according to the method of Bollum[18]. The radioactivities of the dried membrane filters or filter papers were determined in 10 ml of toluene scintillator containing 0.5% (w/v) of 2,5-diphenyloxazole with a Beckman LS 100 liquid-scintillation spectrometer. The radioactivity for zero time was subtracted from each value.

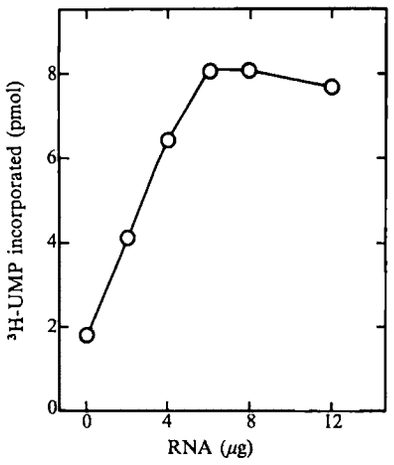


Fig. 1 Dependence of incorporation on concentration of template RNA. The composition of the incubation mixture was the same as described in Table 1 except for the RNA concentration, which varied. RNA and enzyme were from adult rat brain. Incubation was for 10 min.

nearly all homopolymer. When this homopolymer synthesis was investigated it was found, as reported for many other similar systems, that uptake of ^{3}H-UTP was inhibited by the presence of CTP or GTP (Table 4). It is therefore probable that with enzyme from newborn rats presented with all four NTPs nearly all the product was heteropolymer and *a fortiori* for the adult. It was clear that heteropolymer synthesis could be studied more easily using the adult enzyme fraction and homopolymer synthesis using the newborn and we did this, further improving the differentiation by using different concentrations of $MgCl_2$ in the reaction mixtures.

When the added RNA was omitted from the standard assay medium the incorporation fell to about one quarter. Most of this residual activity could be abolished by the addition of RNase which indicates that this also was mainly RNA-dependent RNA synthesis. The reaction was little affected by the addition of 10 μg of DNase and was inhibited by ammonium sulphate at concentrations which augment DNA-dependent RNA synthesis[13,14] (Table) 1. Two micrograms of actinomycin D had no effect. The effectiveness of various nucleic acids as templates was examined by substituting them for the rat brain RNA in the medium (Table 3). Only RNA was effective: DNA from calf thymus produced very little result. RNAs from Qβ, MS2 or GA phages, representatives of different groups of viral RNA, were ineffective and so were tRNA and rRNA from *E. coli* and TMV RNA. Of the homopolymers, poly(A), poly(G), poly(C) and poly(U) only poly(U) had any effect: either the synthesis was occurring by replication or by addition of UMP only to UMP terminals. Rat liver RNA was quite effective but markedly less so than the rat brain RNA. The degree of template specificity, that is, the range of effectiveness of the templates, was similar to that of the RNA-dependent RNA polymerases isolated from leukaemia and ascites tumour cells by Haruna *et al.*[5]. In the case of RNA replicase (RNA-dependent RNA polymerase) isolated from *E. coli* infected by Qβ phage, a basis for recognition of an RNA by an RNA replicase has been postulated by Kamen *et al.*[15] and Nishihara *et al.*[16]. Analysis of the brain RNA by sucrose gradient centrifugation gave the normal pattern for total cell RNA. Under these heteropolymer-promoting conditions the RNA polymerase in the enzyme fraction isolated from newborn rats had properties qualitatively similar to those for the adult but its activity was about three times higher (see Table 1 and Figs 1 and 2; it should be noted that the concentrations of enzyme and RNA differed from those used for the adult). ^{3}H-UTP was incorporated by the adult enzyme at an almost constant rate for the first 10 min of incubation with the template RNA (Fig. 2). During the next 10 min of incubation the rate declined. In the absence of added RNA the incorporated radioactivity reached a steady level after ~ 5 min incubation. With enzyme from newborn rat the activity increased linearly with time up to 30 min (Fig. 2). The maximum activity increased with increasing concentrations of enzyme. An explanation for the differences in time courses is that the adult enzyme fraction was contaminated by RNase or some inhibitor.

Table 2 Effects of nuclease and alkaline treatment on the product of the adult enzyme reaction.

Treatment	^{3}H-UMP incorporated (pmol)
None	9.1
Alkaline hydrolysis (0.3 N KOH; 37° C 18 h)	0.3
RNase 10 μg per 0.25 ml	1.0
DNase 10 μg per 0.25 ml	7.9

Reaction mixtures were as in the legend to Table 1. For the alkaline treatment, after 10 min incubation, sodium dodecyl sulphate and KOH were added to give final concentrations of 0.4% (w/v) and 0.3 N and the mixture was incubated at 37° C for a further 18 h. Ten micrograms of RNase or 10 μg of DNase was added to the incubation mixture after 10 min and incubation continued for a further 10 min. The reaction was terminated by addition of pyrophosphate, 50 μg of carrier albumin and trichloracetic acid. In the case of KOH addition, sodium dodecyl sulphate was added to avoid the degradation of product RNA by RNase contaminant in the enzyme fraction. Adult and 1-d-old rat brains were used to prepare enzyme fraction. Isolated brains containing cerebrum and cerebellum were frozen and thawed once. The brains were minced and suspended in medium (0.1 M Tris-HCl (*p*H 7.4), 0.01 M $MgCl_2$ and 0.5 mM 2-mercaptoethanol) containing 5 μg DNase lm^{-1}. The mixture, after homogenisation for 40 s, was stirred at 4° C for 30 min. The homogenate was centrifuged at 12,000*g* for 30 min and EDTA added to the supernatant to a final concentration of 10 mM. This solution was then centrifuged for 30 min at 12,000*g*. An equal volume of saturated ammonium sulphate (*p*H 7.4) was added to the supernatant and incubated at 0° C for 10 min and the 50% saturated ammonium sulphate-precipitable fraction was collected by centrifugation at 9,000*g* for 10 min and dissolved in medium (0.4 M ammonium sulphate, 9 mM Tris-HCl (*p*H 7.4), 4.5 mM $MgCl_2$ and 0.45 mM 2-mercaptoethanol). 0.2 volume of glycerol was added to the medium. This was the enzyme fraction. Its protein concentration was 16–28 mg ml^{-1} and it was stored at −80° C.

When presented with a single NTP the enzyme fraction from newborn rats synthesised considerable amounts of homopolymer, UTP and ATP being taken up to higher concentrations than GTP (Table 1*b*). Tests made to investigate heteropolymer synthesis by adult enzyme were repeated (Table 4) and showed that poly(U) synthesis was also independent of DNA and the presence of rat brain RNA as a primer was sufficient. It differed

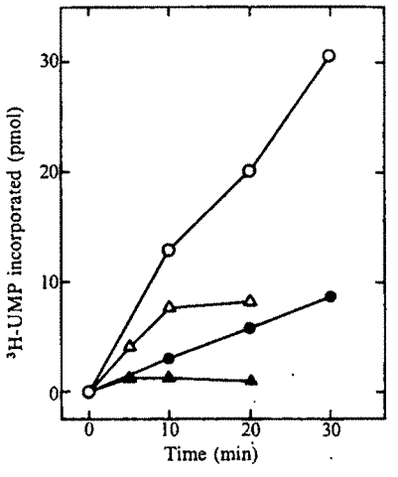


Fig. 2 Time course of RNA synthesis. The reaction mixture and conditions were the same as described in the legend to Table 1. Enzyme and RNA from newborn rat brain: ○, +RNA; ●, −RNA. Enzyme and RNA from adult rat brain: △, +RNA; ▲, −RNA.

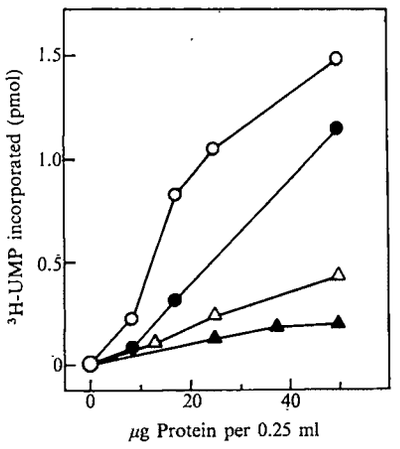


Fig. 3 Dependence of incorporation on concentration of enzyme protein (homopolymer synthesis). Conditions of assay were those described in the legend to Table 4 except for 5 μg of RNA of either adult or newborn brain and 10 minutes incubation. ○, Newborn enzyme with newborn RNA; ● newborn enzyme with adult RNA; △, adult enzyme with newborn RNA; ▲, adult enzyme with adult RNA.

from newborn enzyme in that the optimum $MgCl_2$ concentration was lower, about 4 to 6 mM. The formation of poly(U) may occur either by addition to poly(U) sequences in the template or by terminal addition to an RNA primer. Poly(U) was much more effective as a primer than poly(A) or poly(C), and poly(G) actually inhibited the residual synthesis occurring in the absence of added primer (Table 4). This means that if there was terminal addition it had strict primer requirements. When primed by the same primer RNA the activity of the newborn enzyme was several times higher than that of the adult (Fig. 3).

Table 3 Effect of various nucleic acids offered as template for RNA synthesis by adult enzyme

	Template (μg per 0.25 ml)		^{3}H-UMP incorporated (pmol)
Experiment 1	Brain RNA	10	8.9
	poly(A)	5	1.4
		10	2.1
	Poly(U)	5	7.8
		10	7.1
	Poly(G)	5	2.0
		10	2.4
	Poly(C)	5	2.3
		10	3.6
	−RNA		2.1
Experiment 2	Brain RNA	6	10.1
	Liver RNA	6	6.4
	Qß phage RNA	6	3.1
	GA phage RNA	6	2.4
	MS2 phage RNA	6	1.7
	TMV RNA	6	0.6
	E. coli tRNA	6	2.7
	rRNA	6	2.3
	Calf thymus DNA	7.5	2.8
	−RNA		1.8

Conditions of assay were those described in the legend to Table 1 except that various nucleic acids were used as templates. Qß phage RNA, MS2 phage RNA and GA phage RNA were prepared in our laboratory by the procedure described by Haruna and Spiegelman[1]. TMV RNA, tobacco mosaic virus RNA was kindly supplied by Dr Y. Okada (Tokyo University). RNA was isolated from brains (cerebrums and cerebellums) of adults and 1-d-old Wistar rats. Fresh brain was cut up with scissors and stirred for 30 min at 4° C in a solution containing 0.1 M Tris-HCl (*p*H 7.4) 10 mM $MgCl_2$, 0.5 mM 2-mercaptoethanol, 5 μg ml^{-1} DNase and 0.4% (w/v) sodium dodecyl sulphate and incubated at 20° C for 8 min. An equal volume of phenol saturated with cold water was added and shaken vigorously for 15 min at 4° C. After centrifugation at 12,000*g* for 15 min, the upper aqueous phase was mixed again with an equal volume of water-saturated phenol. Phenol treatment was performed four times and the ether flushed out with nitrogen gas, two volumes of cold (−20° C) ethanol was added and stood overnight at −20° C. The mixture was centrifuged at 9,000*g* for 10 min, the pellet was dissolved in 5 mM $MgCl_2$, 0.01 M Tris-HCl (*p*H 7.4). After ethanol precipitation at −20° C, the pellet was again dissolved in 0.01 M Tris-HCl (*p*H 7.4). The supernatant, after centrifugation at 12,000*g* for 20 min was used as the template and was stored at −20° C.

Table 4 Characterisation of the reaction of ^{3}H-UMP incorporation by newborn enzyme with UTP as substrate

	Assay mixture (μg per 0.25 ml)		^{3}H-UMP incorporated pmol)
Experiment 1	Control		7.9
	+DNase	10	9.4
	+Actinomycin D	2	9.1
	+RNase (+RNA)	10	0.9
	(−RNA)	10	0.5
	−$MgCl_2$		1.5
	−RNA		2.0
Experiment 2	Control		7.2
	+ATP	0.2*	7.6
	+GTP	0.2*	4.0
	+CTP	0.2*	4.1
Experiment 3	Control −RNA		2.5
	+RNA	10	6.6
		20	9.5
	+Poly(A)	10	3.4
		20	3.6
	+Poly(U)	10	5.5
		20	5.6
	+Poly(G)	10	0.6
		20	0.7
	+Poly(C)	10	3.2
		20	3.2
Experiment 4	Control		8.3
	−RNA+C.T. DNA	10	1.6
	−RNA		2.0
Experiment 5	Control (13 mM $(NH_4)_2SO_4$)		7.5
	+0.05 M $(NH_4)_2SO_4$		4.5
	+0.13 M		1.6
	+0.41 M		1.4
	−RNA		1.3

The reaction mixture had a total volume of 0.25 ml and contained 22 μmol of Tris-HCl (*p*H 7.4), 1.5 μmol of $MgCl_2$, 4.5 nmol of 2-mercaptoethanol, 10 nmol of ^{3}H-UTP (2 × 10^6 c.p.m. per 10 nmol) and 34 μg of newborn brain enzyme and 10μg newborn brain RNA. Incubation was for 20 min.

* 0.2 μmol of ATP, GTP or CTP were added to the mixture to test the effect of ribonucleoside triphosphate. Homopolymers were added instead of RNA.

It is not known whether the enzyme fraction contains only one enzyme mediating both types of synthesis or whether there are two or more specialised enzymes. We hope that this point will be clarified by purification of the enzyme fraction. Physiological roles for two enzyme systems are still obscure. It has been reported that homopolyribonucleotide synthesis in embryonic chick retina is involved in selective gene expression[17], and so we assume that our findings that the enzyme activity which is particularly engaged in homopolymeric RNA synthesis in brain tissue is higher in preparations from newborn than adult rats may have physiological significance.

As it is well known that brain RNA changes according to functional activities[11], we suggest that the enzyme reaction may participate in brain function, although we have no direct evidence of physiological function of this enzyme in the brain at present. We are now investigating whether this reaction exists in other tissues.

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1 Haruna, I., and Spiegelman, S., *Proc. natn. Acad. Sci. U.S.A.*, 54, 579 (1965).

[2] Wykes, J. R., and Smellie, R. M. S., *Biochem. J.*, **99**, 347 (1966).
[3] Burdon, R. H., and Smellie, R. M. S., *Biochim. biophys. Acta*, **51**, 153 (1961).
[4] Watson, K. F., and Beaudreau, G. S., *Biochem. biophys. Res. Commun.*, **37**, 925 (1969).
[5] Haruna, I., Ohno, T., Hozumi, N., and Watanabe, I., *Gann Monograph on Cancer Research*, **12**, 203 (1972).
[6] Winters, M. A., and Edmonds, M., *J. biol. Chem.*, **248**, 4756 (1973).
[7] Dravid, A. R., Pete, N., and Mandel, P., *J. Neurochem.*, **18**, 299 (1971).
[8] Hyatt, E. A., *Biochim. biophys. Acta*, **142**, 246 (1967).
[9] Niessing, J., and Sekeris, C. E., *Nature new Biol.*, **243**, 9 (1973).
[10] Wilkie, N. M., and Smellie, R. M. S., *Biochem. J.*, **109**, 485 (1968).
[11] Mandel, P., and Jacob, M., in *Handbook of Neurochemistry*, (edit. by Lajtha, A.) **5**, Part A, 165 (Plenum Press, New York and London, 1971).
[12] Mikoshiba, K., Tsukada, Y., Haruna, I., Ohno, T., and Watanabe, I., *Abstr. fourth Int. Mtg. Int. Soc. Neurochem., Tokyo, Japan*, 290 (1973).
[13] Widnell, C. C., and Tata, J. R., *Biochim. biophys. Acta*, **123**, 478 (1966).
[14] Roeder, R. G., and Rutter, W. J., *Proc. natn. Acad. Sci. U.S.A.*, **65**, 675 (1970).
[15] Kamen, R., Kondo, M., Romer, W., and Weissmann, C., *Eur. J. Biochem.*, **31**, 44 (1972).
[16] Nishihara, T., Haruna, I., Yamaguchi, N., and Watanabe, I., *Nature new Biol.*, **238**, 141 (1972).
[17] Sarker, P. K., Goldman, B., and Moscona A. A., *Biochem. biophys. Res. Commun.*, **50**, 308 (1973).
[18] Bollum, F. J., in *Methods in Enzymology* (edit. by Grossman, L., and Moldave, K.) **12**, Part B, 591 (Academic Press, New York and London, 1968).

No interaction between ultraviolet and X irradiation on chromosome aberrations in cells with trisomy 21

WE have previously shown[1] that ultraviolet irradiation of normal human lymphocytes immediately before or after X irradiation gives an approximately two-fold increase of the frequency of dicentric chromosomes as compared with the yield induced by X rays alone. Ultraviolet light itself induces very few dicentrics. One interpretation of this synergistic reaction might be that lesions induced by X rays are transformed into chromosome breaks by the ultraviolet treatment.

Here we report that no synergistic effect between ultraviolet light and X rays was observed after combined irradiation of blood lymphocytes of individuals with Down's syndrome (trisomy 21). The finding that the chromosome constitution influences the interaction between ultraviolet and X irradiation is of special interest as it has previously been shown that cells with trisomy 21 have a higher sensitivity for chromosome breakage than normal cells after single irradiation with ^{60}Co or X rays[2,3].

Unstimulated lymphocytes resting in the G_0 stage were treated with ultraviolet and X rays in three separate experiments. Peripheral blood from three 47, XY, +21 patients and two 46, XX healthy donors was used immediately after venipuncture. The leukocytes were separated as buffy coats, after which the cells were suspended in a phosphate buffer and irradiated in plastic Petri dishes. The irradiations were made at room temperature and the time interval between the ultraviolet (2537Å, 6.1 erg mm^{-2} s^{-1}) and X-ray (260 kV, 11 mA, HVL 2 mm Cu, 50 rad min^{-1}) exposure in the combined irradiation was less than 30 s. Conventional lymphocyte cultures were prepared and PHA added. The cultures were incubated at 37°C for 50 h (experiments A and B) and 52 h (experiment C), with colchicine added for the last 2 h. Thus, the sampling of cells was made in the first mitotic division, where the aberration yield should not be affected to any appreciable extent by a mitotic delay[4]. After hypotonic treatment and fixation, air dried preparations were made and strained in Giemsa. The slides were screened for consecutive, well-spread metaphases at 10×10 magnification, and the cells were then analysed for dicentric chromosomes (with accompanying fragments) at high-power magnification.

The present results of the induced yields of dicentric chromosomes are given in Table 1. Normal cells exhibit a synergistic effect between ultraviolet light and X rays in agreement with previous data[1]. The 47, +21 cells did not show any significant increase in the dicentric chromosome yield after pre- or post-treatment with ultraviolet as compared with that observed after X-ray exposure alone. The ratio between the number of dicentric chromosomes observed in the combined irradiation to that observed after X irradiation alone is plotted in Fig. 1 for normal cells (including data from ref. 1) and for cells with trisomy 21. The mean values (with standard deviations) of this ratio were $2.07 \pm \frac{0.23}{0.19}$ and $1.15 \pm \frac{0.18}{0.14}$ for normal and trisomy 21 cells, respectively.

In the present experiment the X-ray induced dicentric yield is about 50% higher in cells with trisomy 21 as compared with normal cells. The statistical errors are relatively large, however, and a large significant difference

Table 1 Observed number of dicentrics after single and combined irradiations with ultraviolet (UV) light and X rays.

Experiment	Subject chromosome constitution	Treatment	No. of cells analysed	No. of dicentrics	Dicentrics per cell
A	I : 47, XY, + 21	X rays, 100 rad	100	16	0.16 ± 0.04
		UV, 65 erg mm^{-2} + + X rays, 100 rad	100	20	0.20 ± 0.05
B	II : 47, XY, + 21	UV, 75 erg mm^{-2}	100	2	0.02
		X rays, 125 rad	150	40	0.27 ± 0.05
		UV, 75 erg mm^{-2} + X rays, 125 rad	150	53	0.35 ± 0.06
		X rays, 125 rad + UV, 75 erg mm^{-2}	150	45	0.30 ± 0.05
	III: 46, XX	X rays, 125 rad	150	27	0.18 ± 0.04
		UV, 75 erg mm^{-2} + X rays, 125 rad	150	68	0.45 ± 0.06
C	IV: 47, XY, + 21	X rays, 150 rad	100	40	0.40 ± 0.07
		UV, 55 erg mm^{-2} + X rays, 150 rad	100	41	0.41 ± 0.07
		UV, 75 erg mm^{-2} + X rays, 150 rad	100	42	0.42 ± 0.07
	V: 46, XX	X rays, 150 rad	100	27	0.27 ± 0.06
		UV, 55 erg mm^{-2} + X rays, 150 rad	100	47	0.47 ± 0.07

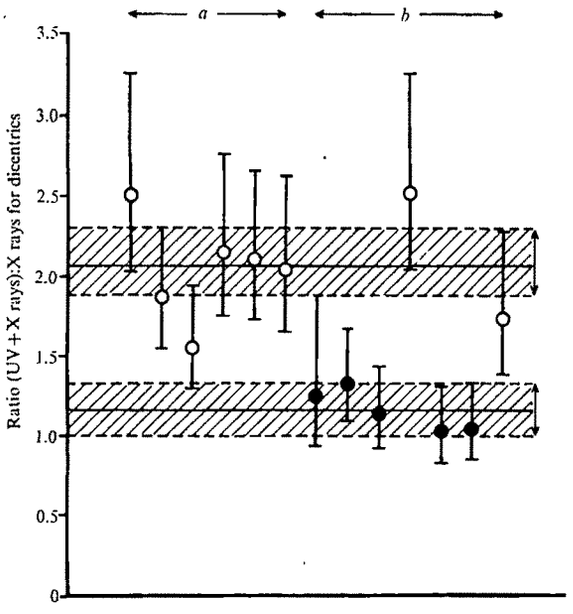


Fig. 1 Ratio between the number of dicentric chromosomes observed in the combined irradiation with that observed after X irradiation alone plotted for male and female normal cells (○) and cells with trisomy 21 (●). *a*, Data from ref. 1; *b*, data from present work.

in radiation sensitivities for the two types of cell is present only in the pooled data of 125 and 150 rad. Sasaki and Tonomura[2] reported an approximately two-fold increase of the radiation (^{60}Co) induced exchange aberration yield in cells with trisomy 21 as compared with normal cells, while the X-ray data by Evans[3] give a yield about 40% higher.

The correlation between the higher yield of X-ray induced chromosome exchange aberrations in cells with trisomy 21 in comparison with controls and the lack of a synergistic effect between ultraviolet and X rays in cells with trisomy 21 cannot be unambiguously explained. The synergistic effect observed in normal cells is, however, (within experimental error) independent of the ultraviolet exposure in the range 50–100 erg mm^{-2}. This correlates with the finding by Evans and Norman[5,6] that the amount of unscheduled DNA synthesis saturates at about 50 erg mm^{-2}. It is not known whether the ultraviolet dose dependence of unscheduled DNA synthesis is different in cells with trisomy 21.

Blood specimens from the individuals with Down's syndrome were provided by Dr J. A. Hedberg, Björnkulla Institution for the Mentally Retarded, Stockholm.

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1 Holmberg, M., and Jonasson, J., *Mutat. Res.* (in the press).
2 Sasaki, M. S., and Tonomura, A., *Jap. J. Human Genet.*, **14**(2), 81 (1969).
3 Evans, H. J., in *Advances in Physical and Biological Radiation Detectors*, 593–609 (Proceedings of a Symposium, IAEA, Vienna 1971).
4 Bender, M. A., and Brewen, J. G., *Mutat. Res.*, **8**, 383 (1969).
5 Evans, R. G., and Norman, A., *Nature*, **217**, 455 (1968).
6 Evans, R. G., and Norman, A., *Rad. Res.*, **36**, 287 (1968).

Injection of foreign substances into single cells by cell fusion

A good technique for injecting a given substance into an individual cell would be of value in cell biology. We here report studies on a method for injection of foreign substances into cells with erythrocyte ghosts as 'syringes', making use of the cell fusion activity of HVJ. Mammalian erythrocyte ghosts seem to be the most suitable disposable 'syringes', since they have no cytoplasm or nucleus, so contamination with cellular materials at the time of injection is minimal. Among the many mammalian erythrocytes tested, only human and dog erythrocytes showed high fusion capacity under the routine conditions used for treatment with HVJ (ref. 1).

As a preliminary step, we examined whether haemoglobin was injected into a culture cell during its fusion with an intact human red blood cell (RBC). Cells of a cloned line of FL cells originating from a human amnion cell were used as host cells. About 1 × 10^5 cells suspended in 8 ml of culture medium minimal essential medium (MEM) (with 10% calf serum) were inoculated into a Petri dish, diameter 5 cm, with several round cover glasses, 1 cm in diameter, at the bottom. The dishes were kept for 12 h to allow the cells to settle on the cover glasses. A suspension of 1.5% RBCs was made with balanced salt solution, BSS$^+$ (ref. 2) and the suspension was kept at 4° C until use. After removal of the culture medium, the cover glasses on which the FL cells had been plated were washed with cold BSS$^-$. To coat the surface of FL cells with HVJ, 1.5 ml of ultraviolet-irradiated HVJ (ref. 3), 1,500 haemagglutinating units[4], suspended in cold BSS$^-$ were added and the culture was kept at 4° C. After 20 min free viruses were washed off with cold BSS$^-$. Then, 2 ml of the RBC suspension were poured into the Petri dish and kept at 4° C for 30 min to allow the RBCs to be adsorbed to the surface of HVJ-coated FL cells. Under these conditions, no cell fusion occurred. After completion of the adhesion of RBCs, the BSS was removed carefully and 5 ml of warm BSS$^+$ was poured into the Petri dish. The culture was then incubated at 37° C for various periods, during which cell fusion between FL cells and RBCs was expected to occur accompanied by haemoglobin injection into the FL cells. The incubation periods used were 0, 20, 45, 90 min, 1 and 2 d; for incubation for longer than 20 min ordinary culture medium was used. After each of these periods a cover glass was taken out and washed with PBS (phosphate buffered saline, *p*H 7.2) to remove free RBCs and then stained with benzidine[5] to detect haemoglobin in the FL cells. The percentages of benzidine positive cells after incubation for 0, 20, 45 and 90 min were 0%, 22%, 34% and 36%, respectively. The injected haemoglobin spread rapidly throughout the cytoplasm. The presence of haemoglobin in FL cells was still clearly demonstrable after incubation for 1 or 2 d. It is significant that all the FL cells into which haemoglobin had been injected looked normal and many dividing cells containing haemoglobin were observable in long-term cultures.

The haemoglobin had to be replaced by other substances without destroying the fusion capacity of the RBCs. For this experiment we chose fluorescein isothiocyanate (FITC) because it is easy to detect. Packed RBCs (1 ml) were haemolysed by addition of 5 ml of cold distilled water. The resulting lysate was centrifuged at 11,000*g* for 10 min and the supernatant discarded. Six-fold diluted PBS was added to the pellet to remove haemoglobin and the mixture was centrifuged in the same way and the supernatant discarded; this procedure was repeated three times. Six ml of a solution of 0.05 mg ml^{-1} of FITC in six-fold diluted PBS were then added to the pellet. The mixture was stirred and centrifuged at 11,000*g* for 10 min and the

supernatant discarded; this procedure was repeated twice. The pellet was then treated similarly with the same concentration of FITC in half-strength PBS and centrifuged at 11,000*g* for 10 min. All these procedures were carried out at 4° C. Finally, the pellet was mixed with the same concentration of FITC in 10 ml of full strength PBS and the mixture was incubated at 37° C for 30 min. It was then centrifuged at 7,000*g* for 10 min and the supernatant discarded. The resulting yellow RBCs were washed with PBS by repeating centrifugation at 1,700*g* for 15 min until the supernatant became colourless. The external appearance of the yellow RBC containing FITC was indistinguishable from that of intact ones and the FITC fluoresced intensely. For preservation, the cells were suspended in cold PBS with 4 mM ATP and 0.1 mM $MgCl_2$. It was estimated that each corpuscle contained about 4.4×10^{-3} pg of FITC. No leakage of FITC from the corpuscles was observable during overnight storage.

Cell fusion between FL cells and the yellow RBCs was tested following the procedure described above. In this case, however, on long-term incubation the FL cells died as a result of the cytotoxic effect of the very small quantity of FITC which leaked from the RBCs during reactions with HVJ, so the presence of FITC in FL cells had to be checked after relatively short incubations of 20 min and 60 min. Cover glasses were removed from Petri dishes, washed with PBS, placed on a thin slide with the cells downwards and enclosed in buffered glycerine. Fluorescence was examined in a fluorescent microscope under a dark field and injected FITC in the FL cells was demonstrated. The frequency of injection was less than that of haemoglobin, however, being lower than 1%. Because FITC is highly cytotoxic, considerable numbers of FL cells may have been killed and detached from the surface of the cover glass immediately after the injection started, so the actual rate of FITC injection may in fact have been much higher. This result is encouraging, however, since intracellular injection of FITC was achieved, albeit at low frequency.

There are several possible advantages of this method. First, it is well known that HVJ causes a cell fusion reaction to a greater or lesser extent in almost all cultured cells, so intracellular microinjection into them will be possible. Second, with further improvement of the method it should be possible to control the dose of substance injected into a single cell. Third, it should be emphasised that after injection of haemoglobin FL cells were healthy and continued to divide, so the injection procedure itself is not basically harmful to the cells.

There are also possible difficulties in the method. There are technical limitations to replacing haemoglobin with other substances. During injection of intracellular substances, the RBC membrane is inevitably transplanted into the plasma membrane of the target cell, but it may have no appreciable effect, since during repeated cell divisions molecules of the transplanted RBC membrane will undergo progressive geometric dilution. Moreover, the transplanted RBC membrane may be a good marker to identify cells which have received an injection, because RBC membrane-specific antigens should be detectable by staining labelled antibody[6].

We plan to examine appropriate conditions for fusion of a single cell with a single RBC.

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1 Okada, Y., Suzuki, T., and Hosaka, Y., *Med. J. Osaka Univ.*, 7, 709–717 (1957).
2 Okada, Y., and Murayama, F., *Expl Cell Res.*, **44**, 527–551 (1966).
3 Okada, Y., and Tadokoro, J., *Expl Cell Res.*, **26**, 98–128 (1962).
4 Salk, J. E., *J. Immun.*, **49**, 87–98 (1944).
5 LoBue, J., Dornfest, B. S., Gordon, A. S., Hurst, J., and Quastler, H., *Proc. Soc. exp. Biol. Med.*, **112**, 1058–1062 (1963).
6 Furusawa, M., and Adachi, H., *Expl Cell Res.*, **50**, 497–504 (1968).

Mechanisms of sensing chemical gradients by polymorphonuclear leukocytes

ALTHOUGH it has been known for nearly 100 yr that polymorphonuclear leukocytes (PMNs) can show oriented locomotion in response to chemical gradients, that is, they display chemotaxis, the way in which these cells detect and respond to chemical gradients is still not understood[1]. These questions are of interest, not only to a study of the inflammatory process, but also to more general problems of oriented cell movements during morphogenesis.

Current studies on bacterial chemotaxis indicate two distinct methods by which an organism can sense a chemical gradient[2,3]. An organism could sense the concentration of a chemotactic substance at one point, move a certain distance and sense it again, comparing this new level with the previous one. This has been termed a 'temporal' mechanism, as the organism compares between two times. In a 'spatial' mechanism the organism compares the concentration of a chemotactic substance at two or more locations on its body at one time. The mechanisms differ in that the temporal mechanism requires only one receptor somewhere on the organism and a simple memory system, whereas the spatial mechanism requires at least two spatially separated receptors on the organism and implies that the organism is capable of sensing the gradient across its own dimensions[2,4]. Analysis of films of horse leukocytes initiating and exhibiting chemotaxis demonstrates that these cells utilise the spatial mechanism. This conclusion is supported by Ramsay's observations of a human PMN responding to a moving chemotactic stimulus[7].

Time lapse films were made of horse blood neutrophilic leukocytes in a thin slide coverslip preparation as they responded to a chemotactic stimulus generated by other leukocytes in contact with a line of aggregated γ globulin that had been previously dried on the slide. The details of the preparation have been described previously[5].

The magnitude of the chemotactic response was quantitated in three ways. First, the number of cells locomoting toward the chemotactic source was compared with that of cells moving away from it. Of the 80 PMNs filmed, 78 moved towards the chemotactic source, that is, into the 180° sector containing the chemotactic source, no cells moved away and two were immobile. Second, the precision of the orientation of cellular locomotion was determined by comparing the orientation of movement of individual cells with the most direct possible path to the chemotactic source. In this analysis the successive positions of 30 cells were traced by projecting frames of the time lapse films onto drawing paper. As the cells tend to move in straight lines for short periods of time, the traced paths could be broken into segments of straight movement (that is movements having less than a 10° change in direction which were separated by relatively abrupt turns of varying magnitude). The tracing of four such cell paths is shown in Fig. 1. From the distribution of straight segments shown in Fig. 2, it was calculated that 65% of segments were oriented within 30°

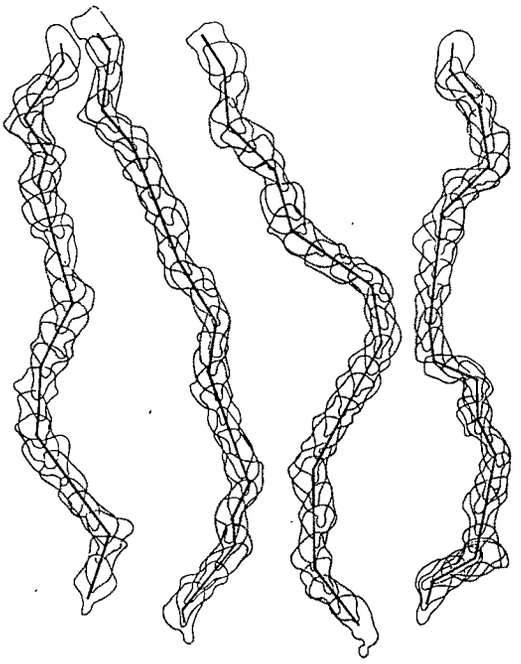


Fig. 1 The tracings of four cell paths is a 16× field immediately adjacent to a line of aggregated γ globulin filmed at 60 frames min^{-1}. The film was projected on paper and at every 30th frame the cell images were drawn. The cells move from the bottom of the figure toward the top; the γ globulin line was parallel to the top edge of the figure. Straight lines were drawn through segments of the tracings which showed less than a 10° change in direction. These lines were then analysed for their orientation relative to a perpendicular to the γ globulin line, their length, and the magnitude and direction of the turns linking the various straight segments.

of the most direct path. Third, the degree of chemotaxis was also evaluated with the chemotropism index of McCutcheon by determining the ratio of the length of the most direct path to the chemotactic source to the path length measured from the film tracings[6]. The chemotropism index ranged from 0.61–0.97 with a mean of 0.85 and s.d.m. of 0.01. The index is higher than those previously published[6–8]. From these observations it can be concluded that PMNs can sense and respond with high accuracy to a chemical gradient. The observed abilities of the PMNs to orient is presumably less than their maximal capability as with a more perfect gradient or a more potent chemotactic substance they could be expected to respond even more accurately.

Studies have shown that bacteria swimming in a gradient of a chemotactic substance have a lower frequency of turning when they are moving up the gradient than when they are moving down[2,3]. This variation in turning behaviour with the orientation of swimming causes the ratio of cells moving up the gradient over those moving down to exceed one. Previous studies on PMNs have suggested that randomly moving cells have a higher frequency of turns and stops than cells exhibiting chemotaxis[3,6]. In my present study the frequency and magnitude of turns were examined with PMNs which were moving with various orientations to a chemotactic source. The distance between turns did not alter as a fuction of the orientation of the locomotion. (coefficient of correlation was -0.169, $N=68$, $P>0.1$). Thus a cell which deviated 40° from the direct path to the source continued in this direction as far as one headed directly towards the source. In the population studied, no cell was moving at an angle more than 100° from the direct path to the source; in situations of less efficient orientation a significant correlation might exist. In this same population, however, a significant correlation was found between the magnitude of a turn and the orientation of that cell immediately before the turn (coefficient of correlation $=0.35$, $N=68$ $P\leqslant 0.01$). The further a cell was oriented from the direct path the greater the magnitude of the next turn tended to be. The correlation, however, was not strong.

Probably an even more important factor in the orientation of moving PMNs than the magnitude of turn is the direction of the turn. Thus 15 out of 16 cases where a cell in the tracings was moving at an angle of 30° or more from the direct path to the chemotactic source, the direction of the next turn by the cell was towards the source; the probability of this bias occurring by chance is less than 0.001.

These behaviour patterns of cells exhibiting chemotaxis are consistent with a spatial mechanism of sensing the gradient. The directional turning response of cells rules out a simple temporal system based on readings at only two points as such a system would not allow the cell to decide in which direction to make its next turn. The data, however, do not eliminate a more complex temporal sensory mechanism which envisions moving cells as having the ability to 'remember' the concentration levels over two preceding intervals as well as the direction of the turn linking the two. This mechanism would give the cell as much information as a spatial mechanism with three or more receptors (knowledge of the concentration at three points in a plane is sufficient to determine the direction and relative steepness of an undirectional gradient in that plane).

To differentiate between spatial and temporal mechanisms I observed the initial orientation of cells placed in a chemotactic gradient. Only a spatial mechanism should allow cells to detect the direction of a gradient while stationary. The direction of initial locomotion of PMNs placed on a slide bearing a streak of aggregated γ globulin was analysed. Filming was begun 3 min after the preparation was made; at this time the PMNs were still rounded and immobile. Gradually the cells began to extend pseudopods, the cells nearest the line of γ globulin first, followed by cells further away. The direction of the initial locomotion was towards the source of the chemotactic agent. In the microscopic field filmed, 18 out of 20 cells which moved either towards or away from the chemotactic source, extended their first pseudopod and made their initial

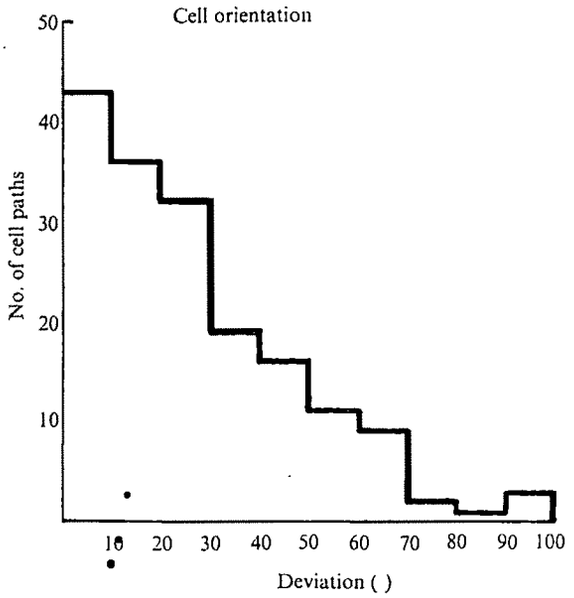


Fig. 2. The number of straight segments of cell paths is plotted against their orientation relative to the perpendicular to the γ globulin line. A total of 172 straight segments taken from the tracings of 30 cell paths were measured.

translocation (of at least ½ their body length) into the 180° sector toward the chemotactic stimulus. This population distribution would occur by chance less than 1 time in 1,000. Thus, the stationary cells are able to sense the gradient across their own dimensions and to respond directionally from the stationary position, an ability characteristic of an organism with a spatial mechanism of detecting gradient (a specialised temporal system where a single receptor is moved over the cells' surface is an alternative, unlikely, possibility).

In response to the chemotactic stimulus the frequency of pseudopod formation increased on the high concentration side of the cell. Before initial migration, the stationary cells did show changes in shape but usually these did not include the formation of structures which could be recognised as pseudopods, either with 16× or 100× microscope objectives. Furthermore, cells greater than 1 cm from the γ globulin line normally remained rounded even after 1.5 h incubation.

How the cell utilises information obtained by the spatial sensory system to selectively form pseudopods is unknown. Possibly there is a time and concentration dependent threshold of stimulation at the membrane which when activated leads to local pseudopod formation and a refractory period inhibiting pseudopod formation on other portions of the cell[10]. Alternatively, the cell could have an internal means of comparing the amount of stimulation of its various sides; for instance local stimulation could affect the distribution of some limiting cofactor required for pseudopod formation. Further studies will be required to distinguish between these and other alternatives.

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1 Leber, T., *Forts chr. Med.*, **6**, 461 (1888).
2 Macnab, R. M., and Koshland, D. E., *Proc. natn. Acad. Sci., U.S.A.*, **69**, 2509 (1972).
3 Berg, H. C., and Brown, D. A., *Nature*, **239**, 500 (1972).
4 Fraenkel, G. S., and Gunn, D. L., *The Orientation of Animals*, **76**, (Dover Publications Inc., New York, 1961).
5 Zigmond, S. H., and Hirsch, J. G., *J. exp. Med.*, **137**, 387 (1973).
6 McCutcheon, M., *Physiol. Rev.*, **20**, 319 (1946).
7 Ramsay, W. S., *Expl Cell Res.*, **70**, 129 (1972).
8 Grimes, G. J., and Barnes, F. S., *Expl Cell Res.*, **79**, 375 (1973).
9 Harris, H., *J. Path. Bact.*, **66**, 135 (1935).
10 Cohen, M. H., and Robertson, A., *Proc. IUPAP Conf. Statistical Mechanics* (edit. by Rice, S. A., Freed, K., and Light, J.), (University of Chicago Press, 1972).

Optimal recovery of lymphocytes and tissue culture cells following rapid cooling

CELLS die if cooled either too rapidly or too slowly during freezing in the presence of dimethylsulphoxide (DMSO)[1-4], but survive at an intermediate optimal cooling rate[5]. We have now obtained improved recovery of lymphocytes, lymphoid cell line cells and Chinese hamster tissue culture cells by interrupting rapid cooling with a timed exposure to a single subzero temperature. This method is simple and here we put forward some theoretical reasons for the improved cell recovery.

Dulbecco's medium containing DMSO (5% v/v) was added to gelatin-separated human peripheral blood lymphocytes immediately before dispensing 0.2 ml aliquots into sterile glass test tubes (10 cm long, 7. mm outer diameter, 5 mm inner diameter). The tubes were immersed either in a constant temperature alcohol bath (± 0.2° C, ref. 6) or in a bath of glycerol solution inside a commercial deep freeze. The bath temperature was measured by a calibrated resistance thermometer[7]. After different times at the chosen subzero temperature, the tubes were plunged into liquid nitrogen. A typical sample reached −25°C in less than a minute from room temperature, the subsequent drop to −196°C took less than 20 s. The samples were thawed in a water bath at 37°C for 20 s. The lymphocytes were diluted in one step with 1.0 ml of serum-gelatin and cultured as previously described[1,5]. Recovery was assessed by uptake of ^{3}H-thymidine (^{3}H-TdR) on the third or fourth day of culture of lymphocytes stimulated with phytohaemagglutinin (PHA).

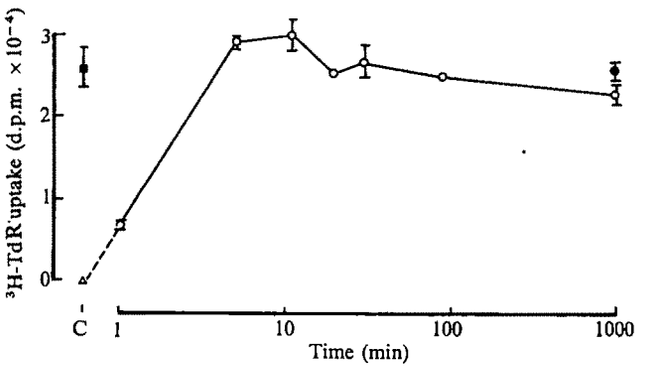


Fig. 1 PHA-stimulated ^{3}H-thymidine uptake (d.p.m.) of unfrozen control lymphocytes without DMSO (■), and thawed cells frozen in DMSO (5% v/v) either directly to −196°C (Δ) or kept at −25.6°C for different times before either thawing (●) or plunging to −196°C before thawing (○).

Figure 1 shows the recovery of cells held at −25.6°C in the deep freeze before immersion in liquid nitrogen. Complete recovery (100%) of the PHA-induced ^{3}H-TdR uptake was obtained with cells held from between 5 min and 18 h at the subzero temperature before cooling rapidly to −196°C. There was no recovery of ^{3}H-TdR incorporation by cells cooled directly to −196°C without exposure at −25.6°C. Using holding temperatures of −22°C, −26°C, −30°C and −40°C the maximum recoveries of PHA-responsive lymphocytes were 18%, 100%, 65% and 40% respectively. Cells held at the sub-optimal temperatures of −22°C and −40°C for 1 to 2 h were damaged on thawing from that temperature; no additional damage was seen when replicate samples were cooled to −196°C before thawing. With DMSO (10% v/v) complete recovery of ^{3}H-TdR uptake in response to PHA was obtained only after 50 min at −26.3°C in the alcohol bath. It was important to cool the sample rapidly to the subzero holding temperature. Even 0.5 ml samples of cells in polypropylene Sterilin tubes showed a slower recovery with time at −25.2°C in the alcohol bath, in comparison with the 0.2 ml samples in the glass test tubes.

Recovery was also observed with two other cell types known to succumb to rapid freezing. Lymphoid cell line cells (Cla 4) showed 100% recovery after 30 min at −26°C before cooling to −196°C. Recovery was assessed by ^{3}H-TdR uptake for 24 h. Chinese hamster lung fibroblast cells (V79-379A) were grown and collected as previously described[8]. Duplicate samples were frozen in the same manner as the lymphocytes in Eagle's minimal essential medium with DMSO (5% v/v). On thawing, the samples were plated for colony assay. Cells frozen directly to −196°C from room temperature showed no colony growth on thawing (Fig. 2). Between 1 min and 10 min at −26.0°C

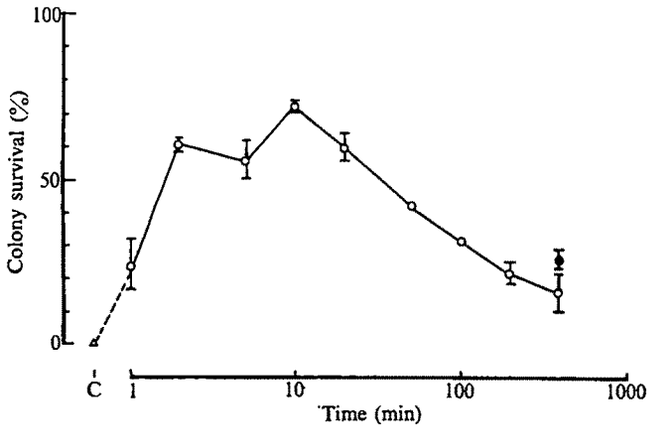


Fig. 2 Colony growth (as % of unfrozen control) of Chinese hamster tissue culture cells thawed after freezing in DMSO (5% v/v) either directly to −196°C (Δ) or kept at −26.0°C for different times before either thawing (●) or plunging to −196°C before thawing (○). The plating efficiency of the unfrozen control was 66.5%.

the survival of tissue culture cells thawed from liquid nitrogen increased to a maximum of 73% at 10 min. With longer times the survival decreased.

These results demonstrate a simple method of obtaining a high survival of different cell types after storage in liquid nitrogen. With human lymphocytes we obtained complete survival of responses to PHA with a low concentration of DMSO (5% v/v). Using conventional optimal cooling rate techniques, this concentration does not allow complete survival[9].

The basis of the present method is the realisation that there is a zone of subzero temperature, exposure to which protects cells against damage due to the subsequent stresses of both rapid cooling to −196°C, and rapid thawing and dilution. This was shown some time ago by Luyet and Keane[10] for bull spermatozoa and by Taylor[11] for chick skin cells. Our results show that the protective properties of different subzero temperatures are affected by physical factors such as the initial concentration of DMSO before freezing. In addition, different cell types respond differently to the same subzero protective environment, thus providing a possible method for their separation.

We propose that this temperature zone conferring protection to cells against subsequent freezing and thawing damage explains the established finding that most cells will survive freezing if cooled slowly (for example at 2°C min^{-1} for lymphocytes in DMSO 5% v/v)[1,5] rather than rapidly. The disadvantage of the conventional optimal cooling rate appears to be two-fold. First, damage may be produced by the initial slow attainment of the best protective temperature (for example the cooling from 0°C to −26°C for lymphocytes in DMSO 5% v/v); second, if slow cooling is continued below the protective zone damage may be caused. The beneficial effects of the two stage cooling rates sometimes used[12,13] can thus be explained. The logical improvement is rapid cooling to the optimal protective temperature, a period at that temperature to confer protection, followed by a rapid cooling to storage temperatures.

We have shown that two separate processes determine the survival of the cells thawed from −196°C; these are damage at the holding temperature and protection against damage on subsequent freezing and thawing.

The mechanism of the protection with time is unknown. It bears one similarity to the thermal shock system[14] where red cells cooled slowly from + 37°C to 0°C in hypertonic sodium chloride are haemolysed less than cells cooled rapidly[15,16]. The rapidly cooled red cells could be protected, however, by increasing the time of exposure to the hypertonic conditions before cooling[16]. The mechanism of protection at the subzero temperature may involve membrane changes induced by the hypertonic conditions, water or solute movements across the cell membrane or the redistribution of ice.

We believe that these results will form the basis for simple improved techniques for cellular preservation.

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1 Farrant, J., Knight, S. C., and Morris, G. J., *Cryobiology*, **9**, 516-525 (1972).
2 Leibo, S. P., Farrant, J., Mazur, P., Hanna, M. G., and Smith, L. H., *Cryobiology*, **6**, 315-332 (1970).
3 Mazur, P., Farrant, J., Leibo, S. P., and Chu, E. H. Y., *Cryobiology*, **6**, 1-9, (1969).
4 Morris, G. J., and Farrant, J., *Cryobiology*, **9**, 173-181 (1972).
5 Knight, S. C., Farrant, J., and Morris, G. J., *Nature new Biol.*, **239**, 88-89 (1972).
6 Pegg, D. E., *Laboratory Practice*, **15**, 772-773 (1966).
7 Pegg, D. E., and Hayes, A. R., *Phys. med. biol.* **15**, 409-416 (1970).
8 McGann, L. E., Kruuv, J., and Frey, H. E., *Cryobiology*, **9**, 494-501, (1972).
9 *La cryoconservation des cellules normales et neoplasiques*, (edit. by Weiner, R. S., Oldham, R. K., and Schwarzenberg, L.) (INSERM, Paris, 1973).
10 Luyet, B., and Keane, J., *Biodynamica*, **7**, 281-292 (1955).
11 Taylor, A. C., *Ann. N.Y. Acad. Sci.*, **85**, 595-609 (1960).
12 Smith, A. U., *Biological Effects of Freezing and Supercooling*, (Arnold, London, 1961).
13 Hors, J., Preud'homme, J. L., Toulze-Zapateria, M. T., Guillet-Bigot, J., Roy, J. P., and Dausset, J., *Transplantation*, **15**, 417-419 (1973).
14 Farrant, J., and Morris, G. J., *Cryobiology*, **10**, 134-140 (1973).
15 Lovelock, J. E., *Br. J. Haemat.*, **1**, 117-129 (1955).
16 Morris, G. J., and Farrant, J., *Cryobiology*, **10**, 119-125 (1973).

Are there differences in contact inhibition of movement between normal and malignant cells

STOKER[1] claimed that the malignant cells transformed from BHK cells by polyoma virus showed a lack of contact inhibition of movement unlike their progenitor strain BHK 21 C 13. One of us[2] reported that measurements made of the degree of contact inhibition shown by these two cell types showed that both displayed the same degree of contact inhibition. Measurements were, however, made at one culture age and population density of cells. It is known that contact inhibition of movement of embryonic chick fibroblasts varies with both population density and culture age[3]. Neither Stoker nor Curtis examined the effects of these factors on the contact inhibition of Py or C13 cells. Thus the disagreement between the results of Stoker and one of us is ripe for further investigation. We report here measurements on the contact inhibition of these two cell types at different culture ages and densities.

BHK C13 and Py cells cultured in this department and originally derived from the Department of Virology, University of Glasgow were obtained from stock cultures by trypsinisation. The cells were grown in Eagles +10% calf serum 10% tryptose phosphate medium. Cultures were started with inoculum densities of 6.4×10^5, 3.2×10^5 and 1.5×10^5(C13) 9.0×10^5, 4.5×10^5 and 1.5×10^5(Py) cells per dish (60 mm Polystyrene) in order to obtain different population densities at any one culture age. Cultures were fixed at 20, 24 and 48 h after inoculation. They were stained with Harris's haematoxylin and contact inhibition measured by

Table 1 Variation of mean overlap ratio with culture age and population density

a Age in culture (h) — x_1

	8	20	24	48
C13	0.183	0.219	0.211	0.298
Py	0.357	0.262	0.255	0.284

b Population density — x_2

	< 70	70–74.9	75–79.9	80–84.9	85+
C13	0.207	0.219	0.259	0.212	0.251
Py	0.456	0.301	0.302	0.294	0.265

c Statistical analysis

	Σx_1^2	Σx_2^2	$\Sigma x_1 y$†	$\Sigma x_2 y$	$\Sigma x_1 x_2$	Σy^2	n
C13	5,061	2,875	14.6	9.90	2,159	0.080	23
Py	2,611	532	−5·4	−3.74	2,665	0.059	20
Pooled	7,672	3,407	9.2	6.16	4,824	0.139	43

Regression coefficient		Deviations from regression		
b_1	b_2		Sum of squares	Mean square
0.0021	0.0019	C13	0.0316	0.0014
−0.0012	−0.0008	Py	0.0496	0.0027
		Sum	0.0812	0.0041
		Pooled	0.0258	0.0006
				$F = 13.32$ (d.f.1:40)

* Expressed as cells per counting area (1.8×10^{-8} m^2).
† y is overlap index.

counting the number of overlaps in a known area of measured cell population. Overlap index[4] was used as a measure of contact inhibition.

The results are tabulated in reduced form in Table 1*a* and *b*. The full results were analysed by multiple covariance analysis in Table 1*c*. The planes of the regression equations for C13 and Py cells are significantly different, F=13.32, d.f.1:40 $P<0.5\%$. Comparing the differences in slope of the regression coefficients for culture age (x_1) alone and for population density (x_2) alone respectively we find that both pairs of coefficients are significantly different. (F=10.29 $P<0.5\%$ for x_1, F=36.8, $P<0.5\%$ for x_2).

Thus the response of Py and C13 cells in their overlap indices to culture age and population density differ. Overlap index increases with population density and age in the normal C13 cells, in other words contact inhibition of movement decreases with these changes. But Py cells show the inverse situation. Contact inhibition of Py cells increases with culture age and population density. In consequence it is possible to choose culture ages and population densities such that cultures of both cell types will show the same degree of contact inhibition of movement; presumably this is what happened in earlier work on these cells. But the results also show that Py cells never show a lack of contact inhibition. It will be important to discover whether similar situations exist in other comparisons that have been made in the contact inhibition of normal and malignant cells.

At the moment we can only speculate about the cause of this difference. It is well known that there are surface and other changes in cells as culture age and population density increase[5–7] though the effects of age and density do not seem to have been separated in such work. Possibly one or more of these changes is related to the changes in contact inhibition we have observed. In many of these situations malignant and normal cells differ in their reaction to culture age and population density.

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1 Stoker, M., *Virology*, **24**, 165 (1964).
2 Curtis, A. S. G., *The Cell Surface: its molecular role in morphogenesis* (Logos Press, London, 1967).
3 Curtis, A. S. G., *J. natn. Cancer Inst.*, **26**, 253 (1961).
4 Abercrombie, M., and Heaysman, J. E. M., *Expl Cell Res.*, **13**, 276 (1954).
5 Bosmann, H. B., Bieber, G. F., Brown, A. E., Case, K. R., Gersten, D. M., Kimmerer, T. W., and Lione, A., *Nature*, **246**, 487 (1973).
6 Dulbecco, R., and Elkington, J., *Nature*, **246**, 197 (1973).
7 Stoker, M., *Curr. Top. dev. Biol.*, **2**, 107 (1967).

New fungal viruses capable of reproducing in bacteria

MANY authors have reported the isolation from fungi of virus particles containing double-stranded RNA[1–5]. It has also been found that extracts of *Penicillium brevi compactum*, *P. chrysogenum* and *P. stoloniferum* can lyse certain bacteria. Fractionation of such extracts and also disruption of spores have revealed the presence of several types of virus particles differing in their biochemical and biological properties. This series of viruses was called PBV, with reference to the host (P) and to their ability to lyse bacteria (B).

Table 1 shows the results of titration of an extract of the mycelium, supernatant and spores of the three species of fungi mentioned above with the cells of *Escherichia coli* C and *E. coli* SK. The infectivity towards bacteria increases as the mycelium grows. No free virus was detected in the supernatant of *P. brevi compactum* after the mycelium was removed, whereas with the other two fungi virus was found in both the mycelium and the culture medium. Viruses which could multiply in bacteria were also found in preparations of the disrupted spores, as well as in preparations of disrupted mycelium not subjected to ammonium sulphate fractionation. In the latter case, the titre of virus in suspension was 1.5–2.0 log lower.

When the fungi were grown in the presence of 250 μg ml^{-1} neomycin, which has a wide spectrum of antibacterial action, the quantity of PBV determined by titration with bacteria does not change as compared with the control without antibiotic. No bacteria were detected at any time in the fungal culture. The results were the same with different strains of *P. brevi compactum* (13 strains have been studied). Figure 1 shows the results of equilibrium centrifugation of mycelial extracts in a Cs_2SO_4 density gradient. The gradient shows several peaks of infectivity to bacteria. The positions of these peaks are different for *P. brevi compactum* and *P. stoloniferum*.

A further investigation has shown that in *P. brevi compactum* extracts there are two types of viruses infective to bacteria (PBV1 and PBV2) in the regions of density 1.38 and 1.40 g cm^{-3}. In the 1.32 g cm^{-3} region[6], a double-stranded RNA-containing mycovirus is present, which seems to be somewhat smaller in comparison with the mycovirus of

P. brevi compactum described by Wood *et al.*[7] (300 Å in diameter as measured by us compared with 400 Å indicated by Wood). In the 1.42 g cm^{-3} region there is a third virus, PBV3. The 'light' zones of the density gradient (1.25–1.32 g cm^{-3}) also show some infectivity with respect to bacteria.

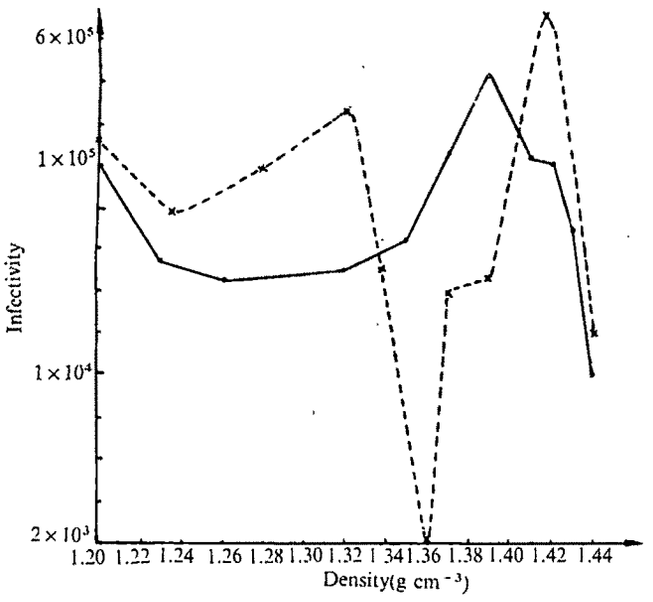


Fig. 1 Equilibrium centrifugation of the extract of *P. brevi compactum* and *P. stoloniferum* mycelium in Cs_2SO_4 density gradient. Preparation and concentration of the extract with ammonium sulphate were carried out as indicated in the legend to Table 1 (the extract of *P. brevi compactum* mycelium was concentrated 10-fold, the extract of *P. stoloniferum*, 1,000-fold). The extracts were layered on the preformed Cs_2SO_4 gradient (1.22–1.44 g cm^{-3}) and centrifuged for 16 h in a 3×5 ml rotor at 35,000 r.p.m. in a Superspeed 50 centrifuge. In the gradient fraction (0.27 ml) infection titre was determined with *E.coli* SK cells by Gracia[8] without dialysis. Under the dilution used the Cs_2SO_4 concentration was low and did not influence the results of titration. ———, *P. stoloniferum* extract; – – –, *P. brevi compactum* extract.

Pure lines of PBV1, PBV2 and PBV3 were obtained (some of their properties are presented in Table 2).

PBV1 and PBV3 comprise icosahedral particles with long noncontractile tails, resembling λ phage; PBV2 is an icosahedral particle with a short tail, resembling T5 phage[6]. The nucleic acid of viruses PBV1 and PBV2, judging by the colour reaction, T_m and buoyant density is a typical double-stranded DNA. PBV1 has an extremely wide spectrum of antibacterial action; it can lyse *E. coli* B, *E. coli* C, *E. coli* K12 and *E. coli* SK; PBV2 lyses only *E. coli* SK. PBV3 was infective towards *E. coli* C, *E. coli* B and *E. coli* SK, and has an abnormally high buoyant density in Cs_2SO_4.

Many facts lead us to believe that titration of mycelium extracts on bacteria did reveal the fungal viruses and not some fungal substances which could induce lysogenic prophages in the bacterial cells. Some of these data are summarised in Table 3. First, definite mycelium fractions after equilibrium centrifugation gave several types of colonies on the same bacteria, differing in size and morphology. From these colonies pure viral lines were isolated which after many passages through bacteria had the same buoyant density and gave the same type of colonies as respective viruses in the fungal extracts. This is evident particularly in the case of PBV3 which has an abnormal buoyant density in Cs_2SO_4.

Second, antisera against three isolated and purified PB viruses grown in bacteria neutralise the infectivity of the fungal fraction towards bacteria. The fraction of density 1.42 g cm^{-3} contains chiefly PBV3 (Table 3) which gives large clear colonies with a halo on *E. coli* SK. PBV1 and PBV2 give large clear colonies without haloes and large turbid colonies respectively and represent an admixture of PBV3 as a result of overlapping zones occupied by these viruses. The same is true for the fractions of density 1.40 and 1.38 g cm^{-3}. Neutralisation experiments with other fractions (1.32 and 1.38 g cm^{-3}) are now under way.

Thus, we conclude that fungal extracts contain virus particles infective towards bacteria. Otherwise *E. coli* C or *E. coli* SK cells would have to contain at least four different prophages which can be induced by fungal substances and at the same time do not restrict the subsequent infection by

Table 1 Titration of Mycelium preparations on *E. coli C* and *E. coli* SK

Fungus species	Time of growth (h)	Infectivity per g biomass *E. coli* C	Infectivity per g biomass *E. coli* SK	Infectivity titre in culture medium *E. coli* C	Infectivity titre in culture medium *E. coli* SK
P. brevi compactum N 192	6	1 × 10^2	3 × 10^2	0	0
	17	8.5 × 10^2	2.6 × 10^3	0	0
	24	1.2 × 10^3	4 × 10^3	0	0
	72	1 × 10^5	2 × 10^5	0	0
	96	2 × 10^6	9 × 10^6	0	0
	Intact spores	0	0	0	0
	Disrupted spores	1 × 10^3	5 × 10^3	0	0
P. stoloniferum 1458	72	2 × 10^3	—	9 × 10^2	—
P. chrysogenum 194	48	9 × 10^2	—	7 × 10^3	—

The sterile growth conditions of the fungus have been described previously[6]. Twice each day the fungus was seeded to detect bacterial flora. The mycelium was separated from the culture medium by filtering through paper filters and then disintegrated either by grinding with quartz powder or by ultrasonic treatment. Many PB viruses are extremely sensitive to temperature, so it must be kept as low as possible during treatment (0.4° C). The disrupted mycelium was extracted with 0.1 M NaCl in 0.05 M phosphate buffer *p*H 7; the virus was then either concentrated by precipitating with 30% ammonium sulphate or directly titrated on bacteria, using the method of Gracia [8]. The spores were treated in a similar way. All the data in the table refer to the culture medium and mycelium extracted per g crude mycelium.

Table 2 Some characteristics of viruses of the PBV series (from *P. brevi compactum*) and their DNAs

Virus species	Buoyant density in Cs_2SO_4 g cm^{-3}	Buoyant density in CsCl g cm^{-3}	Size of head Å*	T_m of DNA 1 SSC	T_m of DNA 0.1 SSC	Buoyant density in CsCl	Buoyant density in Cs_2SO_4
PBV1	1.38	1.49	425 ± 25	88.5°C	72.8°C	1.7078	1.42
PBV2	1.40	1.48	525 ± 25	87°C	72°C	1.7035	1.42
PBV3	1.42	1.51	—	—	—	—	—

The PB viruses were grown according to the standard procedure[8] in a synthetic medium to which broth was added and were then purified by chromatography on DEAE-cellulose, differential centrifugation and equilibrium centrifugation in a Cs_2SO_4 density gradient. DNA was isolated by the phenol method. The DNA preparations were dissolved in 1 SSC. Standard procedures were used for melting of DNA and for reactions with orcine and diphenylamine. The buoyant density of DNA was determined in an analytical centrifuge with known markers (DNA from phage Sd or T5).

*The sizes of the particles are those reported in our preliminary communication[6].

Table 3 Comparison of some properties of PBV originating from fungi and bacteria

Source of Virus*	Buoyant density in Cs_2SO_4 (g cm^{-3})	Colonies on bacteria Type	Diameter (mm)
Extract of *P. brevi compactum*	1.42	Clear with halo ‡	1.5–2.0
		Clear without halo	1.5–2.0
		Turbid	0.6–0.8
The same + antiserum against PBV2	1.42	Clear with halo ‡	1.5–2.0
		Clear without halo	1.5–2.0
The same + antisera against PBV1 + PBV3 †	1.42	Turbid	0.6–0.8
PBV3 (*E. coli* C)	1.42	Clear with halo	1.5–2.0
Extract of *P. brevi compactum*	1.38	Turbid ‡	0.6–0.8
		Clear without halo	1.5–2.0
PBV2 (*E. coli* SK)	1.38	Turbid	0.6–0.8
Extract of *P. brevi compactum*	1.40	Clear without halo ‡	1.5–2.0
		Turbid	0.6–0.8
		Clear with halo	1.5–2.0
PBV1 (*E. coli* C)	1.40	Clear without halo	1.5–2.0
Extract of *P. brevi compactum*	1.32	Different types, clear and turbid	0.5–1.5
Extract of *P. stoloniferum*	1.40	Clear	1.5
Extract of *P. chrysogenum*	—	Turbid	1.0–1.3

*Fungal extracts were obtained and centrifugated in Cs_2SO_4 gradient, as indicated in the legend to Fig. 1. The fractions with indicated buoyant density were titrated on *E. coli* SK. The pure lines of PB viruses were grown on *E.coli* C (PBV1, PBV3) or *E. coli* SK (PBV2) purified as described in Table 2 and titrated under the same conditions as fungal extracts (see Table 1 and Fig. 1).

† The antisera against PB viruses were obtained by usual method of immunisation of rabbits with purified PBV preparations (titre 5×10^{10} plaques ml^{-1}) [10]. The results indicated in the table were obtained with antiserum dilution 1:10, which provided the 100% neutralisation, antisera diluted 1:100 neutralised the infectivity in fungal extracts due to PBV2 (turbid colonies) and PBV1 + PBV3 (clear colonies) for 88% and 90% respectively. Normal rabbit antisera did not influence the PBV infectivity in fungal extracts.

‡ The predominant type of colonies.

infective virus originated from this prophage.

Like the double-stranded RNA mycoviruses, the viruses of the PBV series may be isolated only from the cultures of *P. brevi compactum*, and *P. stoloniferum* with modified morphology and from the strains of *P. chrysogenum*, which produce penicillin. The 'healthy' cultures of these fungi or cultures not producing the antibiotic produce neither mycoviruses nor PB viruses. Attempts to infect 'healthy' cultures with PBVs have so far failed.

In summary, mycelium of *P. brevi compactum*, *P. chrysogenum* and *P. stoloniferum* produces, in addition to known mycoviruses, DNA-containing viruses capable of reproducing in bacteria. The relationship of these viruses to the host fungus is still obscure; it is possible that PBVs have a temperate lysogenic relationship with the fungus.

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1 Ellis, L. F., and Kleinschmidt, W. J., *Nature*, **215**, 649 (1967).
2 Banks, G. T., Buck, K. W., Chain, E. B., Darbyshire, I. E., and Himmelweit, F., *Nature*, **222**, 89 (1969).
3 Banks, G. T., Buck, K. W., Chain, E. B., Darbyshire, I. E., and Himmelweit, F., *Nature*, **223**, 155 (1969).
4 Hollings, M., and Stone, O. M., *Sci. Prog.*, **57**, 371 (1969).
5 Gause, G. F., Maksimova, T. S., Dudnik, Yu.V., Olchovatova, O. A., Velikodvorskaya, G. A., Klimenko, S. M., and Tikchonenko, T. I., *Microbiologid*, **40**, 540 (1971) (in Russian).
6 Velikodvorskaya, G. A., Bobkova, A. F., Maksimova, T. S., Klimenko, S. M., and Tikchonenko, T. I., *Byull. eksp. Biol. Med.*, **73**, 90 (1972) (in Russian).
7 Wood, H. A., Bozarth, R. F., and Miskives, P. B., *Virology*, **44**, 592 (1971).
8 Gracia, A., *Ann. Inst. Pasteur*, **57**, 652 (1936).
9 Velikodvorskaya, G. A., Bobkova, A. F., Petrovsky, G. V., and Tikchonenko, T. I., *Vop. Virus.*, **3**, 332 (1972) (in Russian).
10 Adams, M. H., *Bacteriophages* (Interscience Publishers, Inc., New York and London, 1959).

Estimation of the size of a T-cell-induced lytic lesion

The specific cytolytic activity exhibited *in vitro* by thymus-derived (T) lymphocyte populations from immune animals (see review in ref. 1), is germane to allograft rejection[2,3] and to pathways by which neoplastic tissue can be destroyed *in vivo*[4,5]. The mechanism involved, however, remains controversial (for one viewpoint see ref. 6) and the nature of the lytic lesion is unknown.

T-cell-mediated lysis seems to be unrelated to the complement system, for not only can lysis take place in the absence of serum, but added complement augments neither the rate nor the extent of lysis[7]. Perhaps more pertinently, antisera against complement components C_2, C_3 and C_5 do not interfere with the lytic process[8].

Time-lapse cinematography of lymphocyte-mediated cytolysis revealed lysis as a ballooning phenomenon, in which the antigen-bearing (target) cell became swollen and ruptured after contact with a lymphocyte[9]. Studies from this laboratory on the efflux of material of different molecular sizes from injured target cells have established that the T-cell-induced lytic lesion is progressive[10]. Low molecular weight markers (ATP, ^{86}Rb) were shown to efflux from the damaged target cell before macromolecules (^{51}Cr-protein, ^{3}H-DNA) could pass through the cell membrane. These findings suggest that the original dimensions of the lytic lesion are smaller than the effective diffusion radius of the ^{51}Cr-protein complexes, and are incompatible with 'big-bang' concepts of lymphocyte-induced target cell lysis.

The preferential early efflux of low molecular weight material from target cells suggested that the eventual rupture of the membrane, and the swelling of the target cell observed morphologically, were due to colloid osmotic swelling. This is supported by the findings reported here, for by means of techniques[11,12] used earlier to characterise complement lesions, it was found that when cultures of lymphocytes and target cells were maintained in a solution of high molecular weight (>40,000) dextran, ^{51}Cr-protein release from the target cells could be inhibited while ^{86}Rb efflux continued. Using a series of dextrans of varying molecular size, it was inferred that the initial T-cell-induced lytic lesion in a mastocytoma target cell is of the order of 35–45 Å in radius.

The assay system used, the lysis of DBA/2 mastocytoma cells by spleen cells from alloimmunised C57BL/6 mice, has been described in detail (see legend to Fig. 1)[13,14]. The same system was used to measure specific ^{51}Cr and ^{86}Rb efflux[10]. During the assay times used, specific release of both labels was linear after a lag of 10-15 min for ^{86}Rb and approximately 45 min for ^{51}Cr. The assays were of comparable sensitivity, in that the minimal number of lymphocytes required to cause significant release of label was equal[10]. Typical values for isotope release from 10^5 target cells with 10^7 spleen cells is shown in Table 1.

We have shown[10] that target cell damage after addition of lymphocytes occurs within 15 min, and is characterised by efflux of low molecular weight material. Macromolecules are only released later (> 45 min). If the efflux of high molecular weight material results from osmoregulatory damage, caused by water influx through the initial lesions, then maintaining

Table 1 Release of ^{51}Cr and ^{86}Rb from DBA/2 mastocytoma cells in the presence of immune C57B/6 spleen cells

Time after target cell addition (h)	% ^{51}Cr release		% specific cytolysis	^{86}Rb release			
	Immune	Normal		Time (min)	Normal (c.p.m.)	Immune (c.p.m.)	Specific ^{86}Rb release (△c.p.m.)
1	9.2±1.8	3.6±0.4	5.6	15	541±17	625±20	84
2	22.9±4.0	4.1±0.2	18.8	30	935±22	1,360±18	425
3	40.4±3.7	5.2±0.3	35.2	45	1,294±8	2,124±75	830
4	56.3±5.2	6.5±0.5	49.8	60	1,669±13	2,871±12	1,202

C57BL/6 spleen cells from unimmunised (normal) animals or from mice immunised 10 d previously with 10^7 DBA/2 mastocytoma cells intraperitoneally were incubated with 10^5 ^{51}Cr DBA/2 mastocytoma cells for periods up to 4 h (or with 10^5 ^{86}Rb target cells for periods up to 1h). For the ^{51}Cr release percentage isotope release was calculated relative to the total number of cell-associated counts added to the system. Percentage specific cytolysis merely as the arithmetic difference between release in the presence of immune cells and that with normal cells. Values given are means ± s.d. of quadruplicate assays in one experiment. For the ^{86}Rb assay, because of high turnover of ^{86}Rb in lytic assays and the variability in reporting specific cytolysis from various laboratories (contrast for example the calculation above with that reported in ref. 13), it is considered preferable here to report raw data. The total cell-associated counts at time zero were 4,646±420 c.p.m., the total supernatant counts in the presence of normal and immune lymphocytes is given as c.p.m. ±s.d. of quadruplicate assays. The difference between the mean values was taken as specific ^{86}Rb release. Percentage inhibition of cytolysis in this system was calculated relative to this value.

the target cells in solutions of high molecular weight (non-penetrating) molecules should prevent the progression of the lesion, and prevent macromolecular efflux[11,12].

Using dextrans of various molecular weights, we established that solutions inhibiting cytolysis (^{51}Cr release) were of high intrinsic viscosity. It was considered important, therefore, to establish that inhibition of ^{51}Cr release was due to prevention of macromolecular release from cells bearing a lytic lesion, rather than to inhibition of lymphocyte-target cell interactions by solutions of high viscosity[9,15]. We argued that if inhibition of both ^{86}Rb and ^{51}Cr release was observed, this represented an inhibition of lymphocyte–target cell interaction. If, on the other hand, ^{86}Rb efflux was demonstrable when ^{51}Cr was not, this was regarded as inhibition of the progression of the lesion. Such arguments depend on the following suppositions: (i) that ^{86}Rb and ^{51}Cr are released from injured cells by basically the same mechanism; and (ii) that ^{86}Rb and ^{51}Cr are located either in the same intracellular pool, or that they have equal access to holes in the cell wall.

It follows that inhibitors of cell interactions should affect ^{86}Rb and ^{51}Cr release comparably. The effect of cytochalasin B, an inhibitor of cytolysis[16-19], was thus assayed. Cytochalasin B (0.4-6 μg ml^{-1}) similarly inhibited ^{86}Rb efflux and ^{51}Cr release (Table 2). Additionally, we observed[10] that an inhibitor of the potassium pump (ouabain) affected neither ^{86}Rb nor ^{51}Cr release. These findings are both consistent with the suppositions made above.

Parallel inhibition of ^{86}Rb and ^{51}Cr release was shown when lymphocyte–target cell interactions were made in a dextran solution of mean molecular weight 9,300 (Pharmacia size T10). At concentrations of 20 mM, complete inhibition of specific ^{86}Rb and ^{51}Cr release was found. Decreasing dextran concentrations resulted in a dose-dependent and parallel inhibition of specific ^{86}Rb and ^{51}Cr release (Fig. 1*a*).

Dextrans of mean molecular weight 70,000 (Pharmacia T70) and 231,000 (Pharmacia T250) inhibited specific ^{51}Cr release significantly more than they did ^{86}Rb flux (Figs. 1*b* and *c*). For example, in 0.5 mM, 70,000 molecular weight dextran, specific ^{86}Rb release was suppressed only 6.1% (range 4.2–8.1), whereas ^{51}Cr release was inhibited 38.0% (range 35.0–43.2). Similarly, in 0.1 mM, 231,000 molecular weight dextran, ^{86}Rb release was inhibited 6.5% (range 4.3–9.5) whereas

Table 2 Inhibition of specific ^{86}Rb and ^{51}Cr efflux in the presence of cytochalasin B

Concentration cytochalasin B (μg ml^{-1})	% Inhibition specific cytolysis	
	^{51}Cr	^{86}Rb
0.375	10.0	14.2
0.75	35.2	39.8
1.5	52.0	47.6
3	85.1	79.2
6	97.2	93.4

10^7 immune C57BL/6 lymphocytes (10 d after 10^7 DBA/2 mastocytoma cells intraperitoneally) were incubated with 10^5 ^{51}Cr DBA/2 mastocytoma cells for 4 h at 37°C (or with 10^5 ^{86}Rb target cells for 60 min) in the presence of cytochalasin B. The percentage inhibition of specific cytolysis was calculated relative to control tubes without inhibitor. Data represents mean values of two experiments in each of which the assays were performed in duplicate. Replicate essays in each experiment varied <5%; values from one experiment to the other varied approximately 10%. Cytochalasin B had no effect on the release of either isotope in control cultures.

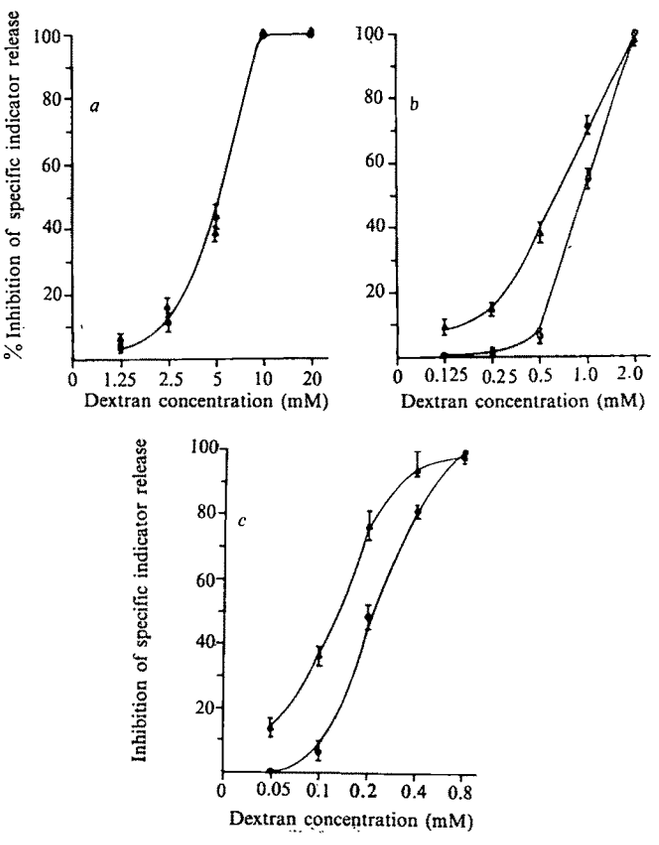


Fig. 1 C57BL/6 spleen cells (10^7) derived from mice immunised 10 d earlier with 10^7 DBA/2 mastocytoma cells intraperitoneally, were incubated with 10^5 mastocytoma cells labelled either with ^{51}Cr(▲) or with ^{86}Rb (●). The cells were mixed in Eagle's minimal essential medium containing 10% foetal calf serum and varying concentrations of dextran. Cytolysis was evaluated 1 h (for ^{86}Rb assays) or 4 h (for ^{51}Cr assays) later. Specific cytolysis was calculated after subtraction of isotope release from control cultures containing 10^7 normal C57B/6 spleen cells in the same concentrations of dextran. Inhibition of specific cytolysis was calculated relative to cultures containing no dextran. The values given are means and range of quadruplicate cultures. Molecular weight: (*a*) 9,300; (*b*) 70,000; (*c*) 231,000.

Table 3 Addition of cytochalasin B or dextran solutions to cytolytic reactions: effect on subsequent ^{51}Cr release

Reacting cells	Inhibitor added after 1 h of incubation†	% Specific ^{51}Cr release in the 2 h following inhibitor addition Expt 1	Expt 2	Expt 3
10^7 immune C57BL/6 spleen cells	None (medium alone)	20.5 (5.2*)	32.3 (3.1*)	28.7 (6.4*)
	Cytochalasin B	7.3	11.5	8.9
	Dextran 9,300	5.8	—	7.5
+	Dextran 70,000	0.2	0.1	0.7
	Dextran 231,000	—	0.5	0.2
10^5 ^{51}Cr DBA/2 mastocytoma cells	BSA	—	3.1	—

* Percentage specific lysis at time of inhibitor addition.
† Inhibitors were added to give a final concentration known to be sufficient to totally inhibit specific ^{51}Cr release if present at time of lymphocyte addition to target cells. These final concentrations were: cytochalasin B, 6 μg ml^{-1}; dextran 9,300, 20 mM; dextran 70,000, 2 mM; dextran 231,000, 0.8 mM; BSA, 25 g%. Values given are means of triplicate assays. Replicates in all cases varied < 10%.
Neither dextran nor cytochalasin B solutions affected spontaneous ^{51}Cr release in parallel cultures containing normal lymphocytes.

^{51}Cr release was inhibited 36.2% (range 33.0–39.1). These findings suggest that the high molecular weight dextrans prevent hole enlargement.

We have previously shown that when cytochalasin B is added to a lytic reaction in progress, ^{51}Cr release continues for a period of approximately 1 h before complete inhibition is demonstrable[17]. It seems reasonable to assume that such release is derived from target cells which bore holes before addition of inhibitor, but that such lesions were not initially large enough to accommodate macromolecular passage. It follows that hole enlargement continued in the presence of cytochalasin B and that inhibition was due to prevention of new lymphocyte–target cell interactions[18].

When low (9,300) molecular weight dextran was added to a lytic reaction already in progress, ^{51}Cr release continued in a manner comparable with that found with cytochalasin B (Table 3). In contrast, addition of 70,000 and 231,000 molecular weight dextrans completely inhibited subsequent ^{51}Cr release. Presumably only those molecules which are too large to penetrate the initial hole can afford osmotic protection against water influx (and the subsequent ^{51}Cr efflux).

Dextran solutions of 40,000 molecular weight also gave protection against ^{51}Cr macromolecular efflux, although less than that obtained with 70,000 molecular weight material. Minimal inhibition of ^{51}Cr release was also obtained by addition of 25 g % bovine serum albumin to an ongoing lytic reaction (for example, experiment 2, Table 3).

The single most important criterion dictating molecular passage across a semipermeable membrane seems to be the molecule's effective diffusion radius (R_{ES}) calculated from the Stokes-Einstein equation[20]. From values in the literature[21] for the molecules used in this study, it would seem that dextran molecular weight 40,000 ($R_{ES} \sim 45$Å) and bovine serum albumin of molecular weight 68,000 (R_{ES} 35.5Å), by affording minimal significant inhibition of ^{51}Cr-protein release from cells bearing a Rb hole, represent the approximate initial dimensions of the T-cell-induced lytic lesion.

These findings show a striking similarity to the nature of the antibody induced complement lesion in erythrocytes[12] and in Krebs ascites tumour cells[11,22]. In these cases too, macromolecular passage across a damaged (K permeable) cell membrane, could be prevented by maintaining the cells in non-penetrating protein[22] or dextran solutions[12]. For reasons advanced earlier, there is good reason to suppose that the complement system is not involved in the T-cell-mediated lytic lesion[7,8]. The nature of the effector molecule remains, however, in doubt.

Currently, it seems reasonable to suppose that the initial lytic lesion in the target cell membrane is inserted during the contact between effector T lymphocyte and its homologous target cell. After this initial lesion, the continued presence of the lymphocyte is unnecessary[23], and the demise of the target cell results from its own inability to handle the augmented influx of water into its cytoplasm.

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Note added in proof: Seeman has recently claimed[24] that the approaches used here to estimate lesion size are invalid. He reports that the efflux of haemoglobin (Hb) from hypotonically lysed cells is suppressed by the presence of extracellular macromolecules, even when the size of the membrane lesion is much greater than the effective diffusion radius of the extracellular molecules. He argues that the macromolecules act simply by reducing the diffusion rate of Hb, a suppression which, he asserts, is dependent on the weight-concentration, and not the molecular weight, of the extracellular macromolecule. The latter assertion makes it unlikely that this is an adequate explanation of the phenomenon described here for dextrans of molecular weight 9,000 and 70,000, used at the same weight concentrations, have very different effects on ^{51}Cr-protein release (Fig. 1).

1 Perlmann, P., and Holm, G., *Adv. Immun.*, **11**, 117 (1969).
2 Berke, G., Yagil, G., Ginsburg, H., and Feldman, M., *Immunology*, **17**, 723 (1969).
3 Rouse, B. T., Wagner, H., and Harris, A. W., *J. Immun.*, **109**, 1282 (1972).
4 Freedman, L. R., Cerottini, J-C. and Brunner, K. T., *J. Immun.*, **109**, 1371 (1972).
5 Rouse, B. T., Wagner, H., and Harris, A. W., *J. Immun.*, **108** : 1353 (1972).
6 Henney, C. S., *Transplant. Rev.*, **17**, 37 (1973).
7 Canty, T. G., and Wunderlich, J. R., *J. natn. Cancer Inst.*, **45**, 761 (1970).
8 Henney, C. S., and Mayer, M. M., *Cell. Immun.*, **2**, 702 (1971).
9 Rosenau, W., *Fed. Proc.*, **27**, 34 (1968).
10 Henney, C. S., *J. Immun.*, **110**, 73 (1973).
11 Goldberg, B., and Green, H., *J. exp. Med.*, **109**, 505 (1959).
12 Scott, J. G., Weed, R. I., and Swisher, S. N., *J. Immun.*, **96**, 119 (1966).
13 Brunner, K. T., Mauel, J., Cerottini, J-C. and Chapuis, B., *Immunology*, **14**, 181 (1968).
15 Wilson, D. B., *J. exp. Med.*, **122**, 143 (1965).
16 Cerottini, J-C., and Brunner, K. T., *Nature new Biol.*, **237**, 272 (1972).
17 Plaut, M., Lichtenstein, L. M., and Henney, C. S., *J. Immun.*, **110**, 771 (1973).
18 Henney, C. S., and Bubbers, J. E., *J. Immun.*, **111**, 85 (1973).
19 Yoshinaga, M., Waksman, B. H., and Malawista, S. E., *Science*, **176**, 1147 (1972).
20 Pappenheimer, J. R., Renkin, E. M., and Borrero, L. M., *Am. J. Physiol.*, **167**, 13 (1951).
21 Grotte, G., *Acta. chir. scand.* (Suppl.) **211** (1956).
22 Green, H., Barrow, P., and Goldberg, B., *J. exp. Med.*, **110**, 699 (1959).
23 Martz, E., and Benacerraf, B., *J. Immun.*, **111**, 1538 (1973).
24 Seeman, P., *Fed. Proc.* (in the press).

Immunosuppression detected in pregnant mice by rosette inhibition test

ATTENTION has recently been drawn to the immunological mechanisms concerned in the nonrecognition of the antigenic status of the foetus during uterine life. Several workers[1–3] have demonstrated that lymphocyte transformation is suppressed during pregnancy. As the rosette inhibition test[4] has been used to show that the lymphocyte activity *in vivo* is modified by immunosuppressive therapy following trasnplantation[5], it suggests that this technique could also be used to investigate possible immunosuppression during pregnancy.

The rosette inhibition titre (RIT) was estimated as described previously[6]. Antilymphocytic serum (ALS) was produced against mouse spleen cells, and was tested for its effect on rosette formation between mouse spleen cells and human red blood cells (HRBC), (H.M., V.H., and G.J.A.C., unpublished). The HRBC were collected fresh each day from the same donor. The ALS was diluted to 1×10^6 and then serially diluted and tested in the range 1×10^6 to $32{,}768\times10^6$. The spleen cells used were from Quackenbush mice aged between 8 and 12 weeks. The RIT was recorded as the highest dilution of ALS at which the number of rosettes formed was 75% or less of the control (Hank's solution without ALS). The RIT of the ALS produced in this series showed significant correlation with the skin graft survival time.

The results of the RIT performed in two mice, a nonpregnant and a pregnant female respectively, are illustrated in Fig. 1, in which the rosette formation as a percentage of the control (without ALS) is plotted against the dilution of ALS. There was a marked difference in RIT, that in the nonpregnant female being 16×10^6 and in the pregnant female $8{,}192\times10^6$. The RIT of ten pregnant females and the normal group of five males and six non pregnant females is illustrated in Fig. 2. The logarithmic mean of RIT $\times10^{-6}$ (to base 2) in the normal group was 3.2 (s.e., 0.42). The RIT of the pregnant group ranged from 32×10^6 to $8{,}192\times10^6$ (log mean 10.2 s.e., 1.05). There was no significant difference between the mean of the titres of the group containing five males and the group containing six nonpregnant females. There was a significant difference between the mean of the titres of the nonpregnant group (five males plus six females) and the pregnant group (ten females) $P< 0.001$. The mean number of spontaneous rosettes formed in the control with spleen cells from five male mice and six non pregnant female mice was 31.2 (s.e., 2.2) rosettes per 1,500 spleen cells, and the mean number in the control with spleen cells from ten pregnant females was 33.9 (s.e., 2.7) rosettes per 1,500 spleen cells. There is no significant difference between the number of rosettes formed in these two groups.

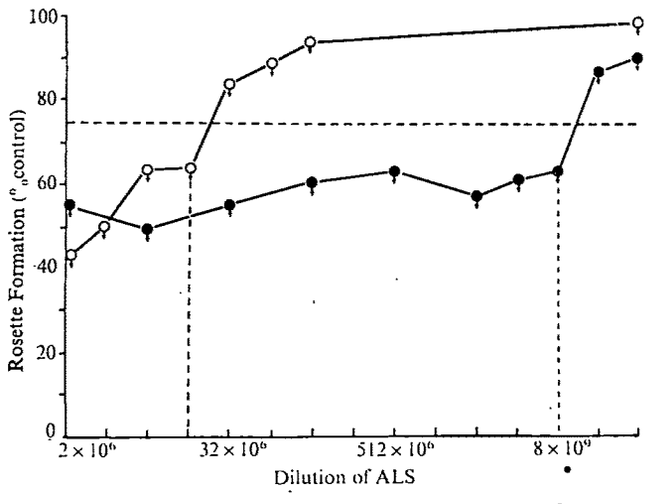


Fig. 1 Rosette formation (as % of the control) with lymphocytes from a pregnant (♀) and a nonpregnant (♀) mouse plotted against ALS dilution, illustrating the high RIT during pregnancy.

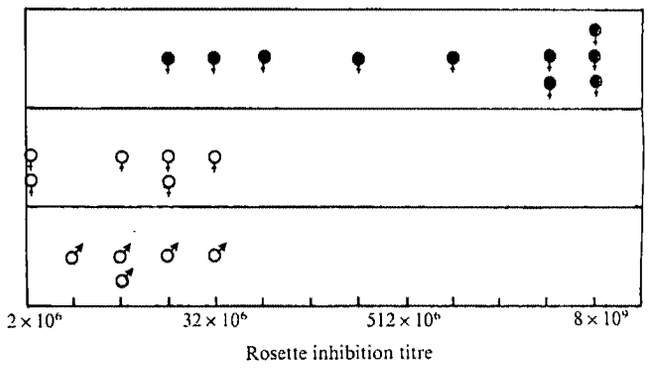


Fig. 2 RIT in male (♂), nonpregnant female (♀), and pregnant female mice (♀), showing rise in RIT in some pregnant females.

To investigate this wide range of RIT in pregnant mice and to follow an apparent change in relation to the stage of pregnancy, mice were killed at different stages after mating and the stage of pregnancy recorded as the crown–rump length of the foetus. A plot of RIT of pregnant mice against crown–rump length of foetus (Fig. 3) indicates

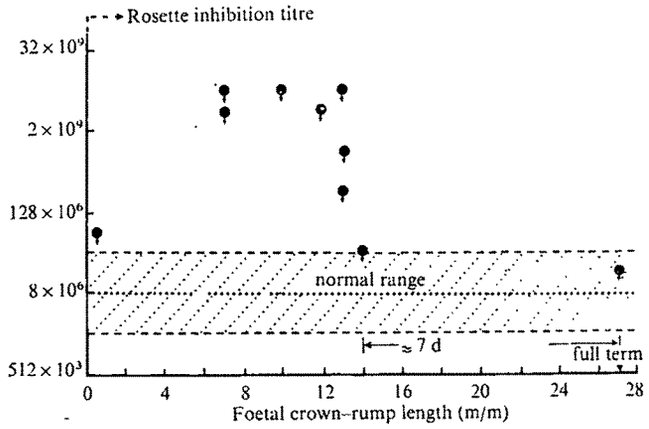


Fig. 3 RIT plotted against stage of pregnancy as measured by crown–rump length, showing a rise in RIT during pregnancy and a fall to normal before delivery.

that the RIT starts to rise early in pregnancy, and then drops to within the normal range approximately 7 d before delivery. The RIT of a mouse tested at full term lay within the normal range.

Our findings support the work of others[1–3] that the immunological competence of material lymphocytes is modified during pregnancy. They found that the phytohaemagglutinin - induced transformation was reduced. Finn *et al.* raised the question whether this was due to a central suppression of the T cells in pregnancy or to fewer T cells present to be stimulated. Our findings support the first of these two hypotheses. If there were fewer T cells to be stimulated then the number of spontaneous rosettes formed in the pregnant group would be less than the number formed in the nonpregnant group. We have found that this is not so, that there was no significant difference between the means of the results obtained from each group.

Contractor *et al.*[3] have concluded from their investigations that HCS and HCG, produced by the placenta, are either binding or competing for the site of PHA stimulation. From our investigations, it seems likely that a similar reaction is occurring. The number of spontaneous rosette-forming cells in pregnancy is not decreased but the number of combining sites on these cells, available for the immunosuppressive activity of the ALS, is decreased. This is shown by the rosette forming activity of these cells being inhibited by much higher dilutions of ALS than those cells from nonpregnant animals. This indicates that the blocking of binding sites on the lymphocyte *in vivo* impairs its immunological competence. We have shown that the RIT begins to return to within normal limits at the beginning of the third week of pregnancy and this lends support to the theory of Contractor *et al.*, that immunorejection may play a part in stimulating parturition.

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1 Purtilo, D. T., Hallgren, H. M., and Yunis, E. J., *Lancet*, **i**, 769 (1972).
2 Finn, R., St. Hill, C. A., Govan, A. J., Ralfs, I. G., Gurney, F. J., and Denye, V., *Br. med. J.*, **3**, 150 (1972).
3 Contractor, S. F., and Davies, H., *Nature new Biol.*, **243**, 284 (1973).
4 Bach, J. F., Dormont, J., Dardenne, M., and Balner, H., *Transplantation*, **8**, 265 (1969).
5 Munro, A., Bewick, M., Manuel, L., Cameron, J. S., Ellis, F. G., Boulton-Jones, M., and Ogg, C. S., *Br. med. J.*, **3**, 271 (1971).
6 Morton, H., Hegh, V., and Clunie, G. J. A., *Clin. exp. Immun.*, **13**, 595 (1973).

Stabilisation of interferons by defensive reversible denaturation

THE stabilities of interferons vary widely, depending on their source and degree of contamination. Even relatively stable interferons seem to become labile as various degrees of purity are attained[1]. In the absence of a more suitable means of stabilisation, some investigators rely on addition of extraneous protein to stabilise interferons during purification[2], thus, a method for stabilising interferons would have considerable value.

The commonly encountered inactivations of interferons (deterioration on storage, repeated freezing and thawing, surface inactivation by shaking or frothing, or exposure to heat) occur under conditions where alterations of primary structurrs of protein are not likely; therefore, such inactivations probably result from denaturations involving only disruptions in secondary and tertiary structures. Theoretically such denaturations should be reversible.

Denatured proteins are more likely to return to native conformations if their amino acid sequence is in a linear random coil[3], that is, a primary structure in which all noncovalent bonds and all cross links (disulphide bonds) have been broken. Since some interferons have been reported to contain disulphide bonds[1,4], attainment of a linear polypeptide should require reduction of such groups. As with some interferons, unfolding of the native protein is required to make cross linkages accessible to attack, an agent to disrupt noncovalent bonds would be required. Presumably, interferon polypeptides maintained in

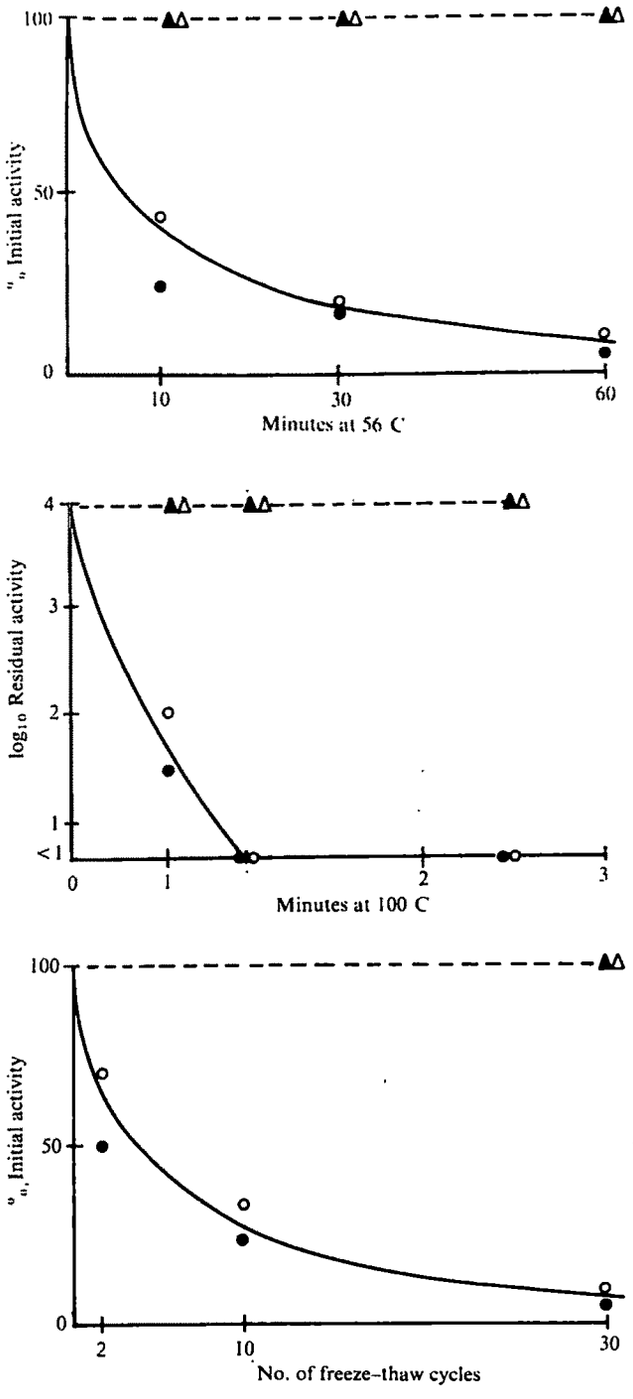


Fig. 1 Stabilisation of interferons by 'defensive' reversible denaturation. Interferons were subjected to the indicated treatment in their native state (○, ●) or in the 'defensively' denatured state (△, ▲). ●, ▲, Mouse interferon; ○, △, human interferon.

such a conformation should be stabilised against inactivation by procedures not inducing covalent changes in the primary structure. To maintain interferon in such a state, we selected a system consisting of urea, 2-mercaptoethanol, and the sodium dodecyl sulphate (SDS). The first two agents should provide random coils, which would theoretically be reversibly denatured, but in practice, oxidation of sulphydryl groups or exchange of sulphydryl and disulphide bonds are almost unavoidable upon their removal[3]. SDS should, however, defend the reactive groups against interactions[5].

Mouse interferon prepared in L_{929} cells induced with Newcastle disease virus was acidified to *p*H 2, extraneous proteins were precipitated by addition of ammonium sulphate to 20% saturation at 20° C, and clarified supernatant fluid was adjusted to *p*H 7.2 by dialysis against 0.01 M Tris HCl buffer. Prepara-

Table 1 Labilities of unfolded and reduced interferons under conditions allowing intra and intermolecular interaction

	Reagent	Initially	$\log_{10}$ Activity After 56° C 30 min	After 100° C 2 min
Mouse interferon	None	3.8	3.0	< 1
	Mercaptoethanol (1.4×10^{-2} M)	3.5	1.5	< 1
	Urea (5 M)	3.3	1.0	< 1
	Mercaptoethanol + urea	3.0	< 1	< 1
Human interferon	None	4.0	3.5	< 1
	Mercaptoethanol	3.8	1.5	< 1
	Urea	3.5	1.5	< 1
	Mercaptoethanol + urea	2.8	< 1	< 1

tions had specific activities of about 10^6 units per mg protein. Human interferon produced in human diploid fibroblast cells induced with poly(I)·poly(C) was dialysed against 0.01 M Tris HCl buffer, *p*H 7.2.

The labilities of mouse and human interferons to various denaturation procedures were remarkably similar (Fig. 1). Heat inactivation kinetics of mouse interferons were independent of the purity of the preparation: preparations with about 10^4, 10^5, 10^6 or 10^7 units per mg protein showed identical inactivation kinetics.

The interferons were only slightly affected by either urea or mercaptoethanol at room temperature and exhibited the same 100° C heat lability in their presence as was seen in their absence; at 56° C, these agents markedly accelerated the inactivation rate (Table 1). The combination of urea and mercaptoethanol caused a greater loss of initial activity and, again, had no stabilising effect on the residual activity. Interferons denatured under these conditions seemingly allow intramolecular and intermolecular interplay.

Addition of SDS to denatured, reduced proteins might be expected to defend against this random, nonselective interplay of reactive groups which presumably contributed to the seemingly irreversible denaturation. Reduced proteins dissociated by urea and SDS seem to stay dissociated in SDS after removal of the unfolding and reducing agents[6,7]. Both human and mouse interferons in the SDS-'defended' state were completely stable to heat and freeze-thaw cycles (Fig. 1). 'Defensive' reversible denaturation was achieved by making the interferons 3.5×10^{-3} M SDS, 1.4×10^{-2} M 2-mercaptoethanol and 5 M urea; in other experiments 3.5×10^{-2} M SDS, 1.4×10^{-1} M 2-mercaptoethanol and 5 M urea gave identical results. Presumably, interferons in this condition are converted to linear peptide chains and after removal of urea, mercaptoethanol and excess SDS by dialysis, the SDS–interferon remains dissociated and returns to an active conformation when incubated with protein-containing medium. Significantly, addition of SDS alone to the interferons reduced their activities by between 60 and 90%, but the residual activity was stabilised in the presence of SDS alone. This suggests that interferons can renature to some extent from configurations that have cross-linked peptide chains, but can renature more efficiently from the linear polypeptide supposedly attained in SDS, urea and mercaptoethanol. Alternatively, this could suggest there are two distinct molecular species in these interferon preparations and that the major component does not renature after treatment with SDS alone, but does so if it is unfolded in SDS and mercaptoethanol, while the minor component is able to unfold in SDS alone and to renature. Since there are no known protein aggregates linked by noncovalent bonds that survive treatment with SDS at 100° C for 1 min (ref. 5), this stabilising process allows the determination of the molecular species of dissociated, denatured and reduced interferons by SDS–polyacrylamide gel electrophoresis and the relative biological (antiviral as against nonantiviral)[8] properties resident in the renatured molecular species of interferons.

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1 Ng, N.H., and Vilcek, J., *Adv. Protein Chem.*, **26**, 173-241 (1972).
2 Stancek, D., and Paucker, K., *Acta virol.*, **14**, 125-134 (1970).
3 Tanford, C., *Adv. Protein Chem.*, **23**, 121-282 (1968).
4 Weil, R., and Dorner, F. in *Selective inhibitors of viral functions* (edit. by Carter, W. D.) 107-121 (CRC Press, Cleveland, 1973).
5 Maizel, J. V., jun., in, *Methods in Virology* (edit. by Koprowski, H. and Maramorosch, K.) 179-246 (Academic New York 1970).
6 Tanford, C., *Adv. Protein Chem.*, **24**, 1 95 (1970).
7 Ray A., Reynolds, J., Polet, H., and Steinhardt, J., *Biochemistry*, **5**, 2606-2617 (1966).
8 Stewart W. E. II, De Clercq, E., De Somer, P., Berg, K., Ogburn, C. A., and Paucker, K., *Nature new Biol.*, **246**, 141-143 (1973).

Surface H-2 antigen concentration requirement of somatic hybrid cells for IgM-mediated cytotoxicity

THE immune response following grafting of allogeneic or semiallogeneic cells in the mouse is characterised by the development of cytotoxic effector lymphocytes and/or antibodies which become cytotoxic *in vitro* in the presence of complement. I have studied the kinetics of the humoral response in DBA/2 inbred mice injected with semiallogeneic somatic cell hybrids and shown that these hybrid cells were unexpectedly resistant to the complement-dependent cytotoxic action of antisera from the initial peak of the biphasic response. The allogeneic parental cells were however highly sensitive to cytolysis under the same experimental conditions. This serological response seems to be directed against the H-2 alloantigens expressed on both the allogeneic parental cells and on the semiallogeneic hybrid cells derived from it. In my attempts to understand this phenomenon, I found that the mouse alloantibodies of the IgM and IgG classes have different efficiencies when tested in the cytotoxic test on cells having a low H-2 antigen concentration on their plasma membrane.

Sera from the first peak (which extended from days 6-9 after injection of Y2C hybrid cells exhibited a clear cytotoxic effect when tested against the allogeneic parental cell C1 1D (sera 1, Table 1). The mean titre, defined as the dilution of serum at which 50% of chromium was released into the supernatant, was 1:32. But when labelled Y2C cells were the target cells, most of these sera did not liberate significantly more chromium than the chromium release background. (Background release was measured in controls where Y2C cells were incubated with complement alone, or with complement and serum from non-immunised DBA/2 mice). Sera from hyperimmunised mice, however, were equally effective against both C1 1D and Y2C cells. The mean titre for C1 1D was 1:128; for Y2C cells, 1:256 (sera 2, Table 1).

This response was directed mainly against the $H\text{-}2^k$ transplantation antigens as was demonstrated by immunoabsorp-

Table 1 Cytotoxic activity of antisera as a function of antibody size and target cell use

Antisera group	Cell injected	Immunised strain	H-2 antigens against which antibodies were made	Antibody size	Target cell	Mean antisera titres
1*	Y2C	DBA/2	H-2^k	19S	C11D	1:32
					Y2C	< 1:4
2†	YC2	DBA/2	H-2^k	19S	311D	1:128
				7S	Y2C	1:256
3	C3H lymphocytes	BALB/c	H-2^k	7S	C11D	1:2,048
					Y2C	1:2,048
4	DBA/2 lymphocytes	C3H	H-2^d	7S	L1210	1:2,048
					Y2C	1:2,048
5	(C3H × DBA/2)F1 lymphocytes	B10.M	H-2^k		L1210	1:4
			H-2^d	19S	C11D	1:4
					Y2C	1:4

* Sixteen individual antisera were tested.

† Produced by nine weekly i.p. injections of 3 × 10^6 Y2C cells. Sera from eight different mice were tested.

For groups 1 and 2 Y2C hybrid clone was derived from the fusion of L1210-R lymphocytic leukaemia cells of DBA/2 mouse origin (H2^d) (ref. 1) with LM(TK$^-$) C1 1D cells, a subclone of C3H mouse L cell line (H2^k) (ref. 2). Cells were fused using ultraviolet-inactivated Sendai virus[3]. The isolation and culture conditions for *in vitro* growth of the hybrid clones have been described elsewhere[4]. C1 1D and L1210 parental and Y2C hybrid cells were propagated in culture in Dulbecco's modified Eagle's medium with 10% heat-inactivated calf serum. The cells (except L1210 cells which grew in suspension cultures) were dispersed with 0.02% versene in Dulbecco's phosphate buffered saline (PBS) rinsed with PBS and suspended in Dulbecco's medium. Three month old DBA/2 mice were inoculated subcutaneously with 3 × 10^6 viable Y2C hybrid cells and bled daily; sera were stored at −20° C until tested. Their activity was measured as complement-dependent cytotoxicity by the chromium release test[5]. One millilitre of a target cell suspension (5 × 10^6 viable cells ml^{-1}) was incubated at 37° C for 30 min with 200 μCi sodium chromate (^{51}Cr) and washed exhaustively with Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated foetal bovine serum. 1 × 10^5 labelled cells were incubated with serial two-fold dilutions of antisera in the presence of rabbit serum (diluted 1:10) as a source of complement, in a final volume of 0.25 ml. At the end of the incubation period (1 h at 37° C in a 5% CO_2 atmosphere), 1.75 ml of ice-cold PBS was added to each tube, and after centrifugation, 1 ml of the supernatant was assayed for released chromium in a well-type gamma radioactivity counter.

tion experiments. Some cytotoxic activity remained after the absorption of hyperimmunised DBA/2 mice sera with C3H mouse splenic lymphocytes; this residual cytotoxicity might be directed against the virus-induced antigens on the L cell line[6], and in hybrid cells in which L cell derivatives (C1 1D) were partners[7–9].

The sizes of the antibodies for the initial and second peak were studied by fractionating the sera at 4° C on a Sephadex G-200 column (1.5×60 cm) equilibrated with PBS. Fractions were concentrated by B 15 Minicon ultrafiltration (Amicon) and tested immediately for cytotoxicity against ^{51}Cr-labelled cells. Only 19S antibodies could be found in the initial peak of the immune response; both 19S and 7S antibodies of approximately equal cytotoxic titres coexisted in the sera from the hyperimmunised mice. When tested for sensitivity to 2-mercaptoethanol, the 19S fraction completely lost its activity but the cytotoxicity of the 7S fraction was only slightly reduced.

In another experiment, both parental and hybrid cells were titred by alloantisera specifically directed against the H-2^d and the H-2^k transplantation antigens. Antisera, made in BALB/c and C3H inbred mice by hyperimmunisation with lymphnode and splenic lymphocytes of C3H and DBA/2 origin, respectively, were tested against target cells. Fractionation of these sera showed that the bulk of the activity was due to molecules of the 7S class. The titres were 1:2,048 when tested against the lymphocytes used in the immunisation. The same high activity was found when titred against the allogeneic parental cell and the Y2C somatic hybrid (sera 3 and 4, Table I) demonstrating that, in accordance with other reports[7–9], the hybrid clone expresses major histocompatibility antigens of both strains of mice from which the parental cells were derived and that Y2C cells were very actively lysed by 7S antibodies. Furthermore, when those antisera were tested on Y2C cells by the indirect immunofluorescence technique, a clear positive reaction was observed on 97% of cells.

Since some reports have stated that mouse 19S antibodies react preferentially with guinea pig complement[10], the cytotoxic test with sera from group 1 was performed in the presence of fresh rat (diluted 1:4) and lyophilised guinea pig sera (1:2) as a source of complement. No improvement was found in ^{51}Cr release when such complements were used instead of rabbit complement.

As antigen masking by sialic acid may occur in both normal cells and tumour cells[11,12], removing sialic acid from Y2C cell surfaces by neuraminidase might improve the cytotoxic activity of IgM. Five million labelled Y2C hybrid cells were incubated with 5 IU of *Vibrio cholerae* neuraminidase (Behringwerke) for 20 min at 37° C in 1 ml of PBS (*p*H 6.5). After washing, the viability was determined by trypan blue dye exclusion and cells were assayed for susceptibility to 19S-mediated cytotoxic effect. Neuraminidase-treated cells became more sensitive to the action of IgM (mean titres changed from < 1:4 to 1:8) presumably as the result of the re-exposure of antigenic determinants, with the concomitant increase in complement-fixing sites.

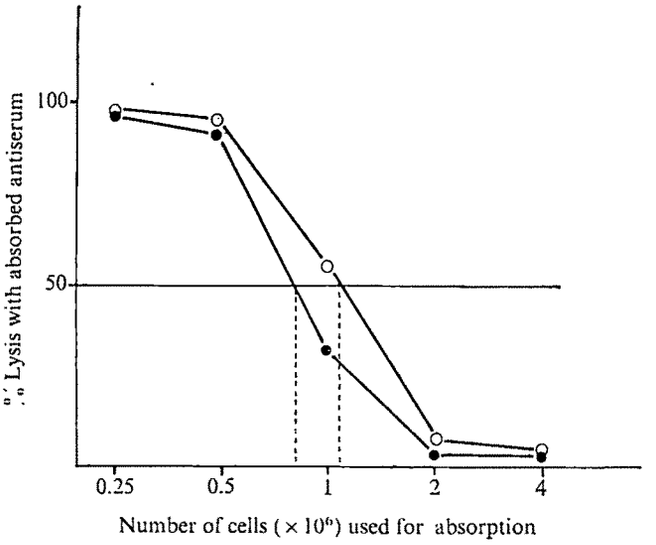


Fig. 1 Antibody binding capacity of C1 1D and Y2C cells. Serum 3, diluted to give a 90–95% cytotoxic activity was absorbed with increasing numbers of cells at 37° C for 30 min and then allowed to stand for 30 min at 4° C. After centrifugation, the remaining activity of the supernatant was tested against the appropriate sensitive cells. The values are based on the results of three experiments. ●, C1 1D; ○, Y2C

When target cells were tested against 19S antibodies from adult B10M inbred mice injected with 8×10^7 splenic cells of (C3H×DBA/2) F_1 hybrid mice and bled 4 d after the injection, a weak but uniform response was found (sera 5, Table 1) the increase in the number of target sites leading to an enhanced susceptibility to lysis in Y2C cells.

The quantitative differences in H-2^k antigen concentration on the plasma membrane from C1 1D and Y2C cells were then investigated by studying the absorption capacity of intact viable cells. Figure 1 shows that the H-2^k antigen concentration on C1 1D cells was approximately 1.5 times greater than on the hybrid cells. Diameters of both cell types were measured with an ocular micrometer (200 cells of each sample), and the ratio of surface area of Y2C cells to that of C1 1D fibroblasts was calculated to be 5:4 (i.e. 20% greater). It seems therefore that the H-2^k antigenic density on Y2C cells is about 55% of that on the C1 1D plasma membrane, if uniform surface smoothness is assumed.

An inverse relationship between the quantitative expression of H-2 and tumour-specific antigens has been demonstrated by some authors[13–15]. In somatic hybrid cells it is possible that all the antigenic determinants of both parental cells are expressed on the cell surface. In addition to the H-2^k antigenic complex and the L antigen, C1 1D express some group-specific murine leukemia antigens[6]. L1210 cells may contribute to the Y2C antigenic constitution with the H-2^d isoantigen complex and also with its tumour-specific transplantation antigens[16]. Thus there is competition for the plasma membrane surface in hybrid cells, resulting in a topographic exclusion of some antigens.

As the immune lysis of mouse leukemic cells by IgM is a 'one-hit' reaction[17], the relative resistance of Y2C hybrid to the 19S antibody-mediated lysis might be due to the great difference between the number of complement-fixing sites and lytic sites available on the plasma membrane. In fact, 3,000 complement C1-fixing sites were necessary to generate one lytic site on the surface of a mouse leukaemic cell, the IgG being more effective in generating C1-fixing sites[17]. The restricted expression of H-2 antigens on the surface of Y2C somatic cell hybrids seems to be responsible for the lack of sensitivity to lysis mediated by IgM antibodies[18]. Moreover, a critically spaced repeating H-2 determinant on the plasma membrane must be required for the induction of complement-dependent cytotoxic effect.

Thus, caution should be exercised in interpreting results of studies carried out with the cytoxicity assay on cell populations with a low density of transplantation antigens (that is, tumour-specific antigens) on the cell surface.

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1 Gresser, I., Brouty-Boyé, D., Thomas, M. T., and Macieira-Coelho, A., *J. natn. Cancer Inst.*, **45**, 1145 (1970).
2 Kit, S., Dubbs, D. R., Piekarski, L. J., and Hsu, T. C., *Expl. Cell. Res.*, **31**, 297 (1963).
3 Jami, J., Failly, C., and Ritz, E., *Expl. Cell. Res.*, **76**, 191 (1973).
4 Jami, J., and Ritz, E., *J. natn. Cancer Inst.*, **51**, 1967 (1973).
5 Wigzell, H. L. R., *Transplantation*, **3**, 423 (1965).
6 Leclerc, J. C., Levy, J P., Varet, B., Oppenheim, S., and Senik, A., *Cancer Res.*, **30**, 2073 (1970).
7 Fenyö, E. M., Grundner, G., Klein, E., and Harris, H., *Expl. Cell Res.*, **68**, 323 (1971).
8 Klein, G., Gars, U., and Harris, E., *Expl Cell Res.*, **62**, 149 (1970).
9 Fenyö, E. M., Grundner, G., Wiener, F., Klein, E., Klein, G., and Harris, H., *J. exp. Med.*, **137**, 1240 (1973).
10 Winn, H. J., in *Complement* (Ciba Foundation Symposium, edit., by Wostenholme, G. E. W., and Knight, J.), 133 (Churchill, London, 1965).
11 Ray, P. K., and Simons, R. L., *Cancer Res.*, **33** 936 (1973).
12 Sandford, B. H., and Codington, J. F., *Tissue Antigens*, **1**, 153 (1971).
13 Haywood, G. D., and McKhann, C. F., *J. exp. Med.*, **133**, 1171 (1971).
14 Cikes, M., Friberg, S., jun, and Klein, G., *J. natn. Cancer Inst.*, **50**, 347 (1973).
15 Ting, C. C., and Herberman, R. B., *Nature new Biol.*, **232**, 118 (1971).
16 Jami, J., and Ritz, E., *Cancer Res.*, **33**, 2524 (1973).
17 Borsos, T., Colten, R. R., Spalter, J. S., Rogentine, N., and Rapp, H. R., *J. Immun.*, **101**, 392 (1968).
18 Möller, E., and Möller, G., *J. exp. Med.*, **115**, 527 (1962).

Subunit structure of HL-A antigens on cell surface

THE major human histocompatibility determinants, the HL-A antigens, are cell surface markers present on lymphocytes and most other nucleated cells (compare ref. 1). Recently, work from two laboratories has indicated that HL-A antigens, released from the cell surface by means of papain digestion, are composed of two polypeptide chains[2,3]. The smaller of the two subunits seems to be invariant regardless of the antigenic specificity carried by the larger HL-A chain[2,3] and occurs in human biological fluids[4].

It had previously been demonstrated that β_2 microglobulin exhibits extensive amino acid sequence homologies with immunoglobulin heavy and light chains[5,6] and that β_2 microglobulin is an integral part of the cell surface[5,7,8]. In a search for its function we showed that β_2 microglobulin was one of the two HL-A polypeptide chains[9]. Independently, Nakamuro *et al.*[10] demonstrated that the small HL-A chain shared some properties with β_2 microglobulin.

Here we present evidence that β_2 microglobulin and the alloantigenic HL-A chain are physically linked to each other on the cell surface of lymphocytes.

Purified lymphocytes were isolated from defibrinated blood by Ficoll-Isopaque gradient centrifugation[11,12]. The blood was obtained from healthy blood donors with known HL-A phenotype. The cells were subjected to the microlymphocytotoxicity test[13] with purified anti-β_2 microglobulin antibodies[14], and cells from all donors were completely lysed regardless of their HL-A phenotype[9,15,16].

It was argued that if β_2 microglobulin and the alloantigen-carrying HL-A chain are indeed parts of the same molecule, antibodies directed against one of the polypeptide chains may interfere with the simultaneous binding of antibodies to the other chain. The results of experiments designed to test this possibility are summarised in Table 1. Fab′ fragments[17] against β_2 microglobulin could completely and specifically inhibit the cytotoxic action of bivalent anti-β_2 microglobulin antibodies. In control experiments, Fab′ fragments against light immunoglobulin chains had no effect. Fab′ fragments directed against various HL-A determinants could specifically inhibit the cytotoxic action of the corresponding bivalent antibodies but not if the anti-HL-A antibodies were directed against some other specificity. A number of monospecific anti-HL-A antibodies could not cause lysis of cells that had been pretreated with Fab′ fragments against β_2 microglobulin (Table 1), whereas their cytotoxic activity was normal when tested against cells treated with Fab′ fragments against immunoglobulin light chains. Cells derived from a doubly homozygous donor, that is, expressing only one serological specificity for each HL-A sublocus, were separately treated with Fab′ fragments directed against one

Table 1 Effects of Fab′ fragments on the lymphocytotoxic effect of anti-β_2 microglobulin and anti-HL-A antibodies*

Specificity of Fab′ fragments†	Specificity of antibodies	Cytotoxic effect‡
— — —	β_2M	++++
β_2 Microglobulin	β_2M	−
Light immunoglobulin chains	β_2M	++++
— — —	HL-A-1	++++
HL-A 1	HL-A 1	−
— — —	HL-A 8	++++
HL-A 8	HL-A 8	−
HL-A 1	HL-A 8	++++
β_2M	HL-A 1	−
Light immunoglobulin chains	HL-A 1	++++
β_2M	HL-A 8	−
Light immunoglobulin chains	HL-A 8	++++
HL-A 1	β_2M	+++
HL-A 8	β_2M	+++
HL-A 1, 8	β_2M	++

* The cells were incubated for 45 min at 37° C in Eagles' MEM with various concentrations of the Fab′ fragments indicated, washed three times in the medium before being subjected to the cytotoxicity test[13]. The concentrations of the Fab′ fragments and the cytotoxic antibodies used were chosen such that a two-fold dilution of the working solutions notably diminished their blocking or cytotoxic activity, respectively.

† The target cells used in the present experiment were from a donor homozygous for the HL-A antigenic specificities 1 and 8. In a number of identical experiments, results similar to those listed were obtained with anti-HL-A antibodies of specificities 2,7,10,11,27, W10, and W15.

‡ The cells were scored ++++ if more than 75%, +++ if more than 50%, ++ if more than 25%, + if more than 15%, and − if less than 15% of the cells were stained by trypan blue.

of the two HL-A specificities. A slight but significant reduction in the cytotoxic activity of anti-β_2-microglobulin antibodies compared with untreated control cells was noted. If, however, the cells were simultaneously treated with a mixture of Fab′ fragments directed against each of the two subloci gene products, the cytotoxicity was substantially reduced. Thus, β_2 microglobulin and the alloantigenic, larger HL-A polypeptide chain are located in close proximity on the cell surface.

Attempts were made to demonstrate simultaneous redistribution of β_2 microglobulin and the alloantigenic HL-A chain on the surface of purified lymphocytes to show that the two polypeptide chains are physically linked to each other.

Fluorescein isothiocyanate (FITC)-conjugated anti-β_2 microglobulin antibodies[18] were incubated together with purified lymphocytes. Details of the materials used have been described elsewhere[19] and the experimental protocol was essentially that of Kourilsky *et al.*[20]. Anti-β_2 microglobulin fluorescence was present on more than 90% of the nucleated cells (compare Table 2). A majority of the cells had patches and a few displayed polar caps. If, however, the FITC-anti-β_2 microglobulin antibody-treated cells were incubated with goat-anti-rabbit immunoglobulin (GARIG) antibodies, rendered specific against the Fc portion of the molecule by thorough absorption with rabbit Fab′ fragments, more than 70% of the stained cells exhibited fluorescent caps. FITC-anti-β_2 microglobulin Fab′ fragments gave diffusely distributed fluorescence both in the absence and presence of GARIG. In control experiments with use of FITC-rabbit-anti-human immunoglobulin (RAHIG) antibodies and GARIG treatment, all fluorescent cells showed caps. This cap formation did not, however, induce any redistribution of FITC-anti-β_2 microglobulin Fab′ fragments, thus establishing that β_2 microglobulin on the cell surface is quite independent from cell membrane-bound immunoglobulins.

FITC-conjugated anti-HL-A antibodies of different specificities gave rise to cells exhibiting patches or caps. The cap formation was more pronounced in the presence of RAHIG. Only diffuse fluorescence was evident on cells treated with anti-HL-A Fab′ fragments whether GARIG had been added or not.

Attempts were made to induce patch and cap formation of FITC-anti-HL-A Fab′ fragments with successive treatment of the cells with anti-β_2 microglobulin antibodies and GARIG. The anti-β_2 microglobulin antibodies, obtained from rabbits immunised with free β_2 microglobulin, did not cause any redistribution of the diffuse fluorescence but rather seemed to displace a large proportion of the fluorescence from the cell surface. This could, however, be explained by the above results on the inhibition of the anti-β_2 microglobulin antibody-induced lymphocytotoxicity (Table 1). Anti-β_2 microglobulin antibodies were therefore obtained from a rabbit immunised with highly purified, papain-solubilised HLA antigens and isolated by immunosorbent purification[21]. These anti-β_2 microglobulin antibodies together with FITC-anti-HL-A Fab′ fragments and GARIG induced caps in all experiments (Table 2).

Cells incubated with FITC-anti-β_2 microglobulin Fab′ fragments, obtained from the rabbit anti-HL-A serum, as described above, were treated in succession first with anti-HL-A antibodies and then with RAHIG. A significent number of cells (> 60%) exhibited patches and with some anti-HL-A antibodies even caps (Table 2). In all experiments, however, a few cells always showed only a diffuse fluorescence.

These experiments clearly demonstrate that β_2 microglobulin and the alloantigenic HL-A polypeptide chain are physically linked on the lymphocyte surface.

As the β_2 microglobulin amino acid sequence is highly homologous to immunoglobulin light and heavy chains[5,6], it has been suggested that it is an evolutionary precursor to regular immunoglobulin polypeptide chains[5]. It now seems possible that at least part of the alloantigens and the immunoglobulins may have arisen from a common evolutionary origin[9,22].

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Table 2 Effects of various antibodies on the redistribution of the antigen-carrying HL-A chain and β_2 microglobulin on the cell surface.

FITC-conjugated component	Additive	Appearance of cell surface bound fluorescence† Diffuse	Patches	Caps
Anti-β_2M antibody	— — —	+	+	−
Anti-β_2M antibody	GARIG	−	+	+
Anti-β_2M Fab′	— — —	+	−	−
Anti-β_2M Fab′	GARIG	+	−	−
Anti-β_2M Fab′	RAHIG+GARIG	+	−	−
RAHIG	GARIG	−	−	+
Anti-HL-A 1 antibody	— — —	+	+	+
Anti-HL-A 8 antibody	— — —	+	+	+
Anti-HL-A 1 antibody	RAHIG	−	+	+
Anti-HL-A 8 antibody	RAHIG	−	−	+
Anti-β_2M-Fab′	Anti-HL-A 1 antibody +RAHIG	+	+	−
Anti-β_2M Fab′	Anti-HL-A 8 antibody +RAHIG	+	+	+
Anti-HL-A 1 Fab′	Anti-β_2M antibody +GARIG	−	+	+
Anti-HL-A 8 Fab′	Anti-β_2M Antibody +GARIG	−	−	+

Approximately 2 × 10^6 cells were incubated for 30 min at 37° C in 0.2 ml of Eagles' MEM containing an appropriate concentration of the FITC-conjugated component (1–4 mg ml^{-1}). At the end of the incubation period, the cells were washed three times with the medium, and examined in a Leitz Ortholux microscope equipped with a HBO 200 W lamp. The Ploem-type vertical illuminator and immersion objectives were used. If further treatment of the cells was required they were incubated with antibodies (3–8 mg ml^{-1}) in Eagles' MEM for another 30 min, washed three times with the medium, examined in the fluorescence microscope or incubated with GARIG or RAHIG (3–5 mg ml^{-1}) for another 30 min in Eagles' MEM before evaluation of the cell-surface bound fluorescence.

† If more than 20% of the fluorescent cells showed a certain type of distribution of the label, they were scored as positive.

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After submission of this manuscript, two papers with data similar to ours have appeared[23,24].

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[1] Reisfeld, R. A., and Kahan, B. D., *Adv. Immun.*, **12**, 117 (1970).
[2] Cresswell, P., Turner, M. J., and Strominger, J. L., *Proc. natn. Acad. Sci., U.S.A.*, **70**, 1603 (1973).
[3] Tanigaki, N., Katagiri, M., Nakamuro, K., Kreiter, V. P., and Pressman, D., *Fedn Proc.*, **32**, 1017 (1973).
[4] Miyakawa, Y., Tanigaki, N., Kreiter, V. P., Moore, G. E., and Pressman, D., *Transplantation*, **15**, 312 (1973).
[5] Peterson, P. A., Cunningham, B. A., Berggard. I. and Edelman, G. M., *Proc. natn. Acad. Sci., U.S.A.*, **69**, 1697 (1972).
[6] Smithies, O., and Poulik, M. D., *Science*, **175**, 187 (1972).
[7] Evrin, P. E., and Nilsson, K., *J. Immun*, **112**, 137 (1974).
[8] Nilsson, K., Evrin, P. E., Berggård, I., and Pontén, J., *Nature new Biol.*, **244**, 44 (1973).
[9] Peterson, P. A., Rask, L., and Lindblom, J. B., *Proc. natn. Acad. Sci., U.S.A.*, **71**, 35 (1974).
[10] Nakamuro, K., Tanigaki, N., and Pressman, D., *Proc. natn. Acad. Sci., U.S.A.*, **70**, 2863 (1973).
[11] Böyum, A., *Scand. J. clin. Lab. Invest.*, **97**, 21 supplement (1900).
[12] Thorsby, E., and Bratlie, A., *Histocompatibility Testing*, 655 (Munksgaard, Copenhagen, 1970).
[13] Kissmeyer-Nielsen, F., and Kjerbye, K. E., *Histocompatibility Testing*, 381 (Munksgaard, Copenhagen, 1967).
[14] Peterson, P. A., Evrin, P. E., and Berggård, I., *J. clin. Invest.*, **48**, 1189 (1969).
[15] Fanger, M. W., and Bernier, G. M., *J. Immun.*, (in the press).
[16] Poulik, M. D., *Immun. Commun.*, (in the press).
[17] Nisonoff, A., Wissler, F. C., Lipman, L. N., and Woernley, D. L., *Archs biochem. Biophys.*, **89**, 230 (1960).
[18] Gennis, L. S., Gennis, R. B., and Cantor, C. R., *Biochemistry*, **11**, 2517 (1972).
[19] Lindblom, J. B., Östberg, L., and Peterson, P. A., *Tissue Antigens* (in the press).
[20] Kourilsky, F. M., Silvestre, D., Neauport-Sautes, C., Loosfelt, Y., and Dausset, J., *Eur. J. Immun.*, **2**, 249 (1972).
[21] Cuatrecasas, P., *J. biol. Chem.*, **245**, 3059 (1970).
[22] Gally, J. A., and Edelman, G. M., *A. Rev. Geneti.*, **6**, 1 (1972).
[23] Poulik, M. D., Bernoco, M., Bernoco, D., and Ceppelini, R., *Science*, **182**, 1352 (1973).
[24] Solheim, A., and Thorsby, E., *Tissue Antigens* (in the press).

Evidence that mitogenic lectins induce changes in lymphocyte membrane fluidity

MITOGENIC plant lectins, such as phytohaemagglutinin (PHA) and concanavalin A (con A), are useful tools to study the control of cell proliferation in lymphocytes. Recent evidence suggests that these mitogenic lectins trigger mitogenesis at the cell membrane[1,2]. It is likely that an understanding of the mechanisms by which these lectins stimulate lymphocytes will provide important clues to more general problems of the regulation of proliferation in eukaryotic cells.

A model has been proposed to explain how modulation of membrane structure may influence cell growth. This model suggests that changes in the fluid characteristics of membrane lipids affect a variety of membrane activities[3–5]. Studies in isolated membranes have shown, for example, that fluidisation of membrane lipids is associated with activation of Na^+, K^+-ATPase[3]. *In vitro* studies have also correlated a fluid state of membrane lipids in transformed cells with high cellular levels of cyclic GMP and low levels of cyclic AMP and ordered membrane lipids in contact inhibited cells with low levels of cyclic GMP and high levels of cyclic AMP[4,6–9].

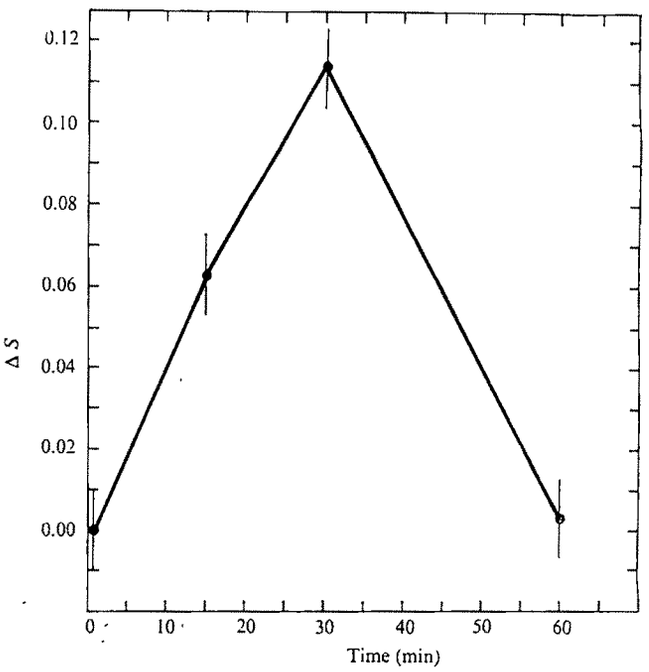


Fig. 1 The effect of an optimal mitogenic dose of PHA-P (Difco, 7.5 $\mu g\ ml^{-1}$) on the fluidity of the human lymphocyte membrane at various times after addition of the mitogen. $\Delta S = S_{control} - S_{PHA}$ and $S_{control} = 0.52$.

In lymphocytes recent observations suggest that mitogens also induce an early increase in levels of cyclic GMP[10]. This nucleotide increases more than ten-fold within 5–20 min of the addition of PHA or con A, followed by a decline to basal levels within 1 h. Other mitogenic agents such as phorbol myristate acetate[8] and high concentrations of insulin[9] also increase considerably the levels of cylic GMP in lymphoid cells. It has been suggested that this increase is part of the signal to initiate cellular proliferation. After binding of mitogenic lectins other early changes in the plasma membrane occur; examples are increased endocytosis[11] and incorporation of orthophosphate into phosphatidyl inositol[12].

According to our membrane model[3–5], lymphocyte mitogens which produce a transient elevation of cellular cyclic GMP should fluidise membrane lipids. The data reported here support this model.

Human peripheral blood lymphocytes, separated using a Ficoll-Hypaque density gradient, were 90% free of contaminating monocytes, erythrocytes and granulocytes. Mouse lymphocytes were obtained from CBA/Ht6 strain spleen cells which were minced and washed three times in buffered saline. The fluid characteristics of the plasma membrane of lymphocytes were probed with the spin label sodium 6-(4′,4′-dimethyloxazolidinyl-N-oxyl)-heptadecanoate[3]. A 0.3 mM solution of the label in buffered saline was incubated with cells for 30 min at 37° C. The cells were then washed five times to remove excess label[13]. The electron paramagnetic resonance (EPR) spectra were recorded at 33° C with a Varian E-3 spectrometer.

The EPR spectra of the spin-labelled fatty acids in natural membranes indicate that the labels have two important characteristics: (1) they undergo rapid anisotropic motion about an axis approximately parallel to the fatty acid chain with a correlation time of $<<10^{-7}$ s[3,14–18] and (2) they undergo rapid lateral diffusion with a diffusion constant of 10^{-8}–10^{-7} $cm^2\ s^{-1}$ (refs 19–22). Since it is improbable that such rapid axial motion or rapid lateral diffusion could occur if the labels were bound to protein, these characteristics suggest that the labels are in a phospholipid bilayer-like environment. While the lateral

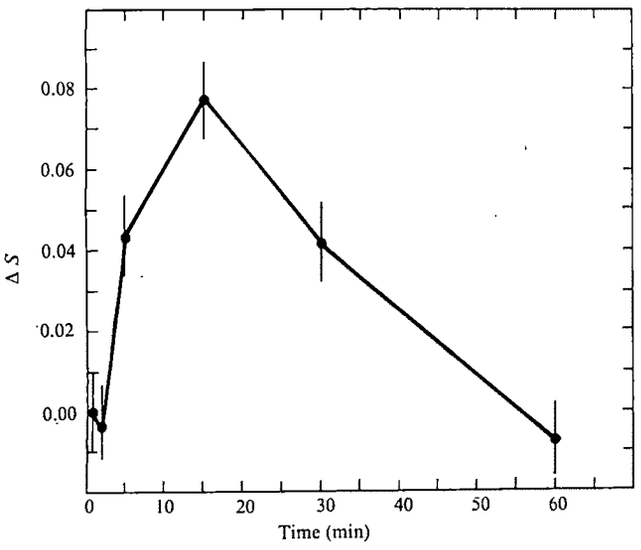


Fig. 2 The effect of con A (Difco, 3 μg ml^{-1}) on the fluidity of the plasma membrane of mouse splenic lymphocytes at various times after addition of the Mitogen. $\Delta S = S_{control} - S_{Con\ A}$ and $S_{control} = 0.52$.

diffusion of surface antigens in mouse-human heterokaryons throughout the cell membrane requires about 40 min[23], that for spin labels of fatty acids should require about 1 s.

Order parameters, S, were calculated from the EPR spectra. Values of S near 1 correspond to an ordered state of the membrane lipids while values near 0 correspond to a fluid state[24]. With the label used in this study, a $\Delta S = 0.03$ would be equivalent to a change in membrane fluidity produced by a 6–10° C temperature change[3].

The data in Figs 1 and 2 clearly demonstrate that treatment of human peripheral lymphocytes with mitogenic concentrations of PHA and treatment of mouse splenic lymphocytes with mitogenic concentrations of con A produce a fluidisation of membrane lipids. Both human and mouse lymphocytes showed maximal fluidisation between 15 and 30 min. The dose-response curve for membrane fluidisation also corresponds well with the dose-response curve for the mitogenic effect of con A (Fig. 3). In contrast, a nonmitogenic lectin, wheat germ agglutinin, had no effect on membrane fluidity.

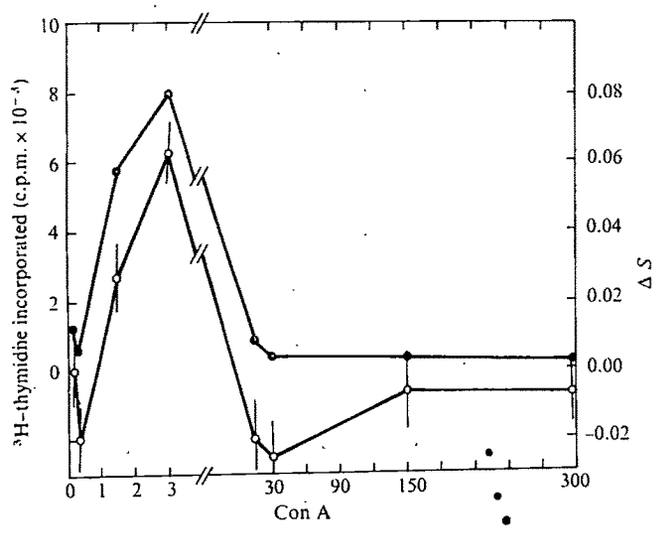


Fig. 3 The dose-response curves for the mitogenic effects of con A (●) and the membrane fluidising effect of con A for a 20 min incubation (○) with mouse splenic lymphocytes. Mitogenesis was determined by incorporation of ^{3}H-thymidine into acid-insoluble precipitate after 72 h of incubation.

Studies are in progress to define precisely the location and distribution of the label in lymphocyte membranes, and to examine other mitogenic agents to determine whether critical membrane fluidisation is a perequisite for mitogenesis in general. The data presented here demonstrate, however, that only mitogenic concentrations of plant lectins produce an early fluidisation of lymphocyte membranes and that the time of membrane fluidisation closely parallels the increase and decrease of cyclic GMP levels in stimulated lymphocytes[10]. It has been suggested that the increase of cellular cyclic GMP levels is a signal for cellular proliferation[7–10] and our data suggests that early membrane fluidisation may be equally important in the triggering process.

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1 Anderson, J., Edelman, G. M., Möller, G., and Sjöberg, O., *Eur. J. Immun.*, **2**, 233 (1972).
2 Greaves, M. F., and Bauminger, S., *Nature new Biol.*, **235**, 67 (1972).
3 Grisham, C. M., and Barnett, R. E., *Biochemistry*, **12**, 2635 (1973).
4 Barnett, R. E., Furcht, L. T., and Scott, R. E., *Proc. natn. Acad. Sci. U.S.A.* (in the press).
5 Barnett, R. E., and Palazzotto, J., *Ann. N.Y. Acad. Sci.* (in the press).
6 Sheppard, J. R., in *Membranes and viruses in immunopathology* (edit. by Day, S. B., and Good, R. A.), 249 (Academic Press, New York, 1972).
7 Goldberg, N. D., O'Dea, R. F., and Haddox, M. K., *Adv. cyclic nucleotide Res.*, **3**, 155 (1973).
8 Goldberg, N. D., Haddox, M. K., Dunham, E., Lopez, C., and Hadden J. W., in *The Cold Spring Harbor symposium on the regulation of proliferation in animal cells*, (edit. by Clarkson, B., and Baserga, R.) (Academic Press, New York, in the press).
9 Goldberg, N. D., Haddox, M. K., Estensen, R., White, J. G., Lopez, C., and Hadden, J. W., in *Cyclic AMP in immune response and tumor growth* (edit. by Lichtenstein, L., and Parker, C.) (Springer, New York, in the press).
10 Hadden, J. W., Hadden, E. M., Haddox, M. K., and Goldberg, N. D., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3024 (1972).
11 Hirschhorn, K., in *Mediators of cellular immunity* (edit. by Lawrence, H. S., and Landy, M.), 3 (Academic Press, New York, 1969).
12 Unanue, E. R., Perkins, W. P., and Karnovsky, M. J., *J. exp. Med.*, **136**, 885 (1972).
13 Kaplan, J., Canonico, P. G., and Caspary, W. J., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 66 (1973).
14 Hubbell, W. L., and McConnell, H. M., *Proc. natn. Acad. Sci. U.S.A.*, **64**, 20 (1969).
15 McConnell, H. M., and McFarland, B. G., *Q. Rev. Biophys.*, **3**, 91 (1970).
16 Seelig, J., and Hasselbach, W., *Eur. J. Biochem.*, **21**, 17 (1971).
17 Hubbell, W. L., and McConnell, H. M., *J. Am. chem. Soc.*, 93, 314 (1971).
18 Jost, P., Libertini, L., Herbert, V. C., and Griffith, O. H., *J. molec. Biol.*, **59**, 77 (1971).
19 Devaux, P., and McConnell, H. M., *J. Am. chem. Soc.*, **94**, 4475 (1972).
20 Träuble, J., and Sackmann, E., *J. Am. chem. Soc.*, **94**, 4499 (1972).
21 Scandella, C. J., Devaus, P., and McConnell, H. M., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2056 (1972).
22 Barnett, R. E., and Grisham, C. M., *Biochem. biophys. Res. Commun.*, **48**, 1362 (1972).
23 Frye, L. D., and Edidin, M., *J. Cell Sci.*, **7**, 319 (1970).
24 Seelig, J., *J. Am. chem. Soc.*, **92**, 3881 (1970).

Hb K Woolwich the cause of a thalassaemia

In general terms, heterozygotes for β thalassaemia have a moderate degree of microcytosis associated with an elevated red cell count, a reduced mean cell volume (MCV) and mean cell haemoglobin (MCH) and a normal, or near normal, mean cell haemoglobin concentration (MCHC). The chief diagnostic parameter of the heterozygous state is, however, the elevated Hb A_2 level which accompanies the condition. This does not occur in other haemoglobinopathies with the exception of those associated with unstable haemoglobins and some heterozygotes for Hb S (ref. 1) and Hb C (ref. 2). At the molecular level β thalassaemia is associated with an imbalance of globin chain synthesis during erythroid cell maturation, as a result of defective β chain synthesis. The extent of the defect parallels the severity of the thalassaemia, ranging from a reduction in the amount of product from the affected β chain locus (β^+ thal) to no product (β^0 thal). In all cases of β^+ thal so investigated, the β chains produced by the affected gene have been indistinguisnable from normal β^A chains.

We here present evidence which compares the inheritance of the β chain gene of Hb K Woolwich (Hb KW) with the inheritance of a β^+ thalassaemia. Also, the interaction of this thalassaemia with an α thalassaemia is demonstrated in a patient of West Indian origin.

Hb KW, first described in a West Indian family, is a stable haemoglobin variant[3]. Table 1 gives the pattern of haemoglobins in seven heterozygotes for Hb KW characterised in this laboratory. The amount of Hb KW is always proportionately less than the other adult haemoglobin (30–40%) and the Hb A_2 is higher than normal in six of the seven cases. The R family (Fig. 1) was available for more detailed haematological investigations. The propositus I–1 had the classical features of β thalassaemia trait; a moderate microcytosis and a high Hb A_2 level in the red cells (Table 1). Investigation of the abnormal haemoglobin identified it as Hb K Woolwich, although β-chain variants usually exceed 50% of the total haemoglobin in double heterozygotes for β thalassaemia and a β-chain variant[2,4]. Nevertheless, the pattern was repeated in members of the family who had inherited the β^{KW} chain gene; they all had high levels of Hb A_2 per red cell (Table 1). The doubly abnormal heterozygote, (Hb KW+Hb S), II–3, had an exceptionally high Hb A_2 but the difference can be accounted for by the additional inheritance of the β^S chain gene, which is often associated with marginally higher levels of Hb A_2 per cell than normal[1] (Table 1).

The conclusion from these observations is that inheritance of the β^{KW} chain gene is associated with a mild β thalassaemia, that is the β^{KW} chain locus is a β^{KW+} thalassaemia gene. This is the first example of a β^+ thalassaemia in which the product of the affected locus is distinguishable from the normal β^A chain.

Since the normal course, in suspected cases of thalassaemia, is an investigation of the *in vitro* biosynthesis of the globin chains, a reticulocyte-rich preparation of red cells was obtained from the blood of the propositus. The results of *in vitro* incorporation of ^{3}H-leucine into the α^A, β^A and β^{KW} chains isolated from the whole globin of these red cells are summarised in Fig. 2. The incorporation of radio-isotope into all chains was linear over the incubation period of 60 min. The total incorporation of ^{3}H-leucine into the β^{KW} chain was always much less than into the β^A chain and averaged less than 50% of the incorporation into the β^A chain (Table 2). The amount of Hb K Woolwich found in the blood of the propositus is therefore an accurate reflection of the amount of this chain synthesised during erythroid cell maturation. This fulfills another criterion required by the definition of β thalassaemia; namely the demonstration of defective synthesis of globin chains by one of the β chain loci.

Table 2 *In vitro* biosynthesis of α, β^A and β^{KW} chains in reticulocytes from propositus

Incubation Time (min)	3	6	9	15	30	60	Mean
$\beta^{KW}/\beta\alpha+\beta^{KW}$	0.29	0.36	0.34	0.31	0.30	0.30	0.30
α^A/β^A	1.30	1.20	1.20	1.10	1.00	1.10	1.20
$\alpha^A/\beta^A+\beta^{KW}$	0.93	0.80	0.78	0.79	0.73	0.74	0.79

The ratios correspond to the relative amounts of each chain synthesised based on the total incorporation of ^{3}H-leucine into the individual chains during *in vitro* incubation of the reticulocytes.

A correlation between the mutation in the $\beta^{K\ Woolwich}$ chain β 132 (H10) Lys–Gln and the lesion responsible for the amount synthesised is important since this could confirm the predictions on one possible cause of β thalassaemia based on a "structure-rate hypothesis"[5]. There are several observations in favour of this explanation: (1) Hb K Woolwich is the only known haemoglobin variant resulting from a mutation at H10 Lys; (2) with the exception of the frog, where it is alanine, in all species so far examined, lysine (H10) is an invariant residue in all α, β^6 and myoglobin chains[6,7]; and (3) a myoglobin variant has been characterised in which H10 Lys is replaced by a residue of asparagine and the amount of the myoglobin variant in muscle extracts is approximately 30% of the total myoglobin[8].

It is possible that the tRNA coding for the β H10 glutamine is in short supply. This would introduce a partial block at position H10 and decrease the rate of translation of the β chain mRNA by the ribosomes. This possibility was investigated by comparing the rate of assembly and termination of the β^A and β^{KW} chains by a modification of the method of Dintzis[9] (Table 3 and Fig. 3). From Fig. 3,

Table 1 haemoglobin pattern and haematological data of the R family and other heterozygotes for Hb KW

Case	% Total haemoglobin				Haematological data				
	Hb KW	Hb S	Hb A_2	Hb F	Hb (g per 100 ml)	RBC ($\times 10^6$ μl^{-1})	MCH (pg)	MCV (fl)	Hb A_2 (pg per cell)
TG	39.2	—	4.8	—	—	—	—	—	—
IB	40.9	—	2.7	—	—	—	—	—	—
T	37.2	—	3.8	—	—	—	—	—	—
RI-1	28.4	—	5.1	0.7	14.3	6.2	23.1	69	1.2
RI-2	—	32.4	3.1	0.7	16.0	5.9	26.6	77	0.9
RII-1	—	—	2.9	0.6	15.1	6.6	22.6	69	0.7
RII-2	38.0	—	4.1	1.8	13.2	4.8	27.4	79	1.1
RII-3	40.0	54.2	5.2	0.6	16.0	7.0	22.6	66	1.2
RII-4	34.2	—	3.9	—	16.3	5.6	28.6	81	1.1
Normal range			2.5–3.5	< 0.8					0.75 (ref. 4)

The haemoglobins were quantitatively estimated by DEAE Sephadex chromatography[15]. Globin prepared from Hb KW (ref. 6), was separated into the individual chains by ion exchange chromotography on CM 23 cellulose[17]. The β^{KW} chain was characterised as β132 (H10) Lys–Gln by fingerprinting the trypticpeptides, elution, hydrolysis and amino acid analysis of the abnormal peptide[3]. Haematological data were obtained from a Coulter S counter.

Table 3 Specific activity of leucine residues in the tryptic peptides of the β^A and β^{KW} chains after 6 and 9 min incubation with ^{3}H-leucine

Tryptic peptide (Tp)		^{3}H-leucine content (d.p.m.)				^{12}C-leucine content (nmol)				Specific activity d.p.m. per 100 nmol			
		β^A6	$\beta^{KW}6$	β^A9	$\beta^{KW}9$	β^A6	$\beta^{KW}6$	β^A9	$\beta^{KW}9$	β^A6	$\beta^{KW}6$	β^A9	$\beta^{KW}9$
1	(1)	70.9	49.3	100.2	52.5	129.4	97.4	104.9	58.6	54.8	50.6	95.5	89.6
2	(1)	—	76.3	76.6	70.9	—	139.9	82.4	75.8	—	54.5	93.0	93.5
3	(1)	80.3	83.1	170.3	—	144.7	144.3	174.7	—	55.5	57.5	97.5	—
4	(2)	90.3	—	—	96.2	161.1	—	—	106.8	56.1	—	—	90.1
5	(1)	86.7	52.5	92.8	80.4	157.9	93.6	97.9	84.2	54.9	56.1	94.8	95.3
9	(4)	256.2	153.7	252.5	218.5	444.6	257.3	267.2	236.8	57.8	59.7	94.5	92.3
11	(1)	—	61.7	110.7	—	—	99.1	112.4	—	—	62.3	98.5	—
12a	(2)	103.7	—	142.3	64.5	177.6	—	141.6	67.9	58.4	—	100.5	95.0
12b	(1)	74.0	59.8	110.3	66.8	125.3	97.9	115.3	68.9	59.1	61.1	95.7	97.1
14	(1)	60.3	24.2	43.3	64.7	97.9	38.1	45.5	67.2	61.6	63.5	99.5	96.3
Blanks	1	11.7	11.6	11.8	12.1	—	—	—	—				
	2	11.1	12.1	11.7	11.9	—	—	—	—				
	3	12.3	11.9	11.9	12.2	—	—	—	—				
Background		11.5	11.3	11.3	11.7								

Experimental data are given in Fig. 3. In column 1, the numbers in parentheses refer to the leucine residues expected in each peptide. Blanks were obtained from three different areas in each fingerprint.

the computed values of the specific activities of the N-terminal and C-terminal amino acids of both chains are approximately the same after 6 and 9 min incubation of the reticulocytes with ^{3}H-leucine. Therefore, the rate of incorporation of isotope into the N-terminal residue relative to the C-terminal residues is the same for both chains, indicating no reduction in the rate of assembly or termination of the β chain as a result of the mutation. Other mechanisms by which the amount of gene product could be reduced are a decreased rate of initiation of β^{KW} chain synthesis on the mRNA and a decreased amount of available mRNA as a result of either instability of the mRNA, or a reduced rate of transcription of the abnormal locus. The available data on translation and transcription in eukaryotes do not offer any reason why a change of nucleotide at this position of the structural gene should result in a reduced amount of product. If we speculate, however, and there is a precedent in known tRNAs (ref. 6) that mRNA normally assumes a stable secondary structure dictated by the nucleotide sequence, then there is a mechanism by which initiation of mRNA could be responsible for the low levels of β^{KW}. For example, the nucleotide sequence around the codon for β 132 (H10) lysine could be important in the maintenance of the proposed secondary structure of the mRNA, such that the initiation codon (AUG) was freely available for reaction with the available ribosomal subunits. The nucleotide change responsible for the mutation Lys–Gln, that is, the transversion A–C, could conceivably alter the secondary structure sufficiently to make the initiation codon less available for interaction. By the same argument, the normal codon could also be involved in the maintenance of a stable secondary structure which contributes to the protection of the mRNA during transport from the nucleus. Then the transversion (A–C) could so reduce the stability of configuration that the normal amount of mRNA is prevented from reaching the cytoplasm and the ribosomes.

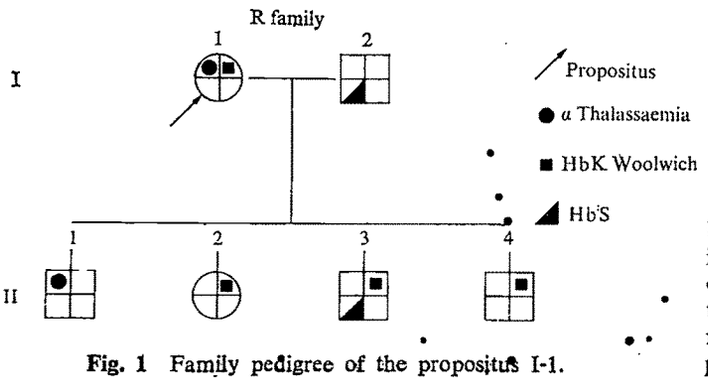


Fig. 1 Family pedigree of the propositus I-1.

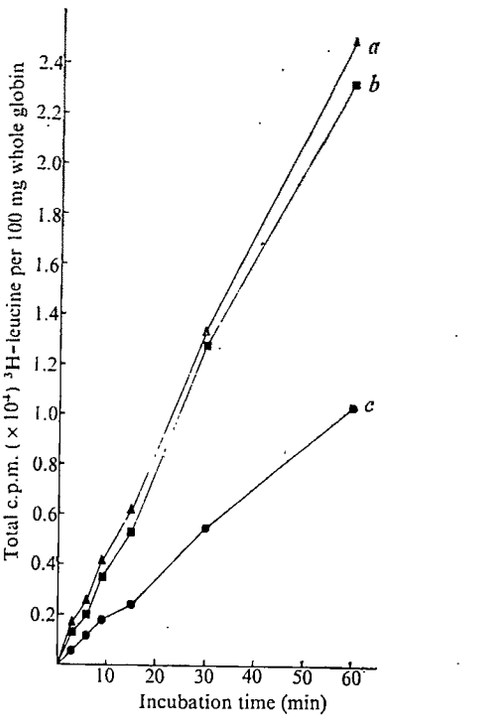


Fig. 2 *In vitro* incorporation of ^{3}H-leucine into the α, β and β^{KW} chains of the propositus. Reticulocyte-rich red cells prepared from the blood of the propositus[12] were pre-incubated for 15 min at 37° C in the medium of Lingrel and Borsook[13]. 250 μCi ^{3}H-leucine (0.007 μmol) were added and samples of cells removed at time intervals from 3–60 min then added to a ten-fold excess of ice cold isotonic saline. The cells were washed several times, lysed and the haemolysate added directly to acid acetone at −20° C. The globin chains from 100 mg whole globin samples were separated on Whatman CM23 cellulose using a linear gradient of Na^+ in 8 M urea[17] *p*H 6.7. The protein fractions corresponding to the α, β and β^{KW} chains were pooled separately and dialysed against 4 × 10 l 0.5% formic acid. The total volume of each solution was measured by weight and aliquots were added to 15 ml of scintillator solution (Triton X100, 500 ml; toluene, 1,000 ml; PPO 5 g) (ref. 14) and counted in a 'Tracerlab Coru Matic 200' liquid scintillation spectrometer. *a*, α chain; *b* β^A chain; *c* β^{KW} chain.

The propositus I–1 had the additional complication that, besides the signs of a β^+ thalassaemia, the overall imbalance in globin synthesis (α:β) was the result of a deficiency in α chain production (Table 2). The ratio $\alpha^A/\beta^A+\beta^{KW}$ was in the range associated with silent α thalassaemia. Since the results have shown an absolute decrease in the amount of product from one β locus, however, it seems that she might

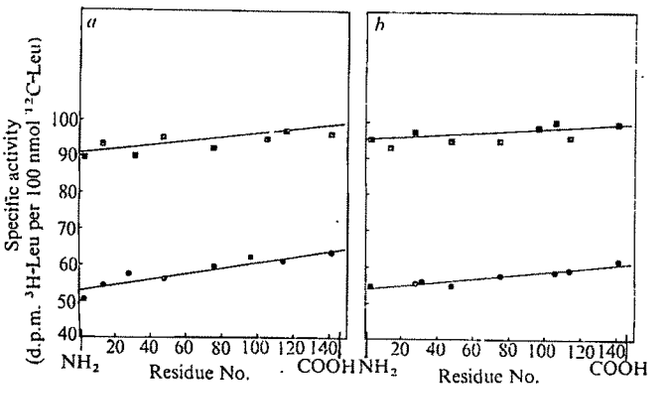


Fig. 3 Comparison of the rates of assembly of the β^{A} and β^{KW} chains. The gradient of radioactivity in the globin chains after 6 (●) and 9 (■) min incubation with ³H-leucine. The method used has been described previously[18]. *a*, $\beta^{K\ Woolwich}$ chain; *b*, β^{A} chain.

have a compensated α thalassaemia; α thalassaemia, β^{K+} thalassaemia. The haematological data for her son II-1 (Table 1) indicated that he has inherited the α thalassaemia gene but, as yet, he has not been available for *in vitro* biosynthetic studies. On diagnosing α thalassaemia, iron deficiency has to be considered. The serum iron levels in this family were all slightly low with a total serum iron binding capacity in the higher range of normal. As they were all similar, one would not expect this to lower the Hb A_2 in II-1 and not in the others; α thalassaemia in association with β thalassaemia has been described[10,11]. The results from the propositus are similar to those described in one family of Italian origin[10]. The amount of Hb KW in the cells of the propositus is lower than in either of her children, but it is recognised that an α thalassaemia can reduce the proportion of an associated β chain variant[4]. As the results of the *in vitro* biosynthetic studies do not implicate assembly or release of the completed chains as a possible point of interaction, it is difficult to understand at what other step in biosynthesis a deficiency of α chains could produce this effect, unless at the level of initiation.

Usually (though not invariably) β^{A} thalassaemia is associated with typical haematological features and these are not seen here. Of the important criteria of thalassaemia, the two found with Hb K Woolwich are the reduction of β-chain synthesis and raised Hb A_2.

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1 Wrightstone, R. N., and Huisman, T. H. J., *Clin. Chim. Acta.*, **22**, 593–601 (1968).
2 Huisman, T. H. J., *Clin. Chim. Acta.*, **40**, 159-163 (1972).
3 Allan, N., Beale, D., Irvine, D., and Lehmann, H., *Nature*, **208**, 658-661 (1961).
4 Weatherall, D. J., and Clegg, J. B., in *The Thalassaemia Syndromes*, **145** (Blackwell, London, 1972).
5 Itano, H. A., *Adv. Prot. Chem.*, **12**, 216–268 (1957).
6 Dayhoff, M. O., *Atlas of Protein Sequence and Structure* (National Biochemical Research Foundation, Washington DC, 1972).
7 Romero-Herrera, A. E., Lehmann, H., Joysey, K. A., and Friday, A. E., *Nature*, **246**, 389–395 (1973).
8 Boulton, F. E., Huntsman, R. G., Romero-Herrera, A. E., Lorkin, P. A., and Lehmann, H., *Biochim, biophys. Acta.*, **229**, 871–876 (1971).
9 Dintzis, H. M., *Proc. natn. Acad. Sci., U.S.A.*, **47**, 247–261 (1961).
10 Kan, Y. W., and Nathan, D. G., *J. Clin. Invest.*, **49**, 635–642 (1970).
11 Knox-Macauley, H. H. M., Weatherall, D. J., Clegg, J. B., Bradley, J., and Brown, M. J., *Br. J. Haematol.*, **22**, 497–512 (1972).
12 White, J. M., Brain, M. C., and Ali, M. A. M., *Br. J. Haematol.*, **20**, 236–275 (1971).
13 Lingrel, J. B., and Borsook, H., *Biochemistry*, **3**, 309–314 (1963).
14 Hunt, T., Hunter, T., and Munro, A., *J. molec. Biol.*, **36**, 31–45 (1968).
15 Huisman, T. H. J., and Dozy, A. M., *J. Chromatog.*, **19**, 160–169 (1965).
16 Winterhalter, K. H., and Huehns, E. R., *J. biol. Chem.*, **239**, 3699–3705 (1964).
17 Clegg, J. B., Naughton, M. A., and Weatherall, D. J., *J. molec. Biol.*, **19**, 91–108 (1966).
18 Lang, A., White, J. M., and Lehmann, H., *Nature new Biol.*, **240**, 268–271 (1972).

Effect of ß-N-oxalyl-L-*a*, ß-diaminopropionic acid on glutamate uptake by synaptosomes

The unusual amino acid β-N-oxalyl-L-α,β-diaminopropionic acid (ODAP), isolated from the seeds of *Lathyrus sativus* is a potent neurotoxin[1–3]. It produces biochemical changes in the brain typical of an excitant amino acid and is implicated in the aetiology of human neurolathyrism caused by eating the seeds of *L. sativus*[4–6]. It may act as a glutamate antagonist: ODAP inhibits glutamate oxidation[7] possibly by inhibiting glutamate uptake in bovine brain mitochondria; it also acts as a competitive inhibitor of glutamate uptake in certain strains of yeast[8], and a similar process might occur at the synaptic level. Any effect of ODAP on glutamate uptake at synapses is significant in view of the neurotransmitter function of glutamate, which seems to be neuroexcitory as well as neurotoxic[9–12]. But Balcar and Johnston[13] have shown with rat brain slices that ODAP does not inhibit the glutamate uptake by the high affinity system.

The high affinity uptake of amino acids such as glutamate, aspartate, glycine and γ-aminobutyric acid by nervous tissue slices and homogenates reflects at least in part their accumulation by the nerve terminals[14–16]. We have studied the *in vitro* effect of ODAP on glutamate uptake by synaptosomes isolated from the brain of 12-day-old rats and from monkey spinal cord. We chose these tissues because ODAP produces convulsions in young rats when injected intraperitoneally and precipitates hind leg paralysis in monkeys when introduced into the cerebrospinal fluid through the lumbar route[1–3].

Synaptosomes were isolated from the whole brain of rats by the procedure of Eichberg *et al.*[17] except that the P2 pellet was isolated by centrifuging the P_1 supernatant at 7,000*g* for 30 min instead of at 10,000*g* for 1 h. This modification by Levitan *et al.*[18] results in a purer preparation of synaptosomes. Synaptosomes were isolated from the monkey spinal cord by the procedure described[19] for the guinea pig spinal cord. Two distinct synaptosomal bands, seen at 1.0–1.2 and 1.2–1.4 M sucrose after density gradient centrifugation in a SW25–1 rotor for 2 h, were collected. These synaptosomal fractions were diluted to 0.4 M and the pellet collected by centrifugation at 15,000*g* for 20 min. It was used for amino acid uptake studies, conducted essentially according to Peterson and Raghupathy[20] (Figs 1–3). The integrity of synaptosomes were ascertained by measuring the lactic dehydrogenase activity of the samples[21] and demonstrating that very little radioactivity

was retained on the filter when the samples after incubation were lysed with water before filtration.

Synaptosomes isolated from rat brain showed a typical biphasic uptake pattern for glutamate (Fig. 1). The low and high affinity uptake systems were characterised by K_m values of 5×10^{-3}M and 2.5×10^{-5}M respectively. We did not take into account glutamate in the synaptosomal preparation but the K_m values obtained were similar to those reported for glutamate uptake in rat and cat nervous tissues[14,15]. Addition of ODAP at 10^{-3}M concentration produced around 15–20% inhibition of glutamate uptake by the low as well as high affinity systems. The Lineweaver–Burk analysis of the data indicates a noncompetitive type of interaction (Fig. 1). The pattern remains essentially the same at ODAP concentration of 5×10^{-3}M or 10^{-4}M.

Monkey spinal cord synaptosomes also exhibited a biphasic pattern of glutamate uptake with K_m values of 1.2×10^{-5}M and 0.5×10^{-3} for the high and low affinity uptake systems respectively (Fig. 2). Similar results have been reported for rat and cat spinal cord slices and homogenates[14,15]. Addition of ODAP to the incubation medium showed a competitive inhibition of glutamate uptake by the high affinity uptake system. ODAP has practically no effect on the low affinity uptake of glutamate.

We examined the specificity of ODAP action by studying its effects on glycine uptake, since a high affinity uptake system for this amino acid has been shown to exist only in the spinal cord preparations but not in those of the cerebral cortex of rat and cat[14–16]. We found that a biphasic pattern for glycine uptake also exists in the monkey spinal cord synaptosomes, but ODAP has very little effect on either the low or high affinity uptake of glycine (Fig. 3).

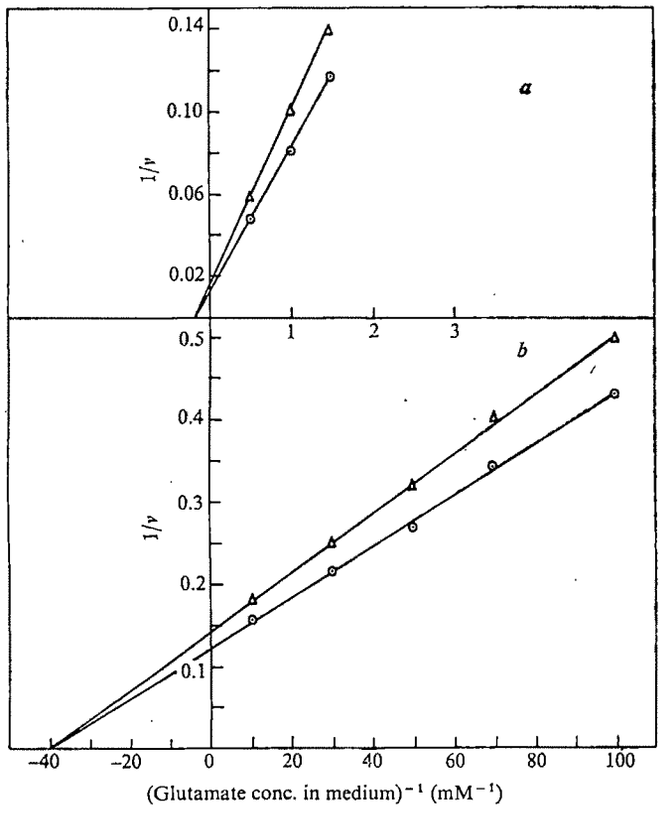


Fig. 1 Effect of ODAP on the uptake of glutamate by young rat brain synaptosomes. Synaptomes (200 μg protein ml^{-1}) were incubated with 0.25 μCi of μ-^{14}C L-glutamate (99 mCi mmol^{-1}) mixed with unlabelled L-glutamate to give the required concentration in a final volume of 1 ml. Incubation were carried out in 5 ml conical flasks at 37° C for 3 min in a Dubnoff shaker. The medium composition was: 10 mM Tris; 150 mM NaCl; 15 mM $MgCl_2$ adjusted to *p*H 7.4. Reactions were started by the addition of synaptosomes. All incubations were terminated by the addition of 2 ml of ice cold medium followed by quantitative transfer to and washing with 30 volumes of the same buffer over Millipore filters (0.45 μm HAWP). The dried filters were then counted in 10 ml of Cocktail (0.5% PPO (w/v) in Toluene) in a Beckman LS-100 scintillation counter. *v* is expressed as nmol glutamate taken up per mg protein per 3 min. *a*, Low affinity uptake; *b*, high affinity uptake; O—O, Control uptake; Δ—Δ, uptake in the presence of ODAP (10^{-3}M).

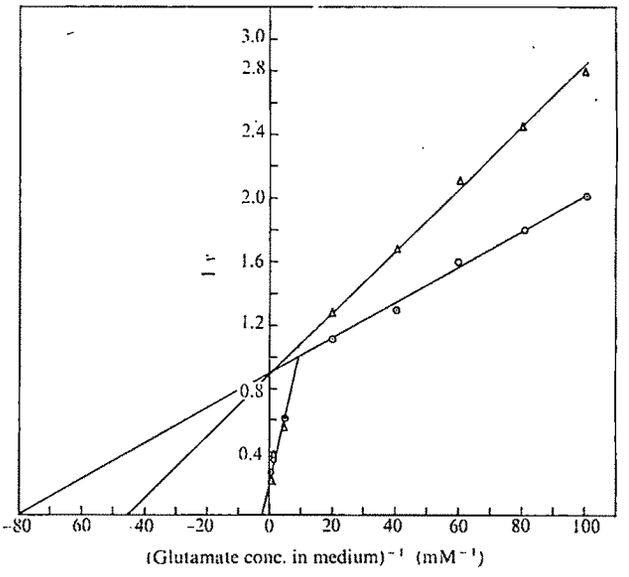


Fig. 2 Effect of ODAP on glutamate uptake by monkey spinal cord synaptosomes. Conditions of incubation as in Fig. 1. O—O, Control uptake; Δ—Δ, uptake in the presence of ODAP (10^{-3}M).

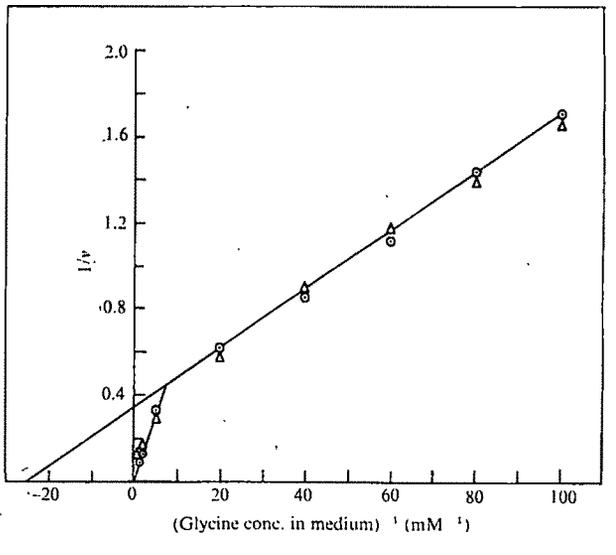


Fig. 3 Effect of ODAP on glycine uptake by monkey spinal cord synaptosomes. Conditions of incubations as for glutamate (Fig. 1) with the following modification. The medium had reduced NaCl (125 mM) but with additional KCl (25 mM). μCi of 2-^{14}C-glycine (27 mCi mmol^{-1}) was used in combination with unlabelled glycine to give the required concentrations in a final volume of 1 ml. *v* is expressed as nmol glycine taken up per mg protein per 3 min. O—O, Control uptake; Δ—Δ, uptake in the presence of ODAP (10^{-3}M).

ODAP does therefore inhibit glutamate uptake by synaptosomes isolated both from young rat brain and from monkey spinal cord. The lesser potency of ODAP and the type of interaction observed in the rat brain synaptosomes might be a species characteristic or could be due to the presence of a more heterogenous population of synaptosomes, with affinity uptake systems for transmitter amino acids different from the monkey spinal cord. The localisation of glutamic and aspartic acids in synaptosomes separable from synaptosomes storing other amino acids has been reported[22].

The high affinity uptake system of synaptosomes is recognised to be important for the removal of amino acid transmitter from the synaptic environment[14,15]. Inhibition of this uptake, even the 15–20% inhibitory effect of ODAP seen for the rat brain synaptosomes, could be significant because of the extremely low concentration of glutamate required to cause excitation[10]. It is also interesting that ODAP inhibits glutamate uptake but not glycine uptake in monkey spinal cord synaptosomes. Since glycine is an inhibitory transmitter at the spinal cord level, these results could mean that the effects of ODAP in causing convulsions in young rats and hind leg paralysis in monkeys are primarily neuroexcitory, and are possibly mediated by glutamate. We have observed similar results with regard to the uptake of L-aspartate (unpublished results). Furthermore, ODAP administered *in vivo* to young rats is localised in synaptosomes and can cause the release of excitory amino acids from synaptosome preparations (manuscript in preparation).

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[1] Rao, S. L. N., and Sarma, P. S., *Biochem. Pharmac.*, **16**, 218 (1967).
[2] Rao, S. L. N., Sarma, P. S., Mani, K. S., Raghunatha Rao, T. R., and Sriramachari, S., *Nature*, **214**, 610 (1967).
[3] Sarma, P. S., and Padmanaban, G., in *Toxic Constituents of Plant Foodstuffs*, 267 (edit. by Liener, I.E.) Academic, New York, 1969).
[4] Cheema, P. S., Malathi, K., Padmanaban, G., and Sarma, P. S., *Biochem. J.*, **112**, 29 (1969).
[5] Cheema, P. S., Padmanaban, G., and Sarma, P. S., *J. Neurochem.*, **17**, 1295 (1970).
[6] Cheema, P. S., Padmanaban, G., and Sarma, P. S., *J. Neurochem.*, **18**, 2137 (1971).
[7] Duque-Magalhaes, M. C., and Packer, L., *FEBS Lett.*, **23**, 188 (1972).
[8] Mehta, T., Hsu, A. F., and Haskell, B. E., *Biochemistry*, **11**, 4053 (1972).
[9] Crewford, J. M., and Curtis, D. R., *Br. J. Pharmac.*, **23**, 313 (1964).
[10] Krnjevic, K., *Nature*, **228**, 119 (1970).
[11] Olney, J. W., *Science*, **164**, 719 (1969).
[12] Johnson, J. L., *Brain Res.*, **37**, 1 (1972).
[13] Balcar, V. J., and Johnston, G. A. R., *J. Neurobiol.*, **3**, 295 (1972).
[14] Logan, W. J., and Snyder, S. H., *Brain Res.*, **42**, 413 (1972).
[15] Balcar, V. J., and Johnston, G. A. R., *J. Neurochem.*, **20**, 529 (1973).
[16] Levi, G., and Raiteri, M., *Life Sci.*, **12**, 81 (1973).
[17] Eichberg, T., Whittaker, V. P., and Dawson, R. M. C., *Biochem. J.*, **92**, 91 (1964).
[18] Levitan, I. B., Mushynski, W. E., and Ramirez, G., *J. biol. Chem.*, **247**, 5376 (1972).
[19] Ross, L. L., Andreoli, V. M., and Marchbanks, R., *Brain Res.*, **25**, 103 (1971).
[20] Peterson, N. A., and Raghupathy, E., *J. Neurochem.*, **19**, 1423 (1972).
[21] Johnson, M. K., *Biochem. J.*, **77**, 610 (1960).
[22] Wofsey, A. R., Kuhar, M. J., and Snyder, S. M., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1102 (1971).

Quasi morphine-abstinence syndrome

ASSUMING that morphine induces dependence in response to continued interaction of the drug with an unidentified biochemical process of neurones, then suitable pharmacological manipulation might produce a paradigm of the morphine abstinence syndrome in experimental animals not exposed to any opiate. If so, the way in which such a quasi-abstinence syndrome was produced might throw light on the endogenous biochemical process involved[1].

In attempting to produce a quasi-abstinence syndrome, we used theophylline, naloxone and 5-hydroxytryptophan (5-HTP). Theophylline was used because it inhibits the phosphodiesterase of rat brain[2] and would therefore preserve brain cyclic nucleotides and because theophylline or cyclic 3′, 5′-adenosine monophosphate (cyclicAMP) intensifies morphine dependence in mice[3]. Naloxone was used because it has been shown to produce an abstinence-like effect in ileum isolated from naive guinea pigs[4]. 5-HTP was used because the ileum from morphine-treated guinea pigs responded more intensely to the product of its enzymatic decarboxylation, 5-hydroxytryptamine (5-HT), than did ileum from naive animals[5].

Male albino rats were treated orally with theophylline (100 mg kg^{-1}) in 20% w/v gum acacia or with the vehicle. At either 1 h or 1.75 h later, rats received 0.9% w/v NaCl in water (saline) subcutaneously or intraperitoneally, or 5-HTP (200 mg kg^{-1}) in saline intraperitoneally, according to the treatment schedule. Two hours after the first treatment with theophylline or vehicle, animals were challenged with a subcutaneous injection of either naloxone hydrochloride (10 mg kg^{-1}) in saline; saline; diamorphine hydrochloride (heroin) (1 mg kg^{-1}) in saline; or heroin (1 mg kg^{-1}) + naloxone hydrochloride (0.2 mg kg^{-1}) in saline. For 15 min after challenge the behavioural responses of the rats were recorded by observers who did not know what treatment each animal had received, as previously described[6]. Before each treatment and at the end of the observation period rats were weighed. For comparison, morphine dependence was induced with a single subcutaneous injection of a sustained-release emulsion containing 150 mg kg^{-1} morphine and abstinence effects were observed 24 h later after subcutaneous challenge with 1 mg kg^{-1} naloxone[6]. The incidence of responses was expressed as the presence or absence of a response in each animal observed. Weight changes were transformed into percentages and group comparisons were made using the Mann-Whitney U test. Comparable weight changes were not available for rats exposed for 24 h to the sustained-release morphine. For all other responses, probabilities were computed by means of the Fisher's exact probability test for a 2×2 contingency table. Weights of drugs are expressed as weights of active base.

Table 1 summarises the responses of rats to the various treatment schedules used. Comparison of responses to theophylline and vehicles with those to vehicles alone shows that theophylline significantly increased the incidence of eight responses — irritability to touch, diarrhoea, ptosis, body shakes, head-shakes, rearing, restlessness and weight loss. Naloxone alone increased the incidence of only two of these responses, irritability to touch and weight loss, as compared with controls receiving vehicles; but, after theophylline, naloxone produced a significantly higher incidence of four further responses (compared with theophylline alone), namely jumping, teeth chattering, irritability to handling and paw tremor and increased the significance of rearing and restlessness.

Table 1 Production of abstinence-like responses in rats by theophylline, 5-hydroxytryptophan and naloxone, without exposure to opiate.

Response	% incidence of responses after treatment schedule							
	(a) V+V+V	(b) V+V+N	(c) T+V+V	(d) T+V+N	(e) V+HP+V	(f) T+HP+V	(g) T+HP+N	(h) M+N[1]
Jumping	3	0	4	22 a b c *e f*	0	0	8	64 *a-g*
Teeth chattering	8	16	17	28 a b e f	4	4	19	75 *a-g*
Irritable to touch	3	36 *a*	35 *a*	31 *a*	23 a	50 *a e h*	58 *a d e h*	17
Irritable to handling	33	56	50	67 *a*	65 a	62 a	73 *a*	69 *a*
Diarrhoea	25	32	48 a	44	50 a	38	31	89 *a-g*
Chewing	33	32	52	44	35	46	46	83 *a-g*
Ptosis	33	37 d	61 a d	25	54	54	46	72 *a d g*
Body shakes	3	0	35 *a b e f g*	17 b	4	4	8	33 *a b e f g*
Head shakes	44	64	83 *a e f* h	75 a *e f*	42	64	73 a e f	58
Paw tremor	0	0	13	14 a	0	4	4	33 *a b* c d *e f g*
Rearing	75 e	84 *e*	94 a *e*	100 *a e*	46	88 *e*	100 *a e*	100 *a e*
Restlessness	44 e	36	71 a *b e*	83 *a b e*	19	88 *a b e*	92 *a b* e c	74 a *b e*
Mean % weight loss	0.6	1.6 a	1.9 *a*	2.3 *a*	0.8	1.8 *a e*	1.9 a e	NT
Maximum no. of rats	36	25	46	36	26	26	26	36
Mean total	25.3	32.8	46.9	45.8	28.5	41.8	46.5	63.9

V, Vehicle corresponding with drug; N, naloxone, 10 mg kg^{-1} subcutaneously (s.c.); N^1, naloxone, 1 mg kg^{-1} s.c.;
T, theophylline, 100 mg kg^{-1} orally; HP, 5-hydroxytryptophan (5-HTP) 200 mg kg^{-1} intraperitoneally;
M, morphine, 150 mg kg^{-1} s.c. in a sustained-release preparation; NT, not tested.
After pretreatment with theophylline, or vehicle (2 h), saline (1 h) or 5-HTP or vehicle 15 min previously, rats were challenged with vehicle or naloxone. After pretreatment with morphine 24 h previously, rats were challenged with naloxone. Responses were recorded during 15 min after challenge. Letters a–h in the body of the Table indicate comparisons between columns where a significantly greater effect was found; where the letter is italic, $P < 0.01$, where it is not, $P < 0.05$.

5-HTP and vehicles, compared with vehicles alone, produced an increased incidence of three responses — irritability to touch, irritability to handling and diarrhoea, but reduced the incidence of rearing and restlessness. Theophylline, 5-HTP and vehicle gave a significantly increased incidence of irritability to touch, rearing, restlessness and weight loss compared with 5-HTP and vehicles. Treatment with theophylline, 5-HTP and naloxone gave a significantly increased incidence of six of the thirteen responses recorded compared with vehicles alone.

Table 1 also gives the abstinence effects observed in a similar way after withdrawal of morphine, precipitated with 1 mg kg^{-1} naloxone, 24 h after administration to rats of a sustained-release preparation of morphine. The incidence of many of the responses is still higher than after theophylline, 5-hydroxytryptophan and/or naloxone.

Table 2 Blockade by heroin of behavioural responses of rats to theophylline and removal of blockade by naloxone.

Response	% incidence of responses after treatment schedule		
	a T+V+V[1]	b T+V+H	c T+V+(H+N)
Jumping	0	10	20
Teeth chattering	0	0	10
Irritable to touch	50 b	0	50 b
Irritable to handling	70 *b*	10	70 *b*
Diarrhoea	80 *b*	10	50
Chewing	70 *b*	0	30
Ptosis	70 *b*	20	40
Body shakes	10	0	10
Head shakes	90 *b*	0	90 *b*
Paw tremor	10	0	0
Rearing	90 *b*	20	100 *b*
Restlessness	90 *b*	0	100 *b*
Mean % weight loss	1.8	1.5	1.6
Maximum no. of rats	10	10	10
Mean total	52.5	5.8	47.5

T, theophylline, 100 mg kg^{-1} given orally at 0 h; V, saline, 10 ml kg^{-1} given subcutaneously (s.c.) at 1 h; V^1, saline s.c. H, heroin, 1.0 mg kg^{-1} s.c. or H + N, heroin 1 mg kg^{-1} + naloxone, 0.2 mg kg^{-1} s.c. at 2 h. Other details as in Table 1.

Table 2 summarises experiments in which heroin (1 mg kg^{-1}) was tested as an inhibitor of responses induced by theophylline. Heroin largely suppressed the quasi-abstinence effects. Naloxone (0.2 mg kg^{-1}) was found to overcome this inhibition by heroin.

Of the various treatment schedules, theophylline with naloxone produced the closest resemblance to a true morphine abstinence syndrome (Table 1); but the presence of theophylline in any schedule led to a high incidence of several quasi-abstinence responses. 5-HTP produced some behavioural responses resembling those of abstinence, but these were fewer than with theophylline. The results of treating naive rats with theophylline resembled in four ways the abstinence syndrome during morphine withdrawal without precipitation by an antagonist: first, many of the same responses increased in incidence; second, naloxone increased the incidence of responses in both conditions; third, heroin lessened the incidence of responses; and fourth, naloxone antagonised this effect of heroin. We therefore apply the term 'quasi-abstinence syndrome' to the behavioural state produced in the naive rat by theophylline.

The ability of theophylline to produce quasi-abstinence effects indicates that cyclic nucleotides may be involved in morphine dependence. This accords with the findings that cyclic AMP or theophylline antagonised the antinociceptive effect of morphine in the rat[7,8] or mouse[8] and enhanced tolerance and dependence in the mouse[3], although other cyclic nucleotides were inactive. This observation is consistent with the suggestion[9] that the raised level of protein kinase found in rat brain after morphine withdrawal may contribute directly to the signs of abstinence.

The contribution of 5-HTP to the quasi-abstinence syndrome reinforces a comment made some years ago[10]. Also, it is consistent with the finding that the ileum isolated from morphine-tolerant guinea pig is more responsive to 5-HT than is normal ileum[5]. That 5-HT inhibits noradrenaline stimulation of adenyl cyclase[11], makes plausible an association between this effect of 5-HT and a cyclic AMP mechanism.

In vivo studies alone cannot determine the precise mechanism of action of a drug, but they can indicate where this mechanism should be sought. The present studies have indeed pointed to a further exploration of the interaction of morphine with cyclic nucleotides; and it has been reported that morphine potently antagonises the activation by PGE_1 or PGE_2 of cyclic AMP formation by rat brain or intestine homogenate[12]. It therefore seems possible that morphine tolerance and dependence may arise through compensatory changes in processes related to this PGE – cyclic AMP interaction.

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[1] Collier, H. O. J., *Pharmacology*, **11**, 58-61 (1974).
[2] Beer, B., Chasin, M., Clody, D. E., Vogel, J. R., and Horovitz, Z. P., *Science*, **176**, 428-430 (1972).
[3] Ho, I. K., Loh, H. H., and Way, E. L., *J. Pharmac. exp. Ther.*, **185**, 347-357 (1973).
[4] Villarreal, J. E., and Dummer, G.E., *Fedn. Proc.*, **32**, 688 (1973).
[5] Schulz, R., and Goldstein, A., *Nature*, **244**, 168-169 (1973).
[6] Collier, H. O. J., Francis, D. L., and Schneider, C., *Nature*, **237**, 220-223 (1972).
[7] Contreras, E., Castillo, S., and Quaida, L., *J. Pharm. Pharmac.*, **24**, 65-66 (1972).
[8] Ho, I. K., Loh, H. H., and Way, E. L., *J. Pharmac. exp. Ther.*, **185**, 336-346 (1973).
[9] Clark, A. G., Jovic, R., Ornellas, M. R., and Weller, M., *Biochem. Pharmac.*, **21**, 1989-1990 (1972).
[10] Collier, H. O. J., *Adv. Drug Res.*, **3**, 171-188 (1966).
[11] Palmer, G. C., Sulser, F., and Robison, G. A., *Neuropharmacology*, **12**, 327-337 (1973).
[12] Collier, H. O. J., and Roy, A. C., *Nature*, **248**, 24-27 (1974).

Neurotrophic control of colchicine effects on muscle?

COLCHICINE, a substance known to block axonal transport[1,2], has been reported to mimic the effects of denervation on mammalian skeletal muscles[3-5]. These effects, which include an increased extrajunctional sensitivity to acetylcholine (Ach) and a fall in resting membrane potential, occur a few days after colchicine is applied locally to the nerve in doses that do not seem to interfere with nerve impulse conduction, neuromuscular transmission, or muscle tension evoked by stimulation of the nerve[3-5]. It seems, therefore, that denervation-like changes may be induced in the presence of normal muscle activity and this suggestion is supported by experiments reported here in which the diaphragms of rats with apparently normal respiration became supersensitive to Ach after injection of colchicine into one of the legs (mean extrajunctional Ach sensitivity of 22 fibres from three rats was 4.6 mV nC^{-1}, range 0–30 mV nC^{-1}). It has been suggested that colchicine produces these changes in muscle by blocking some component of axonal transport, thus interfering with the neurotrophic control of muscle[3,4]. Colchicine also has a systemic effect[5], however, and its effect on muscle might be direct and occur independently of any of its effects on nerve. Here I examine this possibility.

The experiments were performed on young white rats each weighing about 200 g. The sciatic nerve was resected on one side, and two platinum stimulating electrodes were implanted in the denervated leg, one on each side of the soleus muscle. After waiting 1 d for neuromuscular transmission to fail and degeneration of motor nerve terminals to begin[6], the muscle was stimulated directly by intermittent trains of stimuli at 10 Hz until the acute experiment. Such stimulation largely prevents the development of Ach supersensitivity in denervated muscle[7]. Because colchicine is reported to have its greatest effect on muscle after 4 d (ref. 4), it was injected (3.5 µl of a 0.2 M solution) intramuscularly high in the thigh 4 d before the acute experiment. Fourteen days after denervation, the soleus muscles were removed from both sides and placed in a chamber superfused with oxygenated rat Ringer's solution at room temperature. Both the resting membrane potential and the sensitivity to electrophoretically applied Ach were examined in single muscle fibres by conventional techniques[8]. Control animals were similarly treated but received no colchicine; in some of these controls, the unstimulated soleus muscle was also denervated.

Four days after the injection of colchicine, the mean extrajunctional Ach sensitivity of fibres with intact innervation (61 fibres, six muscles) was 3.2 mV nC^{-1} (range 0.2–14.3 mV nC^{-1}) (Fig. 1*a*, open columns). A similar sensitivity and distribution

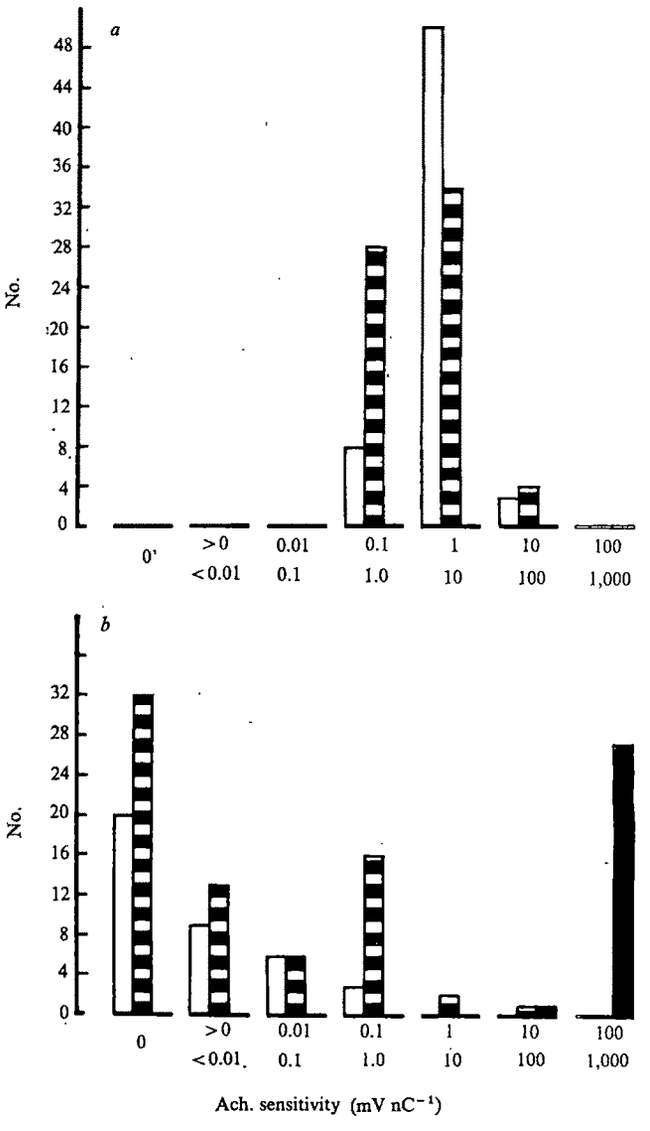


Fig. 1 Extrajunctional Ach sensitivity of soleus fibres from innervated (open columns), denervated stimulated (striped columns) and denervated muscles (solid columns). Denervation lasted 14 d. Stimulation took place from day 2 to day 14 after denervation. Ach sensitivity was measured *in vitro* at 2 mm from the myotendinous junction and is expressed as mV depolarisation per nC charge passed through a micropipette containing 3 M AchCl. Because the supersensitivity following either denervation or colchicine treatment is approximately equal along the membrane lying between the endplate and the myotendinous junction[5,9], these values may be taken as representative of the average extrajunctional sensitivity for the whole fibre. *a*, 270 µg Colchicine injected intramuscularly high in the thigh on day 10, that is 4 d before the Ach-sensitivity was tested; *b*, no colchicine was given.

about the mean (3.2 mV nC^{-1}, range 0.1–15.0 mV nC^{-1}, excluding one fibre with 57 mV nC^{-1}) was found in denervated stimulated soleus fibres (66 fibres) from the opposite leg of the same animals (Fig. 1*a*, striped columns). In the absence of colchicine the same stimulation largely blocked the supersensitivity of denervated fibres (70 fibres, five muscles) so the majority of these were insensitive or only very weakly sensitive (Fig. 1*b*, striped columns) and were indistinguishable from normal fibres (Fig. 1*b*, open columns). In two of the five denervated stimulated muscles, a few fibres had a higher sensitivity than normal. These fibres generally occurred in small groups clearly separated from the insensitive or weakly sensitive fibres in the same muscle. Their high sensitivity may, therefore, be caused by inadequate stimulation or local injurious effects of the stimulation[7]. In contrast, denervated but unstimulated fibres (27 fibres, three muscles) were all highly sensitive with a mean sensitivity of 250 mV nC^{-1} (range 50–525) (Fig. 1*b*, solid columns). It seems that colchicine raises the Ach sensitivity of both innervated and denervated stimulated fibres to about the same extent.

Colchicine had a comparable effect on resting membrane potential. Both innervated and denervated stimulated soleus fibres exposed to colchicine had similar relatively low mean resting membrane potentials; 67.4 ± 4.8 mV (mean ± s.d.) and 67.5 ± 4.2 mV (mean ± s.d.), respectively. In the absence of colchicine the corresponding values were higher: 70.9 ± 4.4 mV and 74.1 ± 4.9 mV. The lowest membrane potentials were recorded in fibres that were only denervated (61.6 ± 5.3 mV). The effect of colchicine on both resting membrane potential and Ach sensitivity was thus similar to that of denervation but less pronounced.

All innervated muscles exposed to colchicine twitched vigorously to stimulation of the nerve and miniature endplate potentials of approximately normal amplitude and frequency were observed in all fibres examined. In denervated stimulated muscles, there was no twitch to nerve stimulation, and no miniature endplate potentials were observed.

The increase in extrajunctional Ach sensitivity and fall in resting membrane potential induced by colchicine in innervated rat soleus muscles confirm other findings[3-5]. In addition my experiments show that similar changes developed in denervated muscles that were kept active by direct stimulation. As direct stimulation in the absence of colchicine has been shown to prevent such changes[7] it seems that colchicine has some effects on the muscle membrane that are not mediated by the nerve. These effects of colchicine on innervated muscle should therefore not be taken as evidence for the neurotrophic control of extrajunctional membrane properties. The mechanism behind the effect of colchicine on muscle is not known, and is in itself of some interest.

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[1] Dahlström, A., *Eur. J. Pharmac.*, **5**, 111 (1968).
[2] Kreutzberg, G. W., *Proc. natn. Acad. Sci., U.S.A.*, **62**, 722 (1969).
[3] Hofmann, W. W., and Thesleff, S., *Eur. J. Pharmac.*, **20**, 256 (1972).
[4] Albuquerque, E. X., Warnick, J. E., Tasse, J. R., and Sansone, F. M., *Exp. Neurol.*, **37**, 607 (1972).
[5] Cangiano, A., *Brain Res.*, **58**, 255 (1973).
[6] Miledi, R., and Slater, C. R., *J. Physiol., Lond.*, **207**, 507, (1970).
[7] Lømo, T., and Rosenthal, J., *J. Physiol., Lond.*, **221**, 493 (1972).
[8] Del Castillo, J., and Katz, B., *J. Physiol., Lond.*, **128**, 157 (1955).
[9] Axelsson, J., and Thesleff, S., *J. Physiol., Lond.*, **147**, 178 (1959).

Pseudorotation mechanism of ATP hydrolysis in muscle contraction

AN analysis of the reaction mechanism of ATP hydrolysis may elucidate how ATP provides energy for muscle contraction. Kinetic studies of the mechanism of ATP hydrolysis catalysed by myosin and its proteolytic subfragments[1,2], revealed at least two intermediates before the rate limiting step: (1) enzyme-bound ATP (M*ATP) and (2) an intermediate (M*ADP·P_i) that yields ADP and P_i when a quenching reagent such as $HClO_4$ is added. Trentham *et al.*[2] proposed that the rate limiting step is the transition from M*ADP·P_i to the Michaelis product complex M·ADP·P_i. That M*ADP·P_i is distinct from the Michaelis product complex is supported by observations made using techniques which include spin labelling[3], difference ultraviolet absorption spectroscopy[4], extrinsic probe fluorescence[5] and intrinsic myosin fluorescence[6]. Each method shows that the conformation of myosin *during* ATP hydrolysis differs from the conformation induced by the binding of ADP and P_i. This conclusion is supported further by the observation that the free energy change for the reversible reaction M*ATP $\rightleftharpoons$ M*ADP·P_i is only −1.3 kcalories per mol (ref. 7). Although comparable in energy with M*ATP, M*ADP·P_i nevertheless seems to be closely related to products because it decays to ADP and P_i when exposed to a quenching reagent. Although this decay to ADP and P_i has been widely interpreted as indicating that ATP is already cleaved in M*ADP·P_i, it could alternatively be interpreted in terms of an uncleaved but labile reaction intermediate.

In the intermediate $P_i \rightleftharpoons H_2O$ exchange[8] ^{18}O must be incorporated into either M*ATP or M*ADP·P_i. Sartorelli *et al.*[9] have shown that when myosin-catalysed ATP hydrolysis is quenched by $HClO_4$ containing $H_2{}^{18}O$, no ^{18}O is detected either in the P_i cleaved from ATP or in the myosin. This implies that H_2O must enter both the hydrolysis and the intermediate exchange reactions before the formation of M*ADP·P_i. Thus M*ADP·P_i cannot be some form of anhydride such as a phosphorylated myosin or metaphosphate.

We show here that a pseudorotation reaction mechanism for ATP hydrolysis can account for both the intermediate and medium[10] $P_i \rightleftharpoons H_2O$ exchanges as well as the characteristics of M*ADP·P_i. A pseudorotation mechanism has previously been proposed for ATP synthesis in mitochondrial oxidative phosphorylation[11–13]. We have recently shown it to be the only mechanism proposed so far that accounts adequately for the mitochondrial oxygen exchanges[14].

Figure 1 presents a schematic representation of the pseudorotation mechanism of ATP hydrolysis. The pseudorotation process transforms the P–O bond cleaved in hydrolysis from a relatively short, strong and nonreactive equatorial bond (Fig. 1*a*) to a relatively long, weak and reactive apical bond (Fig. 1*b*). In Fig. 1*a* the pair of oxoniums bound by H bonds to the enzyme and apical bonds to the phosphorus centre constitute two entry points for H_2O oxygen. The diagram shows that by dynamic reversal of the H_2O binding step before pseudorotation, the two oxygens in the pair of oxoniums could equilibrate with the H_2O oxygens of the medium. This equilibration could introduce up to two labelled oxygens into the P_i cleaved in ATP hydrolysis.

Under certain conditions, however, all four of the oxygens in the P_i cleaved in ATP hydrolysis can be labelled[14,15]. Since the enzyme-bound H_2O exchanges with the medium H_2O, some of the time a H_2O molecule must be missing from the active site. In the absence of H_2O the γ-phosphoryl of ATP might undergo a rotation about the bridge P–O bond in a time comparable with the H_2O exchange time. In this way all four of the oxygens in the P_i cleaved from ATP could be labelled with ^{18}O. It must be emphasised that such a rotation could occur only in the absence of the H_2O molecule. In the pentacoordinate intermediate A the γ-phosphoryl would presumably

be bound so tightly that no rotation could occur. One can also conceive of a mechanism in which complete labelling of P_i occurs into a tightly bound γ-phosphoryl of ATP. For such a mechanism the exchange illustrated in Fig. 1 would be only a prototype.

Since H_2O must enter both the hydrolysis and the intermediate exchange reactions before the formation of M*ADP·P_i, the only alternative to intermediate exchange occurring into M*ATP seems to be the following sequence of events: (1) cleavage of the P–O bond, (2) rotation of P_i in the active site, (3) spontaneous reformation of the P–O bond, and (4) exchange of enzyme-bound H_2O with medium H_2O. If all four of the oxygens of the cleaved P_i were able to interchange their positions in the active site, then all four of the P_i oxygens could be labelled by this mechanism. However, the weak binding implicit in the rotation of P_i in the active site seems to be inconsistent with the evidence that M*ADP·P_i is not a Michaelis product complex but rather is energetically comparable with M*ATP. This alternative therefore seems unlikely.

Thus it seems that exchange occurs directly into the γ-phosphoryl of M*ATP. Excluding an unlikely rotation of the ATP-H_2O adduct in the active site, direct exchange into M*ATP implies at least two entry points for H_2O oxygen. This exceedingly restrictive condition would exclude all mechanisms not of the type depicted in Fig. 1.

Concerning the medium exchange, there is a nucleotide requirement as a cofactor that can be satisfied by nucleotide mono-, di- and triphosphates as well as the ATP analogue adenylyl-methylenediphosphonate which does not undergo hydrolysis[16]. This implies that the medium exchange does not occur through the reversal of hydrolysis. Medium exchange must therefore occur directly into P_i which is analogous to the requirement that intermediate exchange must occur directly into the γ-phosphoryl of ATP by means of two or more entry points of H_2O oxygen. Thus consideration of the medium exchange independently leads to the same conclusion as that reached from consideration of the intermediate exchange.

A medium exchange separate from the reversal of hydrolysis is similar to the mitochondrial $P_i \leftrightharpoons H_2O$ exchange separate from the reversal of phosphorylation[14]. Since the characteristics of this mitochondrial $P_i \leftrightharpoons H_2O$ exchange can be rationalised by a mechanism stereochemically analogous to the mechanism proposed for the mitochondrial ATP $\leftrightharpoons H_2O$ exchange, we propose that the medium exchange can similarly be rationalised by a mechanism stereochemically analogous to the intermediate $P_i \leftrightharpoons H_2O$ exchange.

Equating intermediate *b* in Fig. 1 with M*ADP·P_i accounts for: (1) the difference between M*ADP·P_i and the Michaelis product complex, (2) the 'high energy' nature of M*ADP·P_i, (3) the breakdown of M*ADP·P_i to yield ADP and P_i when a quenching reagent is added, and (4) the breakdown of M*ADP·P_i without incorporation of ^{18}O into either P_i or myosin.

The mechanism proposed here for the intermediate $P_i \leftrightharpoons H_2O$ exchange is identical to that proposed for the mitochondrial ATP $\leftrightharpoons H_2O$ exchange[11] except that the ^{18}O appears in the P_i cleaved from ATP rather than in ATP itself. However, no myosin-catalysed ATP $\leftrightharpoons H_2O$ exchange has been detected[8,9]. The failure to detect a myosin ATP $\leftrightharpoons H_2O$ exchange is consistent with the low value for the dissociation rate of unhydrolysed ATP ($\leqslant 0.02\ s^{-1}$)[7,17] together with the small steady state concentration of the dissociating species M*ATP relative to M*ADP·P_i[7].

Available evidence strongly supports a pseudorotation mechanism of ATP hydrolysis. It is therefore interesting to inquire whether a rationale might be discernible for this mechanism in muscle contraction.

In the model of Lymn and Taylor[18] actin and myosin are not associated in the transition from M*ATP to M*ADP·P_i. This would require that this transition be an approximately energy conserving process, as recently confirmed by Bagshaw and Trentham[7]. As developed elsewhere with regard to ATP

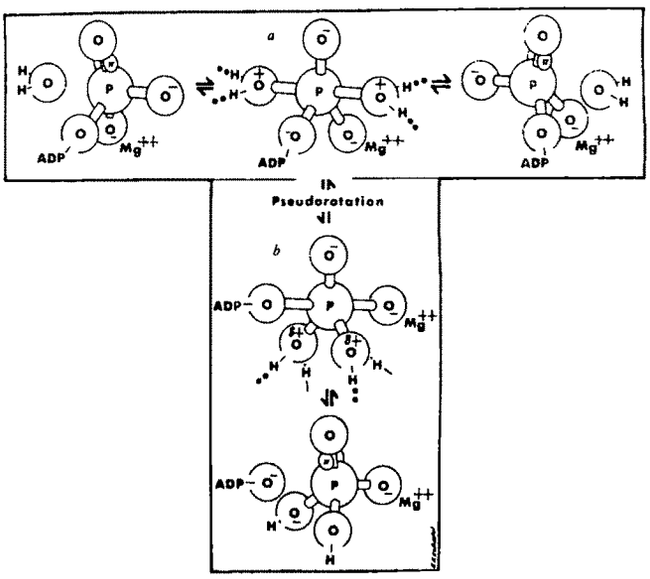


Fig. 1 Pseudorotation reaction mechanism of ATP hydrolysis. Reaction intermediate *a* with ADPO- equatorial has two apical oxoniums. Reaction intermediate *b* depicts ADPO- apical as required for cleavage from the phosphorus centre.

synthesis in oxidative phosphorylation, a fundamental aspect of the pseudorotation mechanism is the pair of oxoniums[11,12]. Its effect is to stabilise intermediate *a* in Fig. 1 relative to intermediate *b*, thereby providing approximate energy parity between the intermediate resembling reactants and the intermediate resembling products. Thus the pair of oxoniums responsible for a straightforward explanation of the oxygen exchanges could also account for the approximate energy parity between these two intermediates. This suggests a mechanistic basis for the correlation between intermediate exchange activity and muscle contraction noted by Yount and Koshland[15].

In the sliding filament model tension is generated by a change in orientation of the myosin head with respect to the actin filament[19]. This implies that actin must initially associate with myosin to give a state other than the thermodynamically most stable state of the actomyosin complex. It seems that actin associates initially with myosin in the M*ADP·P_i state[18,20]. As previously noted, there is considerable evidence that the conformation of myosin in the M*ADP·P_i state differs from that in the Michaelis product complex M·ADP·P_i. Thus these two conformations of myosin might be correlated tentatively with the two orientations of the actomyosin cross bridge invoked by Huxley and Simmonds[21,22] to explain the viscoelastic properties of active muscle fibres. This correlation would also be consistent with the requirement that, because the velocity of muscle contraction decreases with increasing load, the rate limiting step of ATP hydrolysis must be sensitive to tension. Exergonic bond cleavage would therefore occur in the mechanochemically coupled step since in this reaction mechanism cleavage occurs in the transition from M*ADP·P_i to M·ADP·P_i.

These considerations together with the dissociation of actomyosin induced by ATP binding[18] and the refractory state of myosin[20] indicate that the contraction cycle involves a sequence of discrete conformational states of myosin. A sequence of discrete conformational states of myosin as an ATPase enzyme requires a sequence of discrete reaction intermediates in ATP hydrolysis. The intrinsically step-wise character of the pseudorotation mechanism is well suited for providing such a sequence of reaction intermediates with appropriate energetic characteristics.

In view of the apparent similarity between the ATP reaction mechanisms in muscle contraction and oxidative phosphorylation, it is of interest to note that the myosin fragment S-1

appears to be evolutionarily related to the mitochondrial coupling factor F_1 (ref. 23).

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[1] Lymn, R. W., and Taylor, E. W., *Biochemistry*, **9**, 2975-2983 (1970).
[2] Trentham, D. R., Bardsley, R. G., Eccleston, J. F., and Weeds, A. G., *Biochem. J.*, **126**, 635-644 (1972).
[3] Seidel, F. C., and Gergely, J., *Biochem. biophys. Res. Commun.*, **44**, 826-830 (1971).
[4] Morita, F., *J. biol. Chem.*, **242**, 4501-4506 (1967).
[5] Cheung, H. C., *Biochim. biophys. Acta*, **194**, 478-485 (1969).
[6] Werber, M. M., Szent-Gyoergyi, A. G., and Fasman, G. D., *Biochemistry*, **11**, 2872-2883 (1972).
[7] Bagshaw, C. R., and Trentham, D. R., *Biochem. J.*, **133**, 323-328 (1973).
[8] Levy, H., and Koshland, D. E., *J. biol.Chem.*, **234**, 1102-1107 (1958).
[9] Sartorelli, L., Fromm, H. J., Benson, R. W., and Boyer, P. D., *Biochemistry*, **5**, 2877-2884 (1966).
[10] Dempsey, M. E., and Boyer, P. D., *J. biol. Chem.*, **236**, PC6-7 (1961).
[11] Korman, E. F., and McLick, J., *Proc. natn. Acad. Sci. USA*, **67**, 1130-1136 (1970).
[12] Korman, E. F., and McLick, J., *Bioenergetics*, **3**, 147-158 (1972).
[13] Korman, E. F., and McLick, J., *Bioorg. Chem.*, **2**, 179-190 (1973).
[14] Young, J. H., Korman, E. F., and McLick, J., *Bioorg. Chem.* **3**, 1-15 (1974).
[15] Yount, R. G., and Koshland, D. E., *J. biol. Chem.*, **238**, 1708-1713 (1963).
[16] Swanson, J. R., and Yount, R. G., *Biochem. Z.*, **345**, 395-409 (1966).
[17] Mandelkow, E., and Mandelkow, E., *FEBS Lett.*, **33**, 161-166 (1973).
[18] Lymn, R. W. and Taylor, E. W., *Biochemistry*, **10**, 4617-4624 (1971).
[19] Huxley, H. E., *Science*, **164**, 1356-1367 (1969).
[20] Eisenberg, E., Dobkin, L., and Kielley, W. W., *Proc. natn. Acad. Sci. USA*, **69**, 667-671 (1972).
[21] Huxley, A. F., and Simmonds, R. M., *Nature*, **233**, 533-538 (1971).
[22] Julian, F. J., Sollins, K. R., and Sollins, M. R., *Cold Spring Harbor Symp. quant. Biol.*, **37**, 685 (1972).
[23] Weltman, J. K., and Dowben, R. M., *Proc. natn. Acad. Sci. USA*, **70**, 3230-3234. (1973).

In vivo enhancement of tyrosine hydroxylation in rat striatum by tetrahydrobiopterin

THE hydroxylation of tyrosine, the rate-limiting step in the biosynthesis of catecholamines *in vivo*[1], depends on various factors: the concentration of apoenzyme and of cofactor (5,6,7,8-tetrahydrobiopterin, BH_4), the availability of molecular oxygen[2-4] and the activity of the system reducing the oxidised pteridine (dihydropteridine reductase[5]). Calculations based on the rate of tyrosine hydroxylation *in vitro* and on the cofactor concentration in brain suggested that the latter might be limiting[6,7], although its concentration at the enzyme site is not known. Similar conclusions were reached in experiments with cultures of sympathetic chain of chicks in which addition of biopterin increased the catecholamine content[8]. But no evidence exists for a possible role of BH_4 as limiting factor of tyrosine hydroxylation *in vivo*. We describe here the *in vivo* effect of BH_4, the natural cofactor of tyrosine hydroxylase[9], on dopamine formation in rat striatum.

Male albino rats of Wistar origin (Füllinsdorf), 180-200 g, were injected with an aqueous solution of BH_4 into one lateral brain ventricle (30-1,000 μg in 10 μl through a chronically implanted cannula[10]) or intravenously (125 mg kg^{-1}). The solutions contained 1% ascorbic acid. At various time intervals after administration of BH_4 0.18 μg L-2, 3-^{3}H-tyrosine in 10 μl (specific activity) 10 Ci $mmol^{-1}$) were injected intraventricularly. Animals injected with ^{3}H-tyrosine after ascorbic acid served as controls. The rats were decapitated 15 min after ^{3}H-tyrosine injection, the corpora striata immediately dissected and homogenised in 0.4 M ice-cold perchloric acid (1:5 w/v) containing 0.3% ascorbic acid and 10 μg dopamine. The radioactive catechols were absorbed on alumina[11], eluted with 4 ml 0.3 N acetic acid and measured in a liquid scintillation counter. In some experiments the composition of the eluate was checked by paper chromatography (Whatman P81; isopropanol, 0.2 N ammonium acetate, *p*H 6.0, 1:2; 15 h). Dopamine was the main component besides traces of 3,4-dihydroxyphenylacetic acid. The values were corrected for recovery (75 ± 2.3%).

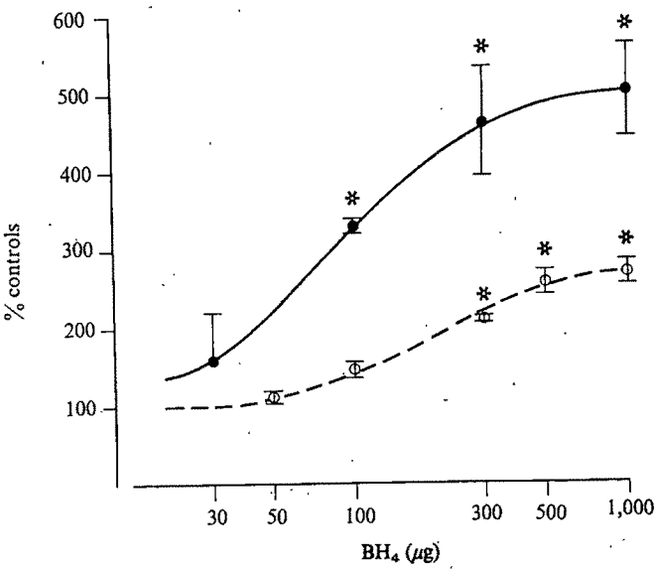


Fig. 1 Concentration of ●, tetrahydrobiopterin (BH_4), and ○, ^{3}H-catechols in the rat striatum following various doses of BH_4. BH_4 was administered into one lateral brain ventricle alone or immediately before 0.18 μg ^{3}H-tyrosine intraventricularly. Rats were killed 15 min after injection(s). The figures represent averages with s.e.m. of values from 12-36 determinations. Each determination was carried out with the corpora striata of one rat. Absolute control values: BH_4 24.5 ± 3.5nmol g^{-1}; ^{3}H-catechols 6.4 ± 0.8pmol g^{-1}. Significance against corresponding controls: $^*P<0.01$; all other values $P>0.05$.

For the measurement of the cofactor concentrations another group of rats received BH_4 (in 1% aqueous ascorbic acid) intraventricularly (i.v.) (see above) at various time intervals before killing. Animals injected with ascorbic acid alone served as controls. The corpora striata from one rat were homogenised in 0.2 M Na-phosphate buffer, *p*H 6.5 (1:5 w/v) containing 0.02 M ascorbic acid, heated for 90 s to 100° C and centrifuged. A 10 μl aliquot of the supernatant was incubated for 60 min at 30° C with: 0.2 M phosphate–citrate buffer, *p*H 6.4, 20 μl; 10 mM ascorbic acid, 20 μl; 0.5 mM L-*p*-^{3}H-phenylalanine (SA 40 mCi $mmol^{-1}$), 20 μl; 70 μg liver phenylalanine hydroxylase (purified according to Kaufman and Fisher[12], first two steps), dissolved in 20 μl 0.2 M Na-phosphate buffer (see above); water to a total volume of 130 μl[13]. Supernatant containing various concentrations of BH_4 (20-100 pmol in phosphate buffer) or phosphate buffer served as standards or blanks, respectively. The reaction was stopped by cooling to 0° C. After addition of 0.5 ml ice-cold Na-acetate buffer (0.2 M, *p*H 5.5), iodination

was performed using 1 mg N-iodosuccinimide[7] in 10 μl dimethylsulphoxide. After 5 min the samples were supplemented with 100 μl 30% trichloracetic acid, centrifuged and passed through columns of charcoal (Norit S×35)/Dowex 50W×4 (10/700 mg) (ref. 7). The effluent (combined with the washing, 1 ml water) containing tritiated water, was measured by liquid scintillation counting. In such a system the amount of tritiated water is proportional to the initial concentration of BH_4 in the incubation medium. In all experiments quenching was corrected by means of internal standards.

Intraventricular administration of increasing amounts (30-1,000 μg) of BH_4 alone and of BH_4 immediately before ^{3}H-tyrosine caused in the striatum a dose-dependent

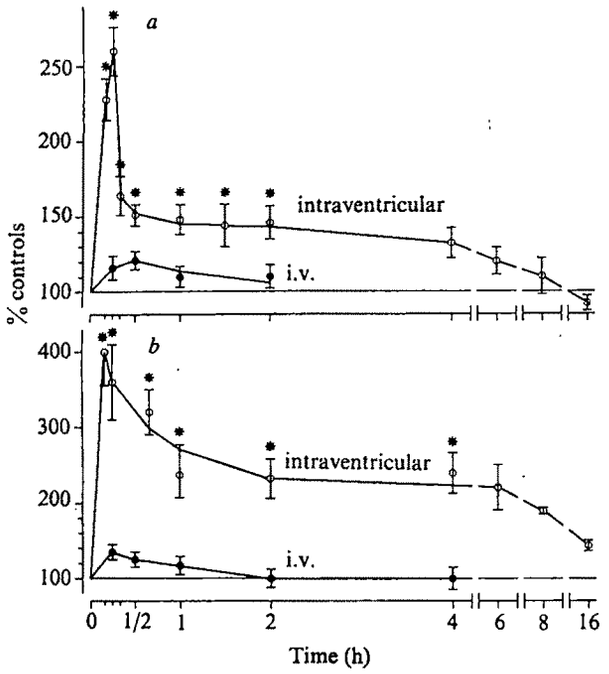


Fig. 2 Time course of *a*, ^{3}H-catechols and *b*, tetrahydrobiopterin (BH_4) in the striatum. BH_4 was administered into one lateral ventricle of the rat brain (0.5 mg per rat) or intravenously (125 mg kg^{-1}) alone or at various time intervals before intraventricular injection of 0.18 μg ^{3}H-tyrosine injected 15 min before killing. Abscissa: time between injection of BH_4 and decapitation. The figures represent means with s.e.m. of values from 12–36 determinations. Each determination was carried out with the corpora striata of one rat. Absolute control values: BH_4 29.0 ± 5.1 nmol g^{-1}; ^{3}H-catechols 7.5 ± 0.8 pmol g^{-1}. Significance against corresponding controls: $*P<0.01$; all other values $P>0.05$.

accumulation of the cofactor and an increased accumulation of labelled catechols, respectively (Fig. 1). The time course of accumulation of striatal BH_4 and increased accumulation of ^{3}H-catechols was similar. Maximal values were found 10 and 15 min respectively, after injection of the cofactor. After 4 h the ^{3}H-catechols had almost reached control levels again, whereas BH_4 was still somewhat increased at 16 h (Fig. 2), possibly due to a continuous recycling of the oxidised cofactor.

Intravenous administration of a large dose (125 mg kg^{-1}) of BH_4 alone or immediately before intraventricular injection of ^{3}H-tyrosine caused a small increase of the cofactor and of the ^{3}H-catechol content in the striatum respectively, which did not, however, reach a significant level (Fig. 2). This small effect may be due to poor penetration of the cofactor into the brain.

The enhanced accumulation of labelled catechols in the striatum which paralleled the increased BH_4 content was probably the consequence of an increased synthesis of the amines from ^{3}H-tyrosine. Thus, 250 mg kg^{-1} α-methyl-*p*-tyrosine (an inhibitor of tyrosine hydroxylase) i.p. 1 h before ^{3}H-tyrosine diminished both the formation of labelled catechols and their enhanced accumulation due to BH_4 (Fig. 3). An impaired elimination of the labelled catechols from the brain is unlikely since in preliminary experiments BH_4 did not enhance the accumulation of ^{3}H-catechols in the striatum of animals administered L-2,5,6-^{3}H-dihydroxyphenylalanine intraventricularly. This result also confirms that BH_4 acts at the level of the hydroxylation of tyrosine.

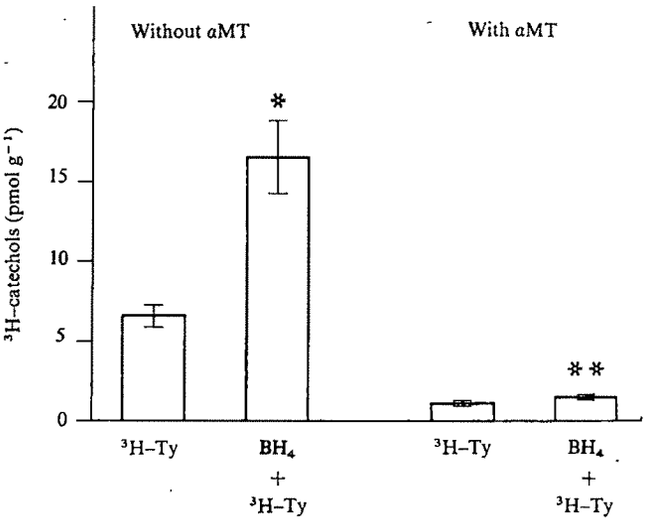


Fig. 3 Effect of α-methyl-*p*-tyrosine (αMT) on the increase of ^{3}H-catechols in rat striatum induced by ^{3}H-tyrosine (^{3}H-Ty) alone or in combination with tetrahydrobiopterin (BH_4). BH_4 (0.5 mg per rat) was injected into one lateral brain ventricle immediately before intraventricular administration of 0.18 μg ^{3}H-Ty. αMT (250 mg kg^{-1}) was administered i.p. 1 h before ^{3}H-Ty. Rats were killed 15 min after ^{3}H-Ty. The figures represent averages with s.e.m. of 4-6 determinations, each carried out with the corpora striata of one rat. Significance compared with corresponding values with ^{3}H-Ty alone: $*P<0.01$; $**P>0.1$.

The striatal content of endogenous dopamine and its major metabolite homovanillic acid (both measured fluorimetrically[11,14]) was not significantly changed 15 min after intraventricular injection of 500 μg BH_4 (dopamine: controls 8.7 ± 0.4, BH_4-treated 9.1 ± 0.2 μg g^{-1}, $P > 0.05$; homovanillic acid: controls 0.84 ± 0.07, BH_4-treated 0.80 ± 0.08 μg g^{-1}, $P > 0.05$; 12 animals per group). These findings indicate that of the total dopamine stored the newly formed amine is only a minor part which may have entered into the small (functional) pool previously claimed to be preferentially filled with the newly synthesised catecholamines[15].

From these results it can be concluded that in the striatum the amount of the apoenzyme of tyrosine hydroxylase is probably not the limiting factor for the hydroxylation of tyrosine *in vivo*. Under physiological conditions, tyrosine hydroxylase apparently does not work to its full capacity due to the limited concentration of BH_4. It is therefore possible that rapid changes in activity of tyrosine hydroxylase may be connected with modifications in the availability of reduced pteridine cofactor at the enzyme site.

The BH_4 was synthesised by Dr K. J. M. Andrews, Roche Products Limited, Welwyn Garden City, UK.

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[1] Levitt, M., Spector, S., Sjoerdsma, A., and Udenfriend, S., *J. Pharmac. exp. Ther.*, **148**, 1 (1965).
[2] Nagatsu, T., Levitt, M., and Udenfriend, S., *J. biol. Chem.*, **239**, 2910 (1964).
[3] Brenneman, A. R., and Kaufman, S., *Biochem. biophys. Res. Commun.*, **17**, 177 (1964).
[4] Kaufman, S., Bridgers, W. F., Eisenberg, F., and Friedman, S., *Biochem. biophys. Res. Commun.*, **9**, 497 (1962).
[5] Musacchio, J. M., *Biochim. biophys. Acta*, **191**, 485 (1969).
[6] Musacchio, J. M., D'Angelo, G. L., and McQueen, C. A., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2087 (1971).
[7] Guroff, G., Rhoads, C. A., and Abramowitz, A., *Analyt. Biochem.*, **21**, 273 (1967).
[8] Coté, L., Benitez, H. H., and Murray, M. R., in *Second Int. Meeting Int. Soc. Neurochem.* (edit. by Paoletti, R., Fumagalli, R., and Galli, G.), 137–138 (Tamburini Editore, Milan, 1970).
[9] Lloyd, T., and Weiner, N., *Molec. Pharmac.*, **7**, 569 (1971).
[10] Hayden, J. F., Johnson, L. R., and Maickel, R. P., *Life Sci.*, **5**, 1509 (1966).
[11] Shellenberger, M. K., and Gordon, J. H., *Analyt. Biochem.*, **39**, 356 (1971).
[12] Kaufman, S., and Fisher, D. B., *J. biol. Chem.*, **245**, 4745 (1970).
[13] Stone, K. J., and Townsley, B. H., *Biochem. J.*, **731**, 611 (1973).
[14] Andén, N.-E., Roos, B.-E., and Werdinius, B., *Life Sci.*, **7**, 4481 (1963).
[15] Kopin, I. J., Bresse, G. R., Krauss, K. R., and Weise, V. K., *J. Pharmac. exp. Ther.*, **161**, 271 (1968).

Increased myocardial cathepsin D activity during regression of thyrotoxic cardiac hypertrophy

SEVERAL types of hormonal or mechanical stimuli may cause cardiac hypertrophy. In many instances, when the stimulus is removed the heart rapidly regresses toward its original size. The cellular mechanism(s) by which this net cardiac catabolism and reduction of myocardial protein mass might be mediated have not been defined. One possibility might be an increase in the activity of intracellular proteolytic enzymes; accordingly, we have investigated the activity of cathepsin D, a lysosomal enzyme that is the major acid proteinase in the heart.

Our experimental model was the hypertrophied heart of the thyrotoxic rat. Exogenous administration of excessive doses of thyroid hormone is well known to lead to marked cardiac enlargement in the rat, and discontinuation of thyroid treatment results in a rapid return of heart weight to normal[1–4]. Indeed, during the first week of recovery from thyrotoxicosis, the heart loses 10% or more of its mass, in comparison with a weekly increase of 10% or more in normal rats[4].

Two-month-old male albino rats were divided into four matched groups. The first group ('control') was untreated for 6 weeks. The second group ('thyrotoxic') received no treatment for 3 weeks followed by daily injections of *l*-thyroxine (1.0 mg kg^{-1} intraperitoneally) for 3 weeks. The third group ('1-week recovery') received a similar 3-week course of thyroxine injections between the second and fifth week of the 6-week experimental period, and the last group was treated during the first 3 weeks only and then remained untreated for 3 weeks ('3-week recovery'). All the animals were killed on the same day by cardiac excision after being stunned by a blow to the head. Total body weights and the weights of the isolated cardiac ventricles were determined. A section of left ventricular muscle was excised immediately, diluted in a solution of 0.1% Triton at 4° C, minced and homogenised vigorously with a Willems homogeniser to disrupt maximally the cells and their organelles. The mixture was spun at 350*g* for 5 min to remove cellular debris, and the supernatant, which contained more than 90% of the total activity of the various enzymes tested, was taken for assay. Activities were measured for the lysosomal enzymes cathepsin D, β-acetylglucosaminidase and acid phosphates[5,6]. The activity of creatine phosphokinase[7], a nonlysosomal enzyme that serves as a good general marker of myocellular viability, was also measured, as was the protein concentration[8].

As others have described, thyroxine therapy caused marked cardiac hypertrophy in the rats. The ratio of ventricular weight to body weight was 0.36±0.01% (s.e.m.) in the control group compared with 0.56±0.02% in the thyrotoxic rats. The difference was due to a significant ($P<0.01$) increase in ventricular weight as well as a decrease in body weight in the treated animals. One week after cessation of therapy, heart weight was significantly reduced and body weight was increased (ratio=0.43±0.02%), and by 3 weeks all values had returned to normal (ratio=0.38±0.02%).

These changes in thyroid state and heart size were accompanied by significant alterations in the cardiac activity of cathepsin D. With thyrotoxic hypertrophy there occurred a 15% decrease in the mean activity of the enzyme, from 68.7 to 58.4 μg tyrosine h^{-1} mg^{-1} protein ($P<0.01$). Subsequently, with discontinuation of thyroid treatment and rapid regression of cardiac hypertrophy, the activity of the enzyme increased by 40% (Table 1) within 1 week. By 3 weeks, when the hearts had already returned to normal size, the activity of cathepsin D was also back to normal (71.3 μg tyrosine h^{-1} mg^{-1} protein).

During the rapid regression of hypertrophy, the increase in catheptic activity greatly exceeded the simultaneous decreases in heart weight and protein content (Table 1). Thus, the measured increase could not have been simply a consequence of slower net breakdown of the cathepsin D molecule compared with other cardiac proteins, which during generalised cardiac catabolism might lead to a relative elevation of the enzyme without an actual increase in its net production. Rather, the data indicate that there was an absolute increase in total cathepsin D activity in each heart, not due to generalised stimulation of all lysosomal enzymes. As Table 1 shows, there were no changes in the activities of glucosaminidase or acid phosphatase. Similarly, the nonlysosomal enzyme creatine phosphokinase was unaffected.

In separate tests, the tissue was homogenised gently in 0.25 M KCl to preserve intact lysosomes as far as possible[9,10];

Table 1 Comparison of ventricular weight, protein, and enzyme activities.

	Thyrotoxic	1-week recovery
Body weight (g)	299±7.6 (s.e.m.)	339±6.5*
Ventricular weight (g)	1.66±0.040	1.43±0.024*
Ventricular protein concentration (mg g^{-1})	102±1.4	102±2.0†
Creatine phosphokinase activity (μmol ATP per min per mg protein)	4.71±0.145	4.92±0.128†
β-acetylglucosaminidase activity (nmol nitrophenol per h per mg protein)	140±3.0	138±3.1†
Acid phosphatase activity (nmol nitrophenol per h per mg protein)	167±5.3	178±5.5†
Cathepsin D activity (μg tyrosine per h per mg protein)	58.4±1.05	81.5±1.77*
Nonsedimentable (free) cathepsin D activity (% of combined free + particulate-bound activity)	30±0.9	35±1.4*

Measurements were made on hypertrophied hearts of 16 thyrotoxic rats and 16 hearts that were undergoing rapid regression of hypertrophy 1 week after discontinuation of thyroxine injections.
* $P<0.01$ compared with thyrotoxic hearts.
† $P>0.10$ compared with thyrotoxic hearts.

subsequent assays revealed altered catheptic activity in the 'free' or nonsedimentable fraction of the cell (which is thought by some to reflect enzyme that is more accessible to intracellular substrates *in vivo*) as well as in the total homogenate (Table 1).

These findings are reminiscent of other instances in which an organ responds to withdrawal of the appropriate hormone by an increase in the activity of lysosomal enzymes and catabolism and atrophy of the tissue[11,12]. Of special interest is the regression of uterine smooth muscle after termination of pregnancy. Removal of the stimulus for uterine growth that is provided by sex hormones leads to rapid regression of the organ, and this is associated with an increase in the activity of uterine cathepsin D[12]. In this condition there are parallel increases in other lysosomal enzymes, although in some other tissues pharmacological or hormonal changes cause specific alterations in cathepsin D without generalised effects on all lysosomal enzymes[12,13].

Our experiments demonstrate that acute changes in the thyroid state are accompanied by specific alterations in the activity of cathepsin D in the heart. Although no cause-and-effect relationship can be established definitively from our data, it seems reasonable to speculate that the observed changes in proteolytic activity are involved in the mediation of the corresponding shifts in protein balance. These findings provide for the first time evidence of an enzymatic alteration that might link an external stimulus for the regression of preexistent hypertrophy to subsequent myocellular catabolism. Whether similar changes accompany regression from other varieties of cardiac hypertrophy remains to be determined.

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1 Gemmill, C. L., *Am. J. Physiol.*, **195**, 385–390 (1958).
2 Sandler, G., and Wilson, G. M., *Q. J. exp. Physiol.*, **44**, 282–289 (1959).
3 Beznak, M., Korecky, B., and Thomas, G., *Can. J. Physiol. Pharmacol.*, **47**, 579–586 (1969).
4 Van Liere, E. J., and Sizemore, D. A., *Proc. Soc. exp. Biol. Med.*, **136**, 645–648 (1971).
5 Wildenthal, K., *Nature*, **243**, 226–227 (1973).
6 Barrett, A. J., in *Lysosomes: a laboratory handbook* (edit. by Dingle, J. T.), 46–135 (North Holland, Amsterdam and London, 1972).
7 Szasy, G., Busch, E. -W., and Faroles, H. -B., *Deutsch. med. Wschr.*, **95**, 829–835 (1970).
8 Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265–275 (1951).
9 Dingle, J. T., and Barrett, A. J., in *Lysosomes in biology and pathology*, **2** (edit. by Dingle, J. T., and Fell, H. B.), 555–566 (North Holland, Amsterdam and London, 1969).
10 Wildenthal, K., in *Lysosomes in biology and pathology*, **4** (edit. by Dingle, J. T., and Dean, R.) (North Holland, Amsterdam and London, in the press).
11 Brandes, D., Gyorkey, F., and Groth, D. P., *Lab. Invest.*, **11**, 339–350 (1962).
12 Woessner, J. F., jun., in *Lysosomes in biology and pathology*, **1** (edit. by Dingle, J. T., and Fell, H. B.), 229–329 (North Holland, Amsterdam and London, 1969).
13 Robinovitch, M. R., *Expl Cell Res.*, **82**, 439–451, (1973).

Vasopressin release from the isolated neurohypophysis induced by a calcium ionophore, X-537A

TEN years ago observations on isolated neurohypophyses prompted the suggestion that action potentials in the hypothalamo-neurohypophysial tract release neurohypophysial hormones by promoting calcium influx and that the appearance of free calcium ions somewhere in the neurosecretory terminals provides the immediate stimulus for hormone extrusion[1-3]. Some time later it was argued that the role of calcium ions in stimulus-secretion coupling in this system and in many other secretory cells was to set in motion a common secretory mechanism, exocytosis[4]. Very recently the evidence supporting this view has been strengthened by light microscopic observations showing extrusion of secretory granules from mast cells on intracellular injection of calcium ions[5]. Meanwhile, electron microscopic[6-11] and chemical[12-16] evidence has borne out the conjecture that neurohypophysial hormones are secreted by exocytosis, and the case for calcium-influx as the initiating event has been strengthened by a number of observations set out in recent reviews[15,16]. These reviews make the further point that the neurohypophysis may be a useful model system for studying stimulus-secretion coupling in nerve cells generally: the involvement of calcium in release of neural transmitters is well-known and may have a similar explanation[17,18].

With the discovery of calcium ionophores a new tool has become available, and our purpose here is to report the stimu-

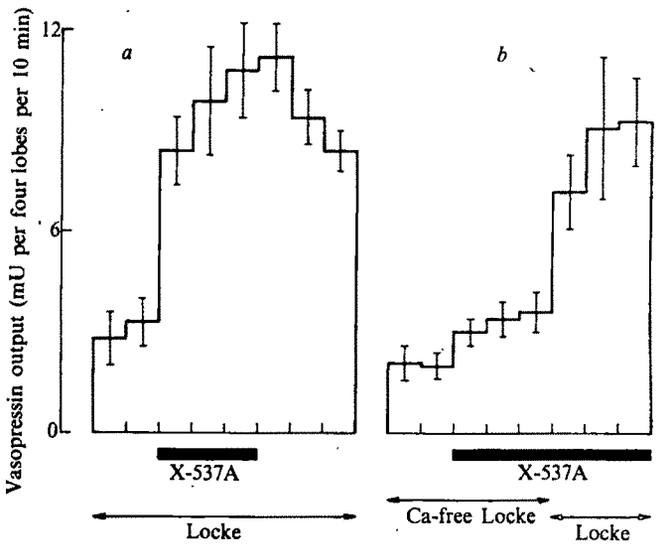


Fig. 1 Stimulant effect of the ionophore X-537A on output of vasopressin from rat neurohypophyses *in vitro* and the inhibitory effect of calcium deprivation. The methods for isolating neurohypophyses, incubating them, and measuring vasopressin output followed the original description[2] except that four neural lobes from male rats weighing about 250 g were used in each experiment and were impaled, after halving, on a fine platinum wire holder[29] facilitating transfer from one incubation chamber to the next. Media containing X-537A were prepared from a stock solution of the drug in dimethylsulphoxide. The final concentration of the solvent never exceeded 0.2% which is without effect on basal or stimulus-evoked output of vasopressin[30]. Drug and solvent did not interfere with the rat blood pressure method used for bioassay of vasopressin. Each of the two curves (*a*) and (*b*) shows vasopressin outputs (means ± s.e.) in successive 10 min incubation periods in three experiments. X-537A (6 $\mu g.ml^{-1}$) was present during the periods indicated by the heavy horizontal bars. Horizontal arrows indicate the incubation medium. Locke's solution contained (mM): NaCl, 150; KCl, 6.0; $CaCl_2$, 2.0; $MgCl_2$, 1.0; $NaHCO_3$, 10; glucose, 10. It was equilibrated with 5% CO_2 in O_2. In calcium-free Locke, $CaCl_2$ was omitted. Records begin after 40 min preincubation with Locke's solution (*a*) or calcium-free Locke's solution (*b*).

Table 1 Effect of calcium deprivation, with or without EDTA, on output of vasopressin in response to X-537A (25 μg ml^{-1})

Preincubation	(*A*) Locke (30 min)	(*B*) Ca-free Locke (60 min)	(*C*) Ca-free Locke + 2 mM EDTA (60 min)	(*D*) Ca-free Locke + 2 mM EDTA (70 min) followed by Mg-free Locke (60 min)
Incubation medium	Locke	Ca-free Locke	Ca-free Locke	Mg-free Locke
Vasopressin output (mU per four lobes per 10 min: mean ± s.e.m. (n))				
Before X-537A	2.0 ± 0.3(5)	2.1 ± 0.3(4)	2.7 ± 0.2(4)	2.2 ± 0.4(4)
During X-537A*				
0–10 min	9.1 ± 1.5(5)	4.8 ± 0.8(4)	3.6 ± 0.4(4)	4.7 ± 0.5(4)
10–20 min	13.5 ± 1.7(5)	4.9 ± 0.6(4)	3.8 ± 0.4(4)	6.8 ± 0.6(4)
20–30 min	16.7 ± 2.3(4)	4.9 ± 0.6(4)	3.8 ± 0.1(3)	8.5 ± 1.2(4)

* All outputs (columns *A*–*D* at each 10 min interval) are significantly higher ($P < 0.05$) than the corresponding values before X-537A.

lant effects, on neurohypophysial secretion, of one of these, X-537A. Like other calcium ionophores, X-537A is a substance that forms a reversible complex with calcium which, by its lipophilic nature, serves as a mobile calcium carrier that will transport calcium ions across biological membranes[19]. It is known to release histamine[20] and, more relevant to the work reported here, to elicit extrusion of secretory granules from mast cells by calcium-dependent exocytosis[21,22].

The methods we have used for studying release of vasopressin from isolated rat neurohypophyses have been described elsewhere (see legend to Fig. 1). In a concentration of 6 μg ml^{-1}, X-537A increased vasopressin output about three-fold in preparations incubated in a conventional, calcium-containing, Locke's solution. The effect outlasted exposure to the drug (Fig. 1*a*). By contrast, this ionophore had little stimulant effect on preparations incubated in calcium-free Locke, although vasopressin output rose substantially when calcium was reintroduced (Fig. 1*b*). This stimulant effect of calcium was clearly attributable to some priming effect of X-537A because calcium had no detectable effect on vasopressin output in each of four experiments where it was reintroduced after the same period of calcium deprivation without exposure to the ionophore, a finding in agreement with earlier observations[2].

In a concentration of 25 μg ml^{-1}, X-537A evoked a still greater output of vasopressin; the response in the presence of calcium (Table 1*A*) being approximately equal to that yielded by a maximally effective concentration (60 mM) of potassium[2]. Omission of calcium (with or without addition of EDTA) inhibited the response (Table 1*B* and *C*). This inhibitory effect was reversed, at least in part, by reintroducing calcium without magnesium (Table 1*D*.)

From these results it is evident that X-537A can stimulate vasopressin output through some calcium-dependent process. The most obvious explanation is that the drug acts on the neurohypophysial terminals as a calcium ionophore, and allows calcium to penetrate these terminals and initiate secretion in line with the original calcium influx hypothesis[1]. But at least one alternative, or additional, explanation deserves consideration, namely that X-537A owes its effect on vasopressin release to its additional ability to complex with and transport monovalent cations[19]. By such an action the drug might depolarise the neurosecretory terminals and set in motion the familiar potential-dependent calcium influx mechanism[3,23].

Further experiment revealed an additional stimulant effect of X-537A on vasopressin output which is not readily explained in terms of depolarisation or calcium entry. Thus, in a concentration of 50 μg ml^{-1} the drug increased vasopressin output from neurohypophyses exposed to a depolarising[2] concentration (60 mM) of potassium in a calcium-free medium (Fig. 2). The response was about half that subsequently obtained in the same preparations 10–20 min after introducing 2 mM calcium in the presence of 60 mM potassium which, as noted above, is the most effective concentration of potassium for evoking vasopressin secretion[2]. In the light of evidence that X-537A can release calcium from sarcoplasmic reticulum[24–26], we suggest that this stimulant effect on output of neurohypophysial hormone may result from mobilisation of cellular calcium and consequent elevation of the concentration of free calcium ions in the neurosecretory terminals. This would be in line with the original suggestion that the appearance of an excess of free calcium ions somewhere in the terminals provides the signal for release of neurohypophysial hormones[1].

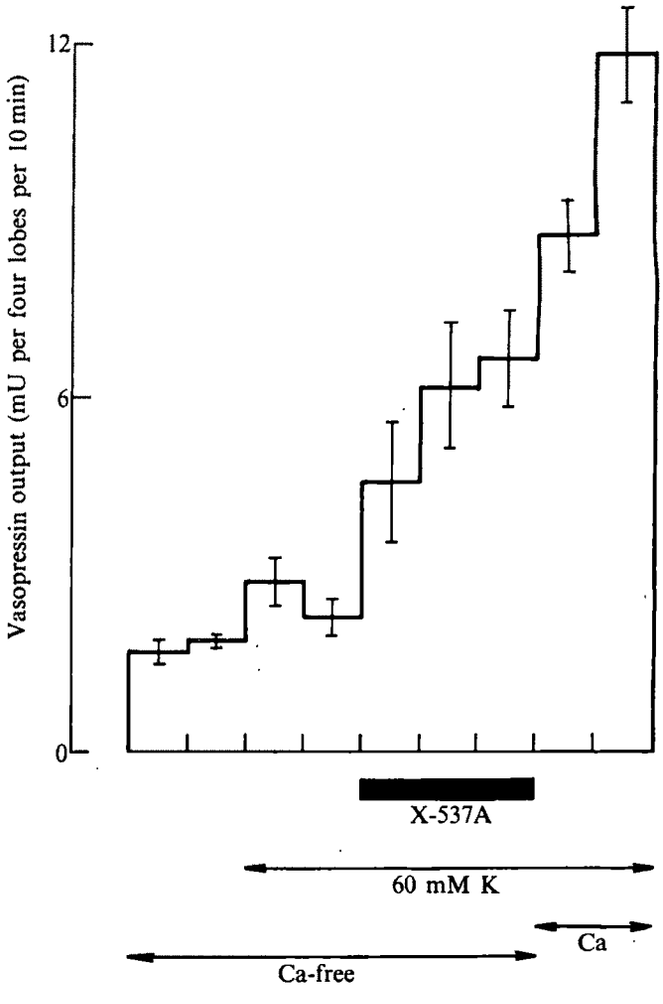


Fig. 2 Stimulant effect of the ionophore X-537A (50 μg ml^{-1}) on vasopressin output from neurohypophyses incubated in calcium-free Locke containing a depolarising[2] concentration of potassium. The record represents the mean (± s.e.m.) of four experiments and begins with lobes in calcium-free Locke (Ca-free) after 40 min preincubation in this same medium. After the first two periods shown, incubation was continued with calcium-free Locke with the potassium concentration raised to 60 mM (' 60 mM K '), the sodium concentration being lowered correspondingly to maintain tonicity[2]. X-537A was present during the period indicated by the heavy horizontal bar. After exposure to the drug, incubation was continued with 60 mM K Locke with the addition of 2 mM calcium (Ca). The secretory response obtained on adding calcium is near maximal for such preparations[2].

Conjecture along these lines is interesting because little is known of the physiological mechanisms for binding or sequestering calcium in secretory systems; yet some such mechanism must exist, as in muscle, if calcium is to function as a mediator in stimulus-secretion coupling. It is conceivable that responses to ionophores obtained from secretory preparations in calcium-free environments may provide a clue. It is possible however that X-537A, especially in the higher concentrations, may be able to release vasopressin independently of cellular as well as extracellular calcium: there are indications that acetylcholine can be released from nerves without involvement of calcium—for example, by high concentrations of ethanol[31].

In contrast to X-537A we have found, in similar experiments on six batches of neurohypophyses, that the calcium ionophore A-23187, in a concentration of 1, 2, 5 or 50 μg ml^{-1}, caused no obvious increase in vasopressin output. Since A-23187 differs from X-537A in having relatively little affinity for monovalent cations[19] this could be interpreted as support for the view that the response to X-537A involves depolarisation as well as calcium. Alternatively, A-23187 may for some reason, fail to act as an effective calcium ionophore in the neurohypophysial preparation as we have studied it. In any event, the behaviour of the neurohypophysis is quite different from that of mast cells studied in this laboratory where A-23187 (2–3 μg ml^{-1}) as well as X-537A causes a prompt release of secretory granules by exocytosis[21,22]. It is thus evident that different secretory cells vary in responsiveness to different ionophores as they do to various other stimuli[16]. Discussion of the possible physiological significance of this difference is best deferred until evidence from various other secretory preparations has been presented. These initial results encourage us to believe, however, that calcium ionophores may be useful new tools for the study of stimulus-secretion coupling in nervous tissue. We would add only, with regard to the exocytosis hypothesis of release of neurohypophysial hormones, that a stimulant effect of calcium after priming with X-537A, such as we have described for the neurohypophysis (Fig. 1*b*), has also been observed in mast cells where light and electron microscopy has shown the response to be extrusion of secretory granules by exocytosis[21,22].

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[1] Douglas, W. W., *Nature*, **197**, 81–82 (1963).
[2] Douglas, W. W., and Poisner, A. M., *J. Physiol. Lond.*, **172**, 1–18 (1964).
[3] Douglas, W. W., and Poisner, A. M., *J. Physiol. Lond.*, **172**, 19–30 (1964).
[4] Douglas, W. W., *Br. J. Pharmac.*, **34**, 451–474 (1968).
[5] Kanno, T., Cochrane, D. E., and Douglas, W. W., *Can. J. Physiol. Pharmac.*, **51**, 1001–1004 (1973).
[6] Nagasawa, J., Douglas, W. W., and Schulz, R. A., *Nature*, **227**, 407–409 (1970).
[7] Douglas, W. W., Nagasawa, J., and Schulz, R. A., *Mem. Soc. Endocr.*, **19**, 353–378 (1971).
[8] Douglas, W. W., Nagasawa, J., and Schulz, R. A., *Nature*, **232**, 340–341 (1971).
[9] Nagasawa, J., Douglas, W. W., and Schulz, R. A., *Nature*, **232**, 341–342 (1971).
[10] Krisch, B., Becker, K., and Bargmann, W., *Z. Zellforsch. Mikrosk., Anat.*, **123**, 47–54 (1972).
[11] Santolaya, R. C., Bridges, T. E., and Lederis, K., *Z., Zellforsch. Mikrosk. Anat.*, **125**, 277–288 (1972).
[12] Matthews, E. K., Legros, J. J., Grau, J. D., Nordmann, J. J., and Dreifuss, J. J., *Nature new Biol.*, **241**, 86–88 (1973).
[13] Edwards, B. A., Edwards, M. E., and Thorn, N. A., *Acta Endocr.*, **72**, 417–424 (1973).
[14] McNeilly, A. S., Martin, M. J., Chard, T., and Hart, J. C., *J. Endocr.*, **52**, 213–214 (1972).
[15] Douglas, W. W., *Prog. Brain Res.*, **39**, 21–39 (1973).
[16] Douglas, W. W., *Biochem. Soc. Symp.*, **39**, 1–28 (1974) (in press).
[17] Hubbard, J. I., *Prog. Biophys. molec. Biol.*, **21**, 33–124 (1970).
[18] Katz, B., *The release of neural transmitter substances*, (Thomas, Springfield, Illinois, 1969).
[19] Pressman, B. C., *Fedn Proc.*, **32**, 1698–1703 (1973).
[20] Foreman, J. C., Mongar, J. L., and Gomperts, B. D., *Nature*, **245**, 249–251 (1973).
[21] Cochrane, D. E., and Douglas, W. W., *Proc. natn. Acad. Sci. USA*, **71**, 408–412 (1974).
[22] Kagayama, M., and Douglas, W. W., cited by Douglas, W. W., in *Secretory mechanisms of exocrine glands*, Alfred Benzon Symposium VII (Munksgaard, Copenhagen, in the press).
[23] Baker, P. F., *Prog. Biophys. molec. Biol.*, **24**, 177–223 (1972).
[24] Entman, M. L., Allen, J. C., Bornet, E. P., Gillette, P. C., Wallick, E. T., and Schwartz, A., *J. molec. cell. Cardiol.*, **4**, 681–687 (1972).
[25] Entman, M. L., Gillette, P. C., Wallick, E. T. Pressman, B. C., and Schwartz, A., *Biochem. biophys. Res. Commun.*, **48**, 847–853 (1972).
[26] Caswell, A. H., and Pressman, B. C., *Biochem. biophys. Res. Commun.*, **49**, 292–298 (1972).
[27] Scarpa, A., and Inesi, G., *FEBS Lett.*, **22**, 273–276 (1972).
[28] Scarpa, A., Baldassare, J., and Inesi, G., *J. gen. Physiol.*, **60**, 735–749 (1972).
[29] Douglas, W. W., and Sorimachi, M., *Br. J. Pharmac.*, **42**, 647P (1971).
[30] Douglas, W. W., and Sorimachi, M., *Br. J. Pharmac.*, **45**, 143-144P (1972).
[31] Quastel, D. M. J., Hackett, J. T., and Cooke, J. D., *Science*, **172**, 1034–1036 (1971).

Capillary permeability and sympathetic activity in canine subcutaneous adipose tissue

Previous studies on the exchange properties of the microvasculature of canine subcutaneous adipose tissue have shown that electrical stimulation of the sympathetic nerves with frequencies within the physiological range, induced vasoconstriction accompanied by an increase in the transcapillary hydrodynamic conductivity expressed by the capillary filtration coefficient (CFC) (ref. 1). Given that CFC is a function of the product of the surface area available for exchange and the permeability of the capillary wall[2,3], these two factors must be separated to establish the functional properties of this vascular reaction. In attempting to do this, the clearance of locally injected $Na^{125}I$ was measured at constant flow perfusion, and was found to be significantly reduced during sympathetic stimulation. Similarly the permeability-surface area product (PS) of the vascular bed measured by the extraction of ^{86}Rb in subcutaneous adipose tissue decreased as a function of sympathetic stimulation (ref. 4 and J. Gainer, B. Linde, and S.R., unpublished).

These data suggest that sympathetic stimulation causes a constriction of the precapillary vessels resulting in a diminished number of capillaries open to blood flow. The increase of CFC could then be a consequence of either an overall increase in permeability of the capillary membrane, or a drastic alteration of the capillary pressure profile, which would enhance the effect of the more permeable venous segments of the microvasculature[5].

The present study was undertaken with the idea that a higher capillary permeability might affect the isovolumetric capillary pressure (P_{oi}) provided that the permeability change is of such a nature that osmotically active molecules become diffusible across the capillary membrane that is, the osmotic reflection coefficient for plasma proteins becomes smaller than one[6].

To test this hypothesis, experiments were performed on 13 female, mongrel dogs, anaesthetised with sodium pentothal

(30 mg kg^{-1}). The inguinal subcutaneous adipose tissue, located between the pubis and mammary glands, was prepared on the right side and placed in a plethysmograph with the artery, vein and nerve passing through a closely fitted opening[1,7]. After the administration of heparin, the artery supplying the adipose tissue was cannulated with plastic tubing and a silicone filled drop recorder was inserted. The plethysmograph was filled with Tyrode's solution kept at 38° C and connected to a volume recorder. Volume changes could be determined with an accuracy of ±0.05 ml. Blood pressures were monitored from the artery and vein supplying the adipose tissue by means of Statham pressure transducers and recorded on a Grass polygraph, together with the blood flow and tissue volume. The vein draining the tissue was cannulated with a widebore plastic tubing, the free end of which could be placed at any desired level above the preparation. In this way the venous outflow pressure, and therefore the venous and capillary pressures in the adipose tissue, could be varied. The isovolumetric capillary pressure (P_{ci}) was determined according to the principle of Pappenheimer and Soto-Rivera[8]. The blood flow was reduced in a stepwise fashion by means of a screw clamp on the artery. The level of the venous cannula was then adjusted to give a hydrostatic capillary pressure which rendered the adipose tissue isovolumetric. The values of arterial and venous pressures at isovolumetric states were then plotted (Fig. 1) and P_{ci} was estimated by extrapolating to zero pressure difference.

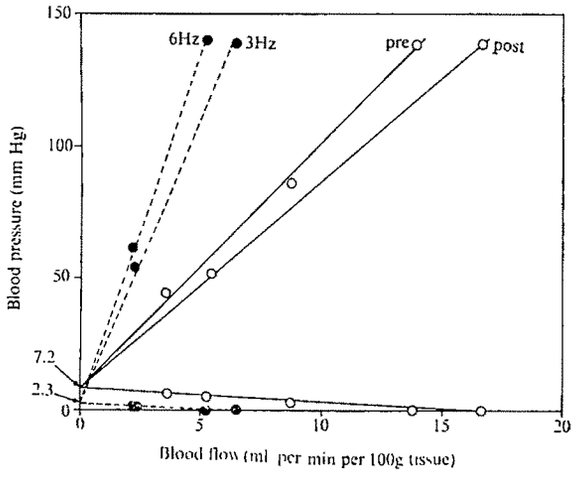


Fig. 1 Determination of isovolumetric pressure in canine subcutaneous adipose tissue. The points indicate arterial or venous pressures at different levels of blood flow at isovolumetric states. O, Determinations made during resting conditions (control), pre- and post-stimulation respectively; ●, determinations make during electrical stimulation of sympathetic nerves with 3 and 6 Hz, respectively. The data are extrapolated linearly to zero pressure difference, which coincides with zero blood flow. P_{ci} is indicated by the intercept on the ordinate. Colloid osmotic pressure 180 mm Hg.

In all experiments the extrapolated arterio-venous zero pressure difference was found to coincide with zero blood flow, as shown in Fig. 1. At zero flow there is no pressure drop along the blood vessels and, consequently, P_{ci} is equal to the pressure at the intercept of the extrapolated pressure-flow relations on the ordinate. The linear relationship between isovolumetric blood flow and isovolumetric arterial and venous pressures, respectively, is evidently a consequnce of the lack of autoregulatory responses in subcutaneous adipose tissue. Therefore, this method can be used with some precision to determine P_{ci} in subcutaneous adipose tissue.

The mean resting isovolumetric capillary pressure was 10.1 mm Hg (range 20.8–3.8, $n=13$), which was found to be lower than the plasma colloid osmotic pressure, 17.6 mm Hg (range 18.3–15.6). (The plasma colloid osmotic pressure was determined by means of a Hansen type osmometer utilised in conjunction with a membrane whose reflection coefficient for albumin was one.) Thus, the hydrostatic pressure which counterbalances all pressures tending to cause fluid absorption at an isovolumetric state is consistently lower than the plasma colloid osmotic pressure by an average of 7.6 mm Hg. This finding may be accounted for by a tissue osmotic pressure of 5–8 mm Hg which corresponds to a tissue protein concentration of 2–3%. This was shown to exist by direct micro-sampling in subcutaneous connective tissue[9], and by measuring the distribution of tagged albumin[10].

Following sympathetic nerve stimulation (3–9 Hz; 2 ms; 7–10 V), which was adjusted to diminish the blood flow to approximately one half, there was a marked reduction of P_{ci} which averaged 5.6 mm Hg (range 10.8–0; $P<0.001$) (Fig. 1). As in the case of resting conditions, in every experiment it was possible to determine P_{ci} by linear extrapolation to zero flow. P_{ci} is estimated by gradually decreasing the arterio-venous pressure difference. In this manner, uneven flow distributions in the vascular bed are diminished. Therefore reduced P_{ci} does not seem to be the effect of a redistribution of blood to areas of higher permeability.

In view of the above mentioned changes in CFC and PS, the reduction of P_{ci} is compatible with an increase in capillary permeability associated with a decrease in the reflection coefficient for plasma proteins. These experiments thus support the hypothesis that the sympathetic nervous system may control capillary permeability in canine subcutaneous adipose tissue[11]. Adrenergic vasomotor control of permeability has not been described previously.

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1 Öberg, B., and Rosell, S., *Acta physiol. scand.*, **71**, 47 (1967).
2 Cobbold, A., Folkow, B., Kjellmer, I., and Mellander, S., *Acta physiol. scand.*, **57**, 180 (1963).
3 Folkow, B., and Mellander, S., *Capillary Permeability*, 614–623, (Munksgaard, Copenhagen, 1970).
4 Ballard, K., and Rosell, S., *Circ. Res.*, **28**, 389 (1971).
5 Zweifach, B. W., and Intaglietta, M., *Microvasc. Res.*, **1**, 83 (1968).
6 Staverman, A. J., *Rec. Trav. chim. Pays-Bas Belg.*, **70**, 344 (1951).
7 Rosell, S., *Acta physiol. scand.*, **67**, 343 (1966).
152, 471 (1948).
8 Pappenheimer, J. R., and Soto-Rivera, A., *Am. J. Physiol.*,
9 Haljamäe, H., and Freden, H., *Microvasc. Res.*, **2**, 163 (1970).
10 Wiederhielm, C. A., *Biological Interfaces: Flows and Exchanges.*, 29–63 (Little, Brown and Company, Boston 1968).
11 Fredholm, B. B., Öberg, B., and Rosell, S., *Acta physiol. scand.*, **79**, 564 (1970).

Intracellular electric responses to sound in a vertebrate cochlea

ELECTRIC potentials generated by the inner ear in response to sound stimulation were first recorded 40 yr ago[1]. Studies

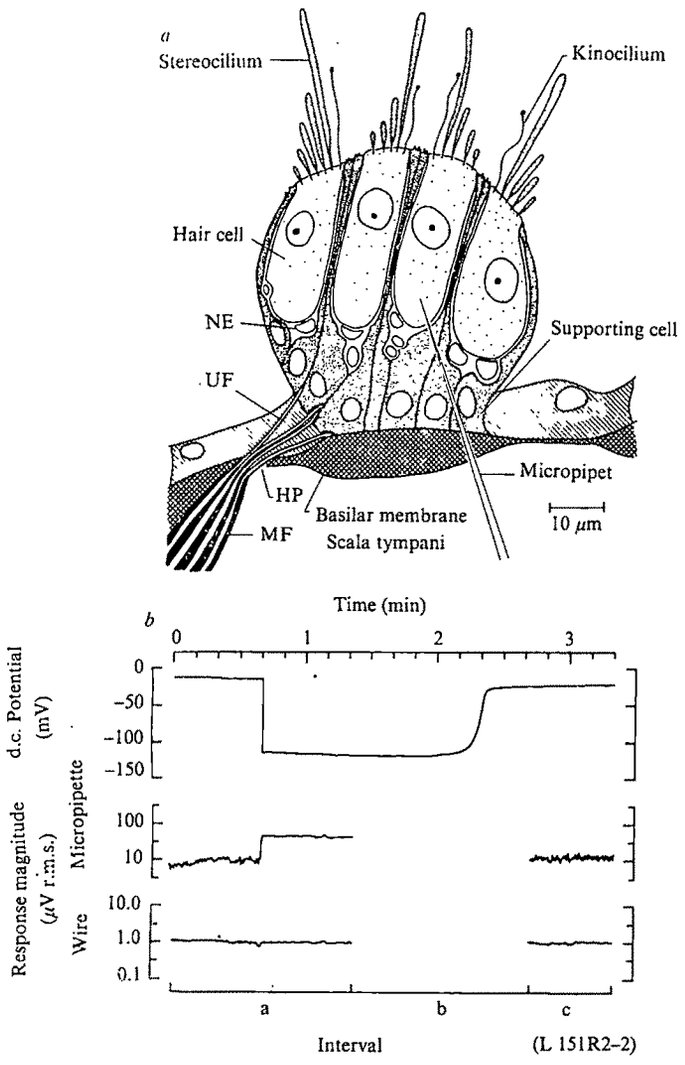


Fig. 1 *a*, Drawing of a transverse section through the basilar papilla of the alligator lizard. The basilar papilla is approximately 50 μm wide by 400 μm long and contains about 150 hair cells innervated by approximately 600 nerve fibres[24–26]. The penetration of the basilar papilla from scala tympani by a micropipette is shown schematically for a typical experiment. HP, habenula perforata; MF, myelinated portion of a nerve fibre; NE, nerve ending; UF, unmyelinated fibre. *b*, Oscillographic records obtained as a micropipette was advanced into the papilla. The top and centre traces were obtained from the potential recorded between the micropipette and a remote reference electrode. The top trace shows the d.c. potential; the centre trace shows the magnitude of the response to a 500 Hz tone at a sound pressure level (SPL) at the tympanic membrane of 75 dB. The bottom trace is the magnitude of the response to the 500 Hz tone obtained from a wire electrode near the round window. For both the centre and bottom traces the potential recorded from the electrode was passed through a narrow-band filter (2 Hz bandwidth) to obtain the fundamental component of the response, the magnitude of which is plotted on a logarithmic ordinate scale[27]. During intervals a and c, the 500 Hz tone was on continuously; during interval b, this tone was turned off and responses to other acoustic stimuli were recorded. When the d.c. potential suddenly dropped to −110 mV, the micropipette tip was assumed to have entered a cell, and the micropipette was held stationary. Coincident with the occurrence of the negative d.c. potential, the response magnitude increased abruptly by at least a factor of 4. After the d.c. potential returned to near zero, the magnitude of the response decreased almost to its previous value. The bottom trace shows that throughout the penetration by the micropipette into the basilar papilla, the response recorded from the wire electrode remained constant.

of these extracellular potentials have led to hypotheses about the role of intracellular potentials in sensory transduction in receptor (hair) cells[2,3]. It is difficult, however, to infer these intracellular potentials from extracellular measurements alone[4–7]; measurements of intracellular potentials from hair cells are clearly desirable. Although intracellular potentials in other hair-cell receptors have been reported recently[8,9], the few previous attempts to record such potentials in the cochlea have not yielded significant results[10]. We report here intracellular responses from single hair cells and supporting cells in the cochlea of the alligator lizard *Gerrhonotus multicarinatus*.

The lizard ear is basically similar to the mammalian ear[11,12]. Sound causes vibrations of the tympanic membrane which are transmitted by a cartilagenous extra-stapes and a bony stapes to the fluids of the inner ear, where the basilar membrane is set in motion. The auditory receptor organ (basilar papilla) consists of a strip of hair cells and supporting cells, which is on the basilar membrane (Fig. 1*a*). The basilar papilla is generally considered to be homologous[13,14] to the mammalian organ of Corti.

In our experiments, some bone and the round window membrane were removed so that the papilla could be visualised with a dissecting microscope. As a glass micropipette (tip diameter less than 0.5 μm) was advanced into the papilla (Fig. 1*a*) sound was delivered to the external ear and electric potentials were measured continuously. An abrupt negative shift in the d.c. potential was taken to indicate that the tip of the micropipette had entered a cell (Fig. 1*b*). (Intracellular d.c. potentials ranged between −25 mV and −125 mV in 90% of 547 cases with a median of −74 mV.) Stable negative d.c. potentials were often recorded for as long as 2 min and in a few cases for 30 min. The maximum displacement of the basilar membrane in these experiments (measured by the Mössbauer method[15]) was estimated to be less than 0.1 μm. Since this displacement is much smaller than the dimensions of the cells, it is not surprising that the micropipette tip could remain intracellular in the presence of this motion. Attempts to mark the cells after recording their responses to sound were made by passing current through dye-filled micropipettes[16,17]. Immediately after the experiment the intact papilla was dissected and the position and type of each dye-marked cell were determined by observation with a light or fluorescence microscope. For more precise localisation of the dye, the basilar papilla was sometimes embedded in Epon and serial sections were examined.

To assess whether the experimental procedures interfered grossly with the normal operation of the basilar papilla, extracellular responses were measured with a wire electrode on the bone near the round window. It was possible to expose the basilar membrane and to advance a micropipette into the papilla without significantly changing this extracellular response (Fig. 1*b*, bottom trace). The electric responses recorded intracellularly apparently did not result from mechanical vibration of the micropipette tip, because injection of sodium pentobarbital into scala tympani greatly reduced both the intracellular responses recorded with the micropipette and the extracellular response recorded with the wire electrode without changing the motion of the basilar membrane.

The intracellular responses to sound recorded in the papilla were graded potentials with a maximum amplitude of 3 mV peak-to-peak, and a short (less than 0.5 ms) latency in response to clicks. Responses to tone bursts had component waveforms that resembled the cochlear microphonic (CM) and summating (SP) potential that have been recorded extracellularly[2,18], suggesting that these intracellular response components (ICM and ISP in Fig. 2*a*) may be sources of the extracellularly recorded responses. Although the ratio of the magnitudes of ISP and ICM varies from cell to cell and is a function of stimulus parameters, ISP

was always a depolarisation. ICM was approximately sinusoidal with a fundamental frequency equal to the tone frequency (Fig. 2*b*).

Electric responses to sounds were of nearly the same magnitude in both hair cells and supporting cells, but the waveforms of the responses to clicks differ in these two types of cells[19]. The largest responses recorded (3 mV) from identified hair cells in the basilar papilla were comparable in magnitude with those reported for identified hair cells in lateral-line organs[8,9]. In contrast, the responses recorded in supporting cells were apparently larger (1.5 mV maximum) than those found in lateral-line organs[8,9]. All-or-none (action) potentials were never observed during penetrations of the basilar papilla, but were recorded during penetrations of the myelinated portion of the cochlear nerve at locations between the ganglion cell bodies and the habenula perforata (Fig. 1*a*).

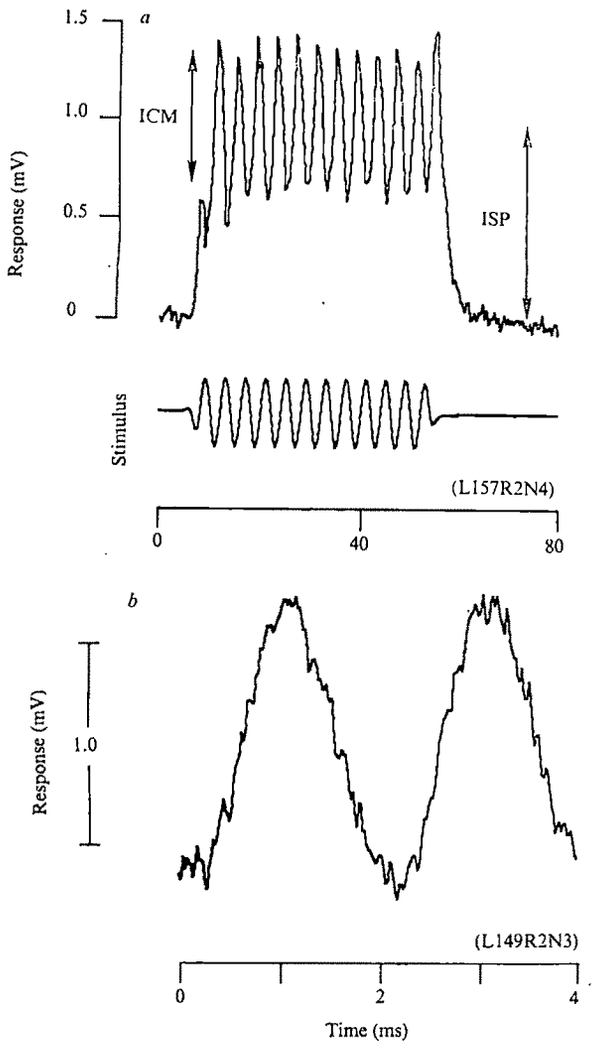


Fig. 2 *a*, Averaged intracellular response to tone-burst stimuli. Deviations of the potential from the resting potential are shown; upward direction is a depolarisation of the cell. The cell in which this response was recorded was identified as a supporting cell based on its response to acoustic clicks[19]. ICM (intracellular cochlear microphonic) is the response component that follows the individual cycles of the tone: ISP (intracellular summating potential) follows the tone burst envelope. The arrows indicate the approximate magnitudes of these components. Tone bursts of 50 ms duration were delivered at a rate of 10 s^{-1}. Rise-fall time was 2 ms. Frequency was 250 Hz and level was 94 dB SPL at the tympanic membrane. The average response was computed from 333 individual responses sampled at 400 μs intervals at 200 points. These points are connected by straight lines. *b*, Averaged intracellular response to a continuous tone recorded from a hair cell (as identified by dye-marking). Tone frequency was 500 Hz and level was 73 dB SPL. This averaged response was computed from 310 individual responses sampled at 20 μs intervals.

We should like to make some general comments about our results: (1) The existence of intracellular response potentials which are similar in waveshape but larger than the extracellular cochlear nucrophonic and summating potentials is consistent with the widely-held view that the cells of the sensory organ are sources of these extracellular potentials. The sodium pentobarbital-induced reduction of both intracellular and extracellular potentials is also consistent with this view. (2) The occurrence of responses of comparable magnitude in hair cells and supporting cells suggests either that supporting cells respond directly to sound and/or that hair cells are sources of currents which produce potentials in supporting cells. Thus supporting cells may play some role in the transduction process[19]. (3) The hair-cell afferent nerve junction is probably a chemical synapse[3]. Studies of chemical synapses indicate that presynaptic potentials of a few millivolts can produce changes in the release of transmitter substances[20,21]. Thus the receptor potential in hair cells may modulate transmitter release. (4) Studies of action potentials in single fibres of the auditory nerve[22,23] have demonstrated that during a tone burst (*a*) the mean rate of occurrence of action potentials is increased, and (*b*) action potentials tend to occur in synchrony with individual cycles of the tone. The fact that intracellular potentials in receptor cells have components (ISP and ICM) with waveshapes that are similar to the observed action-potential patterns is consistent with the view that these intracellular potentials may be a causal step in the excitation of action potentials in auditory nerve fibres.

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1 Wever, E. G., and Bray, C. W., *Proc. natn. Acad. Sci. U.S.A.*, **16**, 344 (1930).
2 Davis, H., *Physiol. Rev.*, **37**, 1 (1957).
3 Flock, Å., in *Handbook of sensory physiology*, **1** (edit. by Loewenstein, W. R.), 396 (Springer, New York, 1971).
4 Békésy, G., *J. acoust. Soc. Am.*, **23**, 18 (1951).
5 Whitfield, I. C., and Ross, H. F., *J. acoust. Soc. Am.*, **38**, 126 (1965).
6 Kohllöffel, L. U. E., *Acta otolaryngol.*, Suppl., **288** (1971).
7 Weiss, T. F., Peake, W. T., and Sohmer, H. S., *J. acoust. Soc. Am.*, **50**, 602 (1971).
8 Harris, G. G., Frishkopf, L. S., and Flock, Å., *Science*, **167**, 76 (1970).
9 Flock, Å., Jørgensen, M., and Russell, I. J., in *Basic mechanisms in hearing* (edit. by Møller, A. R.), 273 (Academic Press, New York, 1973).
10 Fex, J., in *Handbook of sensory physiology* (edit. by Keidel, W. D., and Neff, W. D.), 5 (Springer, New York, in the press).
11 Retzius, G., *Das gehörorgan de wirbelthiere*, 2 (Samson and Wallin, Stockholm, 1884).
12 Romer, A. S., *The vertebrate body*, 525 (Saunders, Philadelphia and London, 1946).
13 Baird, I., in *Biology of the reptilia* (edit. by Gans, C., and Parsons, T. S.), **2**, 193 (Academic Press, New York, 1970).

[14] Shute, C. C. D., and Bellairs, A. d'A., *Proc. zool. Soc. Lond.*, **123**, 695 (1953).
[15] Gilad, P., Shtrikman, S., Hillman, P., Rubinstein, M., and Eviatar A., *J. acoust. Soc. Am.*, **41**, 1232 (1967).
[16] Potter, D. D., Furshpan, E. J., and Lennox, E. S., *Proc. natn. Acad. Sci. U.S.A.*, **55**, 328 (1966).
[17] Stretton A. O. W., and Kravitz, E. A., *Science*, **162**, 132 (1968).
[18] Dallos, P., *The auditory periphery* (Academic Press, New York, 1973).
[19] Weiss, T. F., Mulroy, M. J., and Altmann, D. W., *J. acoust. Soc. Am.*, **55**, 606 (1974).
[20] Liley, A. W., *J. Physiol.*, **134**, 427 (1956).
[21] Katz, B., *Proc. R. Soc.*, **B155**, 455 (1962).
[22] Tasaki, I., *J. Neurophysiol.*, **17**, 97 (1954).
[23] Kiang, N. Y. S., Watanabe, T., Thomas, E. C., and Clark, L. F., *Discharge patterns of single fibres in the cat's auditory nerve* (MIT Press, Cambridge, 1965).
[24] Wever, E. G., *J. aud. Res.*, **5**, 331 (1965).
[25] Wever, E. G., *J. aud. Res.*, **11**, 160 (1971).
[26] Mulroy, M. J., Dissertation, Univ. California, San Francisco (1968).
[27] Sohmer, H. S., Peake, W. T., and Weiss, T. F., *J. acoust. Soc. Am.*, **50**, 572 (1971).

Behavioural and physiological bases of drinking inhibition in water deprived rats

FLUID balance is normally precisely maintained. Yet, following water deprivation, neither rat nor man drink enough water to correct the incurred fluid deficit. Fluid balance is corrected, however, when food is also made available. Adolph[1] termed this phenomenon voluntary dehydration.

In this communication, experiments that identify the physiological and behavioural bases of voluntary dehydration are described. In preliminary analyses, to be reported elsewhere, we found first, that only 79% of the fluid lost by rats deprived of water for 24 h was restored by drinking; second, that water intake stopped in spite of a persistent intravascular deficit (estimated to be 20% from haematocrit and plasma protein measures[2]); and third that the cessation of drinking was closely associated with the development of cellular overhydration (based upon Leaf's treatment[3] of Na as an estimator of plasma volume). Specifically, 15 min after the start of drinking, 79% of drinking was complete and the cellular phase was estimated to be 1.5% overhydrated ($t = 2.217$, $d.f. = 15$, $P < 0.05$).

These correlational observations indicate the following alternatives regarding the determinants of voluntary dehydration. Intake may be exclusively controlled by the hydrational status of the cellular phase. That is, cellular overhydration may be the necessary and sufficient event to prematurely terminate drinking. This predicts that the manner in which cellular overhydration is attained by drinking compared with gavage, is irrelevant. Absorption may allow the mouth and stomach to control drinking either by metering fluid intake or by reducing the urge to drink. Both oral and postingestional information may be critical and drinking may stop because the ingestive act is closely related in time to its postingestive consequences.

To distinguish among these alternatives, ten adult female Sprague Dawley rats were each studied on nine occasions (including two baseline tests where sham preloads were given).

Table 1 Outline of deprivation, preloading and drinking schedule followed in this study

0 h	23 h	24 h	25 h
Water deprived	Preload	Preload	Preload
(Purina pellets available)	Intubate or drink water or 0.153 M NaCl*	Intubate or drink water or 0.153 M NaCl*	Drink water or 0.153 M NaCl for 5 h

* The amount given was 27.5% of the water loss.

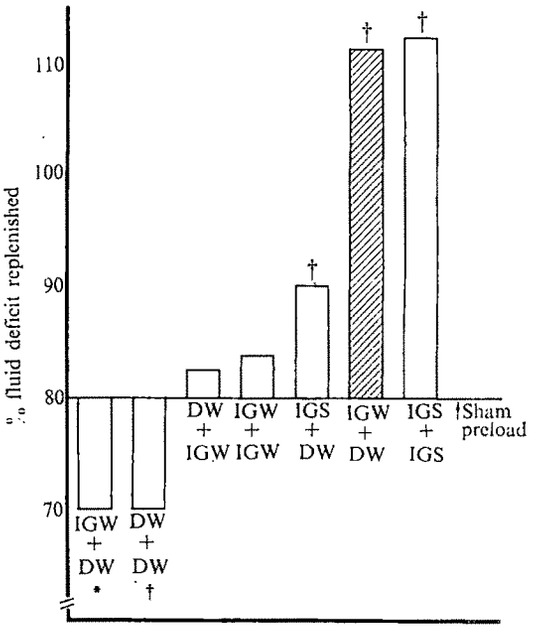


Fig. 1 Percentage of fluid deficits replenished in each of the eight conditions. % replenished = [(preload (ml) + test intake (ml))/water loss (ml)] × 100 The spontaneous drinking of sham loaded rats is presented as the baseline at 80%. IGW, Intragastric water; DW, drinking water; IGS, intragastric saline; DS, drinking saline. ☐ Water drunk during test; saline drunk during test. ▨ The first open column, for example, indicates that the first preload was water delivered intragastrically, water was drunk as the second preload and water was drunk during the 5 h test. * $P < 0.05$ † $P < 0.01$

Tests were separated by a mean of 7 d (range 4–10 d) and each rat was tested under a different semirandom order of preload and drinking conditions. The general procedure is presented in Table 1. On a given test, following 23 h of water (but not food) deprivation, food was removed and each rat drank, or received by gavage, one half of its fluid loss (approximated to be 67% of body weight loss[1]). One hour later the second half was either drunk or intubated. After another hour, either tap water or isotonic saline (0.153 M) was made available for 5 h. Food and water were then made continuously available until the next test.

Figure 1 compares total intakes (that is, the amount of preload and amount drunk during the drinking test) to the amount drunk spontaneously following sham loadings. In all but two conditions, total intake equalled or exceeded the amount drunk spontaneously following sham loadings. Total intake was actually reduced in the two exceptional cases (Fig. 1) and it was only in these two conditions that the second water preload was drunk following an initial water preload. Absorption of this volume of water was sufficient to produce cellular overhydration. Thus, only in situations in which development of cellular overhydration was closely paired with drinking, was premature termination of drinking exaggerated.

Cellular overhydration *per se* was not the critical event terminating drinking. If it were then the outcomes of the first four conditions, in which identical water preloads were delivered, and comparable haemodilution occurred, should not have differed one from another.

Neither the amount of the preload drunk nor the time spent in its drinking were the determining factors. If they were, then intake following the DW+DW sequence (see Fig. 1) should have been the most suppressed. Drinking here did not differ however, from the IGW+DW condition where only one half of the preload was drunk. Moreover, if oral metering *per se* were the critical factor, then intake following IGW+DW should not have differed at all from that following the DW+IGW sequence. As seen in Fig. 1, however, intakes differed markedly.

The IGS+DW condition (Fig. 1) indicates that the prevailing factor in the first two conditions was not the memory of having recently drunk water. Here the preload of DW followed intragastric isotonic saline. That is, drinking occurred in the absence of cellular overhydration. This emphasises that when water intake is divorced from cellular overhydration, drinking continues.

The shaded column of Fig. 1 (the test solution isotonic saline) suggests that the efficacy of treatment IGW+DW resided in the inhibition of water ingestion and not in thirst reduction. If it were the elimination of thirst, then no drinking should have occurred during the test hour regardless of the proffered solution. Yet a considerable volume of saline was ingested presumably in response to the prevailing extracellular deficit. This is an important aspect of the phenomenon for it raises the possibility that inhibition of drinking is specific to the solution drunk during the development of cellular overhydration. The final column emphasises the importance of cellular overhydration. When the entire preload was isotonic saline, a solution which has little initial effect on cellular balance, water intake was very substantial (9.4 ml).

The only conditions that exaggerated voluntary dehydration were those in which water intake occurred in contiguity with cellular overhydration; namely, drinking the entire water preload or intubating the first half of the preload and drinking the second half. Intubation of the entire preload or drinking the first half and tubing the second half did not have this effect.

These data provide the behavioural and physiological bases for the natural occurrence of voluntary dehydration in rats and indicate that: as drinking ensues, water is quickly cleared from the stomach and absorbed[4]; the cellular phase becomes rapidly overhydrated, and this occurs while the rats are actively engaged in drinking; the water remaining in the stomach and intestine is cleared, absorbed, and excreted in a dilute urine. The exaggeration of voluntary dehydration is likely a result of allowing ample time for complete absorption following the preloads and thereby avoiding any lag in the system.

The above theory predicts therefore, that even if the entire amount drunk were to be preloaded by gavage, drinking would still occur. This is precisely what happened. Ten rats preloaded a mean of 14.7 ml (the gross intake of a previous test) drank an additional 3.0 ml (range 1.0–5.0 ml) despite the fact that the preload caused substantial overhydration ($\bar{X}=7.1\%$).

The implications of this report may extend beyond voluntary dehydration to include other ingestive phenomena. For example, Nachman and Valentino[5] have shown that adrenalectomised rats, although intubated with the amount of NaCl required to satisfy their salt deficit, drank a considerable amount of 0.3 M NaCl. There have also been reports that water delivered by gavage is not nearly as effective as water taken by mouth in reducing intake to either intravascular depletion by hyperoncotic colloid[6] or to intracranial angiotensin[7]. According to Fitzsimons, rats infused with their normal daily water requirement still drink significant amounts[8]. Janowitz and Grossman[9] have shown that loading food intragastrically 20 min before regular feeding failed to depress intake in dogs. Baille *et al.*[10] reported that intragastric intubation of glucose inhibited feeding only when the load was delivered concurrently with feeding. If the load was delivered 30 min in advance of feeding, there was no suppression. Miller[11] has presented extensive evidence that rats work for more food or water following intragastric preloads than following oral preloads. These data have, in general, been intrepreted from the 'drive stimulus reduction' point of view, or with the idea that the mouth in some way meters the amount of ingesta appropriate to the animals' requirement. In light of the present evidence, these interpretations might be re-evaluated and the influence of ingestion in contiguity with its postingestional consequences explored.

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[1] Adolph, E. F., Barker, J. P., and Hoy, P. A., *Am. J. Physiol.*, **178**, 538 (1954).
[2] Stricker, E. M., *Physiol. Behav.*, **3**, 379 (1968).
[3] Leaf, A., *New Engl. J. Med.*, **267**, 24 (1962).
[4] O'Kelly, L. I., Falk, J. L., and Flint, D., *J. comp. Physiol. Psychol.*, **51**, 16 (1958).
[5] Nachman, M., and Valentino, D. A., *J. comp. Physiol. Psychol.*, **62**, 280 (1966).
[6] Stricker, E. M., *Am. J. Physiol.*, **217**, 98 (1969).
[7] Rolls, B. J., and McFarland, D. J., *Physiol. Behav.* (in the press).
[8] Fitzsimons, J. T., *J. Physiol. Lond.*, **138**, 39P (1957).
[9] Janowitz and Grossman, *Am. J. Physiol.*, **159**, 143 (1949).
[10] Baille, C. A., Zinn, W., and Mayer, J., *Physiol. Behav.*, **6**, 537 (1971).
[11] Miller, N. E., *Science*, **26**, 1271 (1957).

Optical polarisation indicates linear arrangement of rhodopsin oligosaccharide chain in rod disk membranes of frog retina

RHODOPSIN, the visual pigment of the retinal rods, is a doubly conjugated protein constituting about 80% of the total protein of the rod disk membrane in the frog[1]. The two prosthetic groups of rhodopsin are the visual chromophore retinal, and a single oligosaccharide chain comprising six sugar residues[2,3].

Electron microscope[4,5,6] and X-ray diffraction[7–10] studies offered no evidence for the orientation of the chromophore molecules or the oligosaccharide chains of rhodopsin with respect to the plane of the disk membrane. The negative, dichroism of the outer segments with the greater absorption for light polarised perpendicularity to the rod axis[11,12] indicated that the chromophore molecules are oriented with their long axis parallel to the plane of the disk membranes. That no dichroism in the axial direction could be observed, indicates that the chromophore molecules are scattered randomly in all directions with their lengths in the plane of the membrane. It was found recently[14,15] that when a flash of linearly polarised light passed along the lengths of the rods, axial dichroism was induced as a result of bleaching of the retinal molecules lying parallel to the plane of polarisation

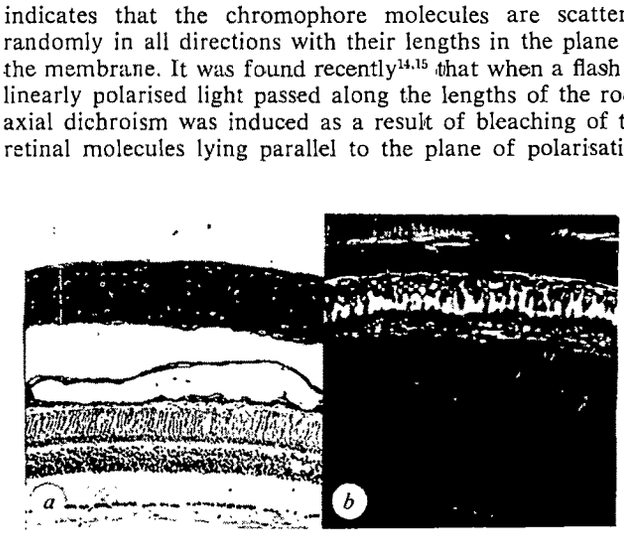


Fig. 1 Paraffin section of formaldehyde-fixed frog retina, depigmented with H_2O_2 and stained with 0.1% toluidine blue at *p*H 3.0 for 5 min. After blotting off the dye solution, the staining effect was stabilised with 2% potassium ferricyanide[20] and mounted in gum arabic which was allowed to dry; *a*, in the light microscope, at this *p*H, only the cell nuclei appear stained the rod outer segments show no dye binding. At the top the basophilic scleral cartilage is seen; *b*, the same field with the polaroids crossed. The ground substance of the cartilage shows strong toluidine blue induced birefringence[20]. Above the cartilage birefringent collagen fibres are seen.

of the light used. This dichroism, however, was very short lived and rapidly decayed with a relaxation time of about 12 ms, but was more persistent in glutaraldehyde-fixed rods. It was concluded that in the native state rhodopsin molecules were free to undergo rotational (Brownian) motion, however, with the chromophore remaining in the plane of the disk membrane. This would indicate a relatively fixed rotation axis of the rhodopsin molecules perpendicular to the plane of the membrane.

Thus rhodopsin seems to be highly oriented in the photoreceptor membrane[16,17], which seems to be of great functional importance[18]: it secures the greatest possible absorption of incident light of all possible vibration planes by way of the membrane parallel orientation of the chromophore molecules and by the rotational motion of rhodopsin.

The question can be raised whether the hydrophilic oligosaccharide chain of rhodopsin has a definite form and arrangement with respect to the plane of the membrane, indicating its possible contributory role in the maintenance of the order of rhodopsin in the disk membrane. So far no information about the steric arrangement of the oligosaccharide chain of rhodopsin has been available.

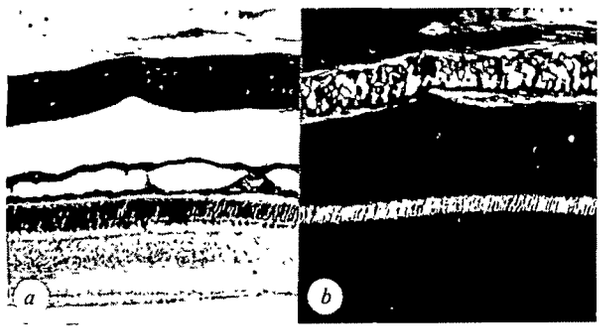


Fig. 2 After depigmentation and preoxidation with 0.1% HIO_4 (30 min) the slice was kept in aqueous saturated sodium bisulphite (30 min) and then stained with toluidine blue at *p*H~1.0, stabilised with potassium ferricyanide. *a*, At this *p*H the cell nuclei remained unstained but there is strong basophilia of the outer segments of the retinal rods, sharply delineated against the unstained inner segments. (The basophilic granules—with weak mosaic-like birefringence in (*b*)—seen around the cell nuclei in the scleral row of the outer nuclear layer were glycogen granules and could be removed by diastase.) At the top the basophilic scleral cartilage is seen; The same area as in, (*a*) with the polaroids crossed. The basophilic outer segments reveal a strong toluidine blue-induced negative birefringence with respect to their lengths, with retardations of about 30 nm at 600 nm. The cartilage shows strong toluidine blue induced birefringence[20].

Since we have developed a polarisation optical method for demonstrating linearly ordered glycol groups in polysaccharide chains, we have used this method in an attempt to decide whether the oligosaccharide chains of rhodopsin have a definite orientation with respect to the plane of the disk membrane. The method is based on the preoxidation by periodic acid of the vicinal OH groups, or amino–alcohol groups, of carbohydrates to dialdehyde groups, which after addition of sodium bisulphite, become negatively charged and able to bind toluidine blue at *p*H 1.0. This results in strong basophilia of the reacting compounds as reported for glycogen[19] and, as we have found, in a strong toluidine blue induced birefringence: negative in character with respect to the oligosaccharide chains when they are present in a linear order (as in neutral mucins, basement membranes and starch granules). As all the carbohydrate residues of the oligosaccharide chain of rhodopsin (three glycosamine, two mannose and one galactose) may be expected to react with periodate[20] as confirmed by the strong periodic acid Schiff reaction of the rod outer segments, the method proved promising for the investigation of whether or not the carbohydrate chain of rhodopsin was arranged in a definite order with respect to the plane of the disk membrane.

Dark-adapted retinae of the frog (*Rana esculenta*) were fixed by perfusion from the aorta in 10% formaldehyde solution, in a mixture of formaldehyde (5%), and glutaraldehyde (2%), and in Zenker fixative. Good polarisation optical results were obtained with formaldehyde and Zenker. Glutaraldehyde, a bifunctional cross-linking agent markedly depressed the optical effect, but not the induced basophilia, apparently because of the extensive cross linking of the underlying structure, which inhibits oriented dye binding. Paraffin slices were investigated in the light and polarisation microscopes. Data on the periodic acid bisulphite addition (PA-BSA) reaction and toluidine blue staining are included in the legend of Fig. 1. In Fig. 1*a*, the rod outer segments do not stain with toluidine blue at *p*H 3.0 and are isotropic in the mounting medium used (Fig. 1*b*). After PA-BSA reaction and toluidine blue staining (Fig. 2*a*) the outer segments reveal strong basophilia sharply delineated against the inner segments and corresponding to the staining with the PAS reaction, both indicating the presence of vicinal OH groups in great density in them. After the PAS reaction, however, the outer segments are optically isotropic, which means that this histochemical reaction of the vicinal OH groups gives no information about the molecular structural order of the underlying carbohydrate component in the photoreceptor membranes. On the other hand after PA-BSA reaction and toluidine blue staining the basophilic outer segments appear strongly birefringent (Fig. 2*b*) with a dispersion curve of their birefringence as a function of the wave length of the light as shown in Fig. 3. In it a diagram is included to show the underlying molecular mechanism of the PA-BSA reaction and the oriented toluidine blue binding of the carbohydrate chain with the resulting anisotropy.

It is obvious that optical anisotropy as a result of oriented molecular textures[22]. Therefore, the toluidine blue-induced anisotropy of the outer segments after PA-BSA reaction cannot be explained as arising either on randomly

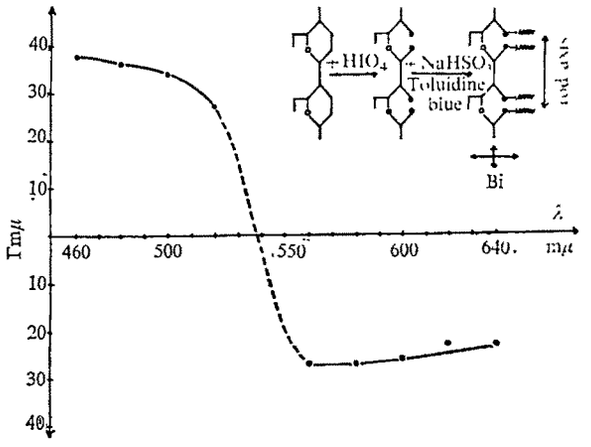


Fig. 3 Dispersion curve of the toluidine blue-induced birefringence of the rod outer segments of Fig. 2 *b*, which are negatively birefringent for light of wave length over λ 540 nm and positive under λ 530 nm. In the zone of immersion (· · · · · · · · · ·) no exact measurement of birefringence was possible. The inversion of birefringence is the result of anomalous dispersion of the refractive indices of toluidine blue when present in an oriented system[21]. As a result of the dispersion of the toluidine blue induced birefringence anomalous colours were seen: blue at additive, and red at subtractive compensation. The negative birefringence for the long-wavelength side of the spectrum indicates membrane-parallel orientation of the dye molecules[21] and in turn, membrane perpendicular orientation of the reacting oligosaccharide chains of rhodopsin.

arranged straight oligosaccharide chains or on irregularly bended oligosaccharide chains of the rhodopsin molecules. Thus, from these polarisation optical findings it may be concluded that the oligosaccharide chains of rhodopsin have a straight linear form oriented perpendicularly to the plane of the disk membrane.

It seems reasonable to assume that these straight hydrophilic carbohydrate chains of the rhodopsin molecules protrude into the aqueous area of the disk membranes. In this way they may form an axis for each rhodopsin molecule, which may well act as an axis for their rotational motion as inferred from the polarisation optical studies of Brown[13] and Cone[14], and suggested by Vanderkooi[23] in his membrane model. It is interesting to note that Heller[2], who was the first to demonstrate the oligosaccharide chain as an integral part of rhodopsin, suggested that by hydrophilic interactions the carbohydrate moiety probably served as an orientation factor for the rhodopsin molecules in the membrane. Corless[24] even assumed that the carbohydrate chains might serve as rotation axes for the rhodopsin molecules, although there had been no evidence for a definite orientation of these side chains with respect to the plane of the disk membrane. Our polarisation optical observation indicating a straight form of the oligosaccharide chains of rhodopsin with perpendicular orientation to the plane of the disk membrane, strongly supports such a view on the structural-functional significance of this part of the rhodopsin molecule.

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1 Heitzmann, H., *Nature new Biol.*, **235**, 114 (1972).
2 Heller, J., *Biochemistry*, **7**, 2906 (1968).
3 Heller, J., and Ostwald, T. J., *Ann. N.Y. Acad. Sci.*, **195**, 439 (1972).
4 Godfrey, A. J., *J. ultrastruct Res.*, **43**, 228 (1973).
5 Nilsson, S. E. G., *J. ultrastruct Res.*, **10**, 390 (1964).
6 Nilsson, S. E. G., *J. ultrastruct Res.*, **12**, 207 (1965).
7 Blasie, J. K., *Biophys. J.*, **12**, 191 (1972).
8 Blaurock, A. E., and Wilkins, M. H. F., *Nature*, **236**, 313 (1972).
9 Webb, N. G., *Nature*, **235**, 44 (1972).
10 Worthington, C. R., *Ann. N.Y. Acad. Sci.*, **195**, 293 (1972).
11 Schmidt, W. J., *Z. Zellforsch. mikrosk. Anat.*, **22**, 485 (1935).
12 Denton, E. J., *Proc. R. Soc.*, **B150**, 78 (1959).
13 Liebman, P. A., *Biophys. J.*, **2**, 161 (1962).
14 Brown, P. K., *Nature new Biol.*, **236**, 35 (1972).
15 Cone, R. A., *Nature new Biol.*, **236**, 39 (1972).
16 Cone, R. A., and Brown, P. K., *Science*, **156**, 536 (1967).
17 Blasie, J. K., *Biophys. J.*, **12**, 205 (1972).
18 Wald, G., *Science*, **162**, 230 (1968).
19 Malinin, G. I., *J. Histochem. Cytochem.*, **18**, 834 (1970).
20 Gedigk, P., *Klin. Wschr.*, **30**, 1057 (1952).
21 Romhányi, Gy., *Acta histochem.*, **15**, 201 (1963).
22 Romhányi, Gy., and Deák, Gy., *Acta morph. hung.*, **17**, 245 (1969).
23 Vanderkooi, G., *Ann. N.Y. Acad. Sci.*, **195**, 6 (1972).
24 Corless, J. M., *Nature*, **237**, 229 (1972).

Adaptation to invisible gratings and the site of binocular rivalry suppression

THE visual system can be described usefully as a hierarchical series of functional stages, the initial stage being physical impingement of the stimulus and the terminal stage being visual perception. Psychophysical investigations typically provide rigorous specification of the first and last stages in the sequence, but a more complete explanation clearly must include information about intermediate stages as well. At present, however, very few techniques are available for analysing inferentially the intermediate stages[1]. By incorporating two separate phenomena, binocular rivalry and visual aftereffects, into one paradigm, we describe here another technique for inferential analysis of the stages leading to vision. This technique indicates the sequence of stages underlying both phenomena.

Binocular rivalry refers to the transitory, phenomenal suppression of each eye's view produced when the two eyes receive different stimulation[2]. Even though a rivalry-inducing stimulus continuously impinges on the retina, it is only intermittently visible. Several characteristics of rivalry indicate that it is a reflexive process, the suppression state resembling in many ways a central scotoma[3–7]. Visual aftereffects refer to temporary perceptual distortions produced by prolonged exposure to a stimulus, some familiar examples being the waterfall illusion[8], figural aftereffects[9], and the more recently discovered McCollough effect[10]. Up to a limit, the magnitude of most aftereffects increases with duration of exposure. This property of aftereffects, coupled with the unique dissociation of physical stimulation and phenomenal viewing characteristic of rivalry, provides the keystone to the technique reported here. Imagine that an aftereffect-inducing stimulus impinges on an eye for a certain duration, but at the same time is phenomenally suppressed for a substantial portion of that time because of binocular rivalry. If the inducing stimulus remains effective while suppressed, the magnitude of the aftereffect will correspond to the duration of physical stimulation. But if the stimulus is rendered ineffective by suppression the aftereffect will be weakened, and its magnitude will correspond to the duration of phenomenal viewing. We have applied this technique using two spatial frequency aftereffects, both of which are induced by prolonged exposure to a grating pattern of repetitive light and dark bars. One aftereffect is an elevation in threshold contrast[11], and the other is a shift in apparent spatial frequency[12]. Both aftereffects are limited to gratings similar in spatial frequency and orientation to the adaptating grating. We have found that suppression exerts no influence on the growth of either of these aftereffects, and take this as evidence that the stage where suppression occurs is located subsequent to the site of these aftereffects.

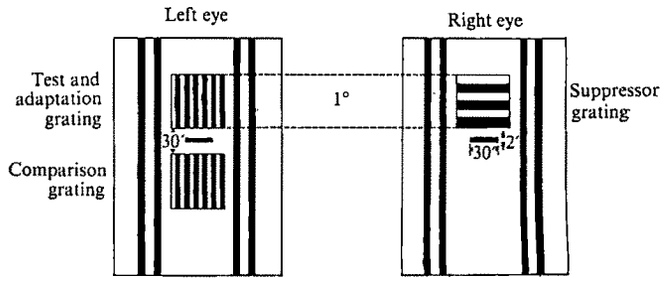


Fig. 1 The haploscopic stimulus arrangement used to induce and measure the spatial frequency after effects. Spatial frequency (number of cycles of the grating per degree of visual angle) and contrast (difference between the maximum and minimum intensities in the grating divided by their sum) could be altered, and the gratings turned off or on, without disturbing the mean luminance, 17 cd m^{-2}.

The displays used in our experiments are illustrated in Fig. 1. Squarewave gratings, masked to 1° square by transilluminated plastic sheets, were generated on cathode-ray tubes[13] mounted onto the arms of a large haploscope. Subjects viewed the displays from a distance of 91.6 cm through 3 mm artificial pupils, with head movements minimised by a bite-board and headrest. Space-average luminance of each grating was 17 cd m^{-2}, independent of spatial frequency or contrast, and the luminance of the masks was 5.1 cd m^{-2}. Each grating could be turned off or on without changing the average luminance. With the two displays

fused, the horizontal suppressor grating appeared in binocular coincidence with the upper left-eye grating, with a small fixation bar appearing immediately below. Thin vertical stripes on each side of the gratings facilitated fusion of the two displays.

We used a matching procedure to measure the aftereffects. While staring at the fixation bar, subjects quickly adjusted the test grating, using a potentiometer, to match the comparison grating on the basis of either contrast (experiment 1) or spatial frequency (experiment 2), offsetting the potentiometer after each adjustment. During these adjustments the right-eye suppressor grating was turned off. Adjustments were made before and immediately after exposing the left eye to an adaptation grating which temporarily replaced the test grating. During this adaptation period the subject gazed back and forth along the fixation bar to discourage formation of an afterimage. Each experiment consisted of four conditions of adaptation: (*a*) condition T—the adaptation grating was continuously present and visible for t s, with the right-eye suppressor grating turned off; (*b*) condition 2T—the adaptation grating was continuously present and visible for $2t$ s, with the suppressor grating off; (*c*) condition BR—the adaptation grating was physically present for $2t$ s but was seen for a total of no more than t s due to rivalry suppression produced by the suppressor grating presented to the right eye; and (*d*) condition RM—the adaptation grating was physically present and visible for t s distributed intermittently over $2t$ s, intermittency being produced by alternately exposing the left-eye adaptation grating and the right-eye suppressor grating following a pattern mimicking the fluctuations of rivalry. Each trial thus involved a preadaptation match of the test grating to the comparison grating, exposure to one of four adaptation conditions, followed immediately by a postadaptation match of the test grating (which fell on adapted retina) to the comparison grating (which fell on unadapted retina). Seven trials were devoted to each adaptation condition, the order of trials being haphazard. On each trial of condition BR, subjects continuously reported on rivalry fluctuations using a switch connected to an event recorder. Subjects rested at least 5 min between trials, until the aftereffect had dissipated.

Before an experiment, two adaptation durations, different by a factor of 2, were selected for each subject to maximise differences in the magnitude of the aftereffect under study. Only durations of 60 s or less were selected, for beyond this period the magnitude of the aftereffects no longer increased. Also, the spatial frequency and contrast of the adapting grating were adjusted for each subject to insure at least 50% suppression of the adaptation grating. Of three subjects, two were naive regarding the purpose of the experiments.

In experiment 1, the comparison grating was set for each subject at threshold contrast, and the spatial frequencies of the test, comparison, and adaptation gratings were identical. For two subjects the left-eye gratings were 3 cycles per degree, while for the remaining subject they were 6 cycles per degree. For each subject the contrast of the adaptation grating was approximately 1 log unit above threshold. With these spatial frequency and contrast values, the adaptation grating was suppressed for at least 50% of the adaptation period on each trial of condition BR, as determined from the graphic records of rivalry. The duration of adaptation t (condition T) varied from 15 to 30 s among subjects. The results from this first experiment are summarised in the graphs of Fig. 2*a*, which show for each subject the magnitude of the elevation in threshold contrast produced by each of the four conditions of adaptation. While all conditions produced some adaptation, the aftereffect for condition BR was significantly greater ($P<0.01$, t test) than either condition T or RM and was at least as large as that for condition 2T. Control trials were included to determine (i) if the suppressor grating alone influenced the visibility of the test grating, (ii) if the effects of the adaptation grating spread to the comparison grating, and (iii) if the aftereffect was limited to frequencies similar to that of the adaptation grating. Results confirmed that the aftereffect was frequency specific and unconfounded by either the suppressor grating or by interaction between the adaptation and comparison gratings. Considered together, this pattern of results demonstrates that suppression exerts no influence on the growth of the threshold elevation aftereffect.

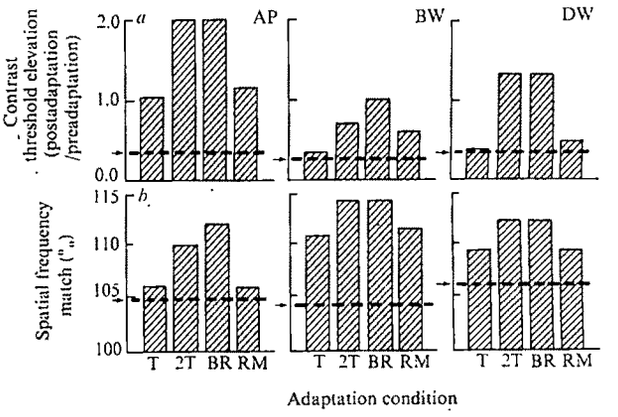


Fig. 2 The three bar graphs in (*a*), one for each subject, show the magnitude of the elevation in threshold contrast for four conditions of adaptation. Each value represents the mean ($n=7$) ratio of the adapted threshold to the unadapted threshold, minus 1.0. No aftereffect is thus represented by a value of 0.0. The arrow and dashed horizontal line indicate for each subject the level of significant threshold elevation (a ratio value equivalent to $2\sqrt{2}$ times the standard error of the unadapted thresholds). The average standard error for adapted thresholds was only about 0.03 log units. The three bar graphs in (*b*) show the magnitude of the shift in apparent spatial frequency, expressed as the mean ($n=7$) percentage difference from unadapted frequency matches. No aftereffect would yield a value of 100. Again the arrow and dashed lines mark the level of significant aftereffect, based on unadapted standard errors.

In experiment 2, in which we studied the size aftereffect[12], the spatial frequency of the comparison grating was 6 cycles per degree and the adaptation grating was 8 cycles per degree; all gratings were 1 log unit above threshold contrast and the durations t varied from 15 to 30 s among subjects. Results from this experiment, shown in Fig. 2*b*, follow the same pattern of results and significance found in the previous experiment. Because this aftereffect involves a change only in spatial frequency, not in contrast, these results extend the generality of those obtained in the first experiment.

In both instances the magnitude of the aftereffect was determined solely by the duration of physical impingement, phenomenal suppression exerting absolutely no influence. Yet from other data we know that suppression exerts potent inhibitory effects, at both the psychophysical[3–5] and electrophysiological level[6,7]. Thus the failure of suppression to weaken either aftereffect must mean that spatial frequency information arrives at the locus of the aftereffects even during suppression. This in turn implies that the neural mechanism responsible for suppression resides within the visaul system after the site of the aftereffects which is generally presumed to be visual cortex[11,12,14]. Our conclusion is based, of course, on the quite plausible assumption that pattern information is processed in serial fashion from eye to cortex.

Our experiments have helped to clarify the interaction between pattern adaptation and rivalry suppression. The technique of functionally uncoupling stages of the visual system by means of suppression, however, seems generally

applicable to other phenomena involving time-dependent effects and suppressible stimuli.

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[1] Julesz, B., *Foundations of cyclopean perception* (University of Chicago Press, Chicago, 1971).
[2] Breese, B. B., *Psychol. Monogr.*, **3**, No. 11 (1899).
[3] Fox, R., and Check, R., *J. exp. Psychol.*, **78**, 388–395 (1968).
[4] Lack, L., *Percept. Psychophys.*, **13**, 374–378 (1973).
[5] Blake, R., and Fox, R., *Vision Res* (in the press).
[6] Lansing, R. W., *Science*, **146**, 1325–1327 (1964).
[7] McKay, D. M., *Nature*, **217**, 81–83 (1968).
[8] Wohlgemuth, A., *Br. J. Psychol.*, Monogr. No. 1 (1911).
[9] Gibson, J. J., *J. exp. Psychol.*, **16**, 1–13 (1933).
[10] McCollough, C., *Science*, **149**, 1115–1116 (1965).
[11] Blakemore, C., and Campbell, F. W., *J. Physiol. Lond.*, **203**, 237–260 (1969).
[12] Blakemore, C., and Sutton, P., *Science*, **166**, 245–247 (1969).
[13] Campbell, F. W., and Green, D. G., *J. Physiol. Lond.*, **181**, 576–593 (1965).
[14] Blake, R., and Fox, R., *Nature new Biol.*, **240**, 76–77 (1972).

Memory in enzyme membranes

THE problem of information storage in the phase of 'short term memory', has attracted considerable attention. A plausible mechanism for the process is based on an all-or-none process, assuming the existence of metastable states[1,2]. Such phase transitions were thought to require structural changes in macromolecular components or in the membrane permeability of the information storage unit[3–5].

The rapid development in the techniques of manufacturing synthetic enzyme membranes[6] provides a new tool for demonstrating the existence of hysteretic phenomena which do not occur as a result of structural changes in a system.

The idea is based on studies by chemical engineers in which cooperative effects resulting from a nonlinear temperature dependence of rate coefficients were investigated from the point of view of the stability of chemical reactors. The existence of multiple stationary states was demonstrated for a reaction mixture having different reaction rates for the chemically controlled region and the diffusion-controlled region under the same external parameters. As a result the possibility of hysteresis and oscillations in internal parameters of the systems when coupled with thermal flows arose and was discussed[7].

In analogy to these results, we demonstrate here the existence of hysteretic phenomena, through simple kinetic mechanisms which are shared by most enzymes. We refer to the two simple cases; autocatalysis by the product, and inhibition by excess substrate. Both are examples of systems which do not satisfy the Le Chatelier-Braun principle, and the phenomenon never takes place along the whole range of concentration values, thus resulting in a maximum in the activity. When coupled with diffusion of the product of substrate respectively, there is, because of the nonlinearities in the reaction rate, the possibility of three stationary states, of which two are stable and one unstable, for a limited range of parameter values[8].

Two enzymatic reactions were studied. The hydrolysis of benzoyl arginine ethyl ester (BAEE) by papain (EC3.4.4.10) and the oxidation of uric acid by uricase (EC1.7.3.3). In both systems displaced far from equilibrium (namely, high *p*H for the papain system (of which the product is a strong acid, *p*H=3.38) or excess of substrate for the uricase system) an autocatalytic effect of the product, or substrate inhibition, respectively is seen. When the reagents are subject to diffusion limitation (for example when bound to a membrane matrix), the description 'stationary state' implies equal rates of supply and consumption through the diffusional flows and the chemical processes, at any point in the reaction space. The rate of hydrolysis is a function of BAEE concentration, hydrogen ion concentration and their diffusivities, and the rate of oxidation is a function of uric acid concentration, oxygen concentration, and their diffusivities. Under zero order kinetics for the oxygen or BAEE, a degree of freedom is lost, thus eliminating the occurrence of oscillatory behaviour, demonstrated previously[9,10], and forcing the systems to remain in one of the two stable stationary states, depending on its history.

Two different methods were used in these studies. The papain membrane was produced on the surface of a rotating *p*H glass electrode, by pouring a solution of collodion (3%, w/v), in ether (48%), ethanol (50%) and water (2%), on the surface of the electrode bulb (Metrohm, 'combined', while rotating horizontally around its revolution axis at 100 r.p.m. After 2–3 min, the membrane-electrode was introduced into

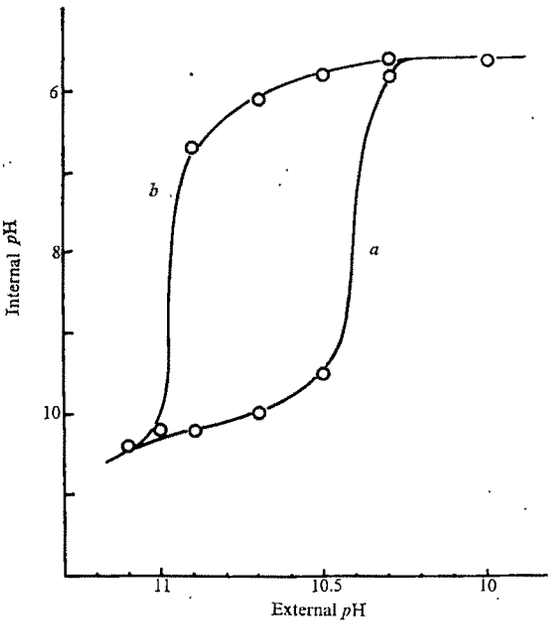


Fig. 1 Papain membrane-coated electrode. Stationary state description of internal *p*H as a function of increasing (*a*), and decreasing (*b*), the external *p*H values. The experiment started with decreasing the *p*H and then increasing. (See text for details.)

a solution of nonactivated papain (1 mg ml^{-1}), 0.1 M KCl for 3 d in 4° C (ref. 11), and then introduced to a solution of glutaraldehyde (0.5%) in phosphate buffer (0.02 M, *p*H 6.8), for 3 h at room temperature. The immobilised enzyme was activated by immersion in a solution containing EDTA (1 mg ml^{-1}), cysteine (1 mg ml^{-1}) and 0.1 M KCl, and then introduced in water, at 60° C, for 90 min, to cause partial denaturation, and to reduce the activity. It was then reintroduced to the cysteine EDTA solution, substrate was added and the *p*H at the electrode-membrane interface was recorded as a function of time and external *p*H. The results are given in Fig. 1, showing a hysteresis loop with two steep transitions, from *p*H 9.7–5.8 and from *p*H 6.7–10.2. Each point describes a quasistationary state of the system under constant external *p*H, controlled by a *p*H-stat appa-

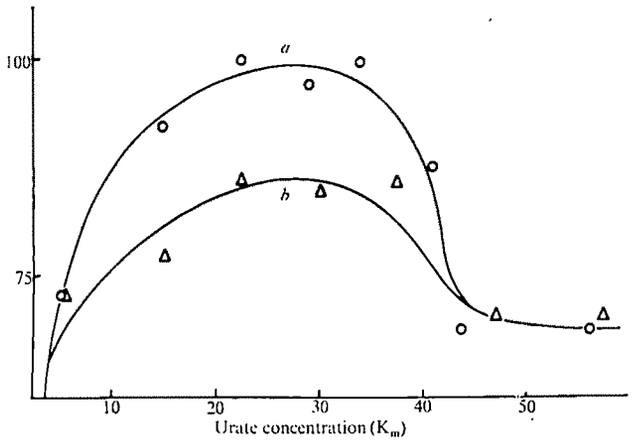


Fig. 2 Uricase membrane immersed in a solution of substrate. Enzyme activity (IU) as a function of the substrate concentration in the bulk solution. Results are given for increasing (*a*) and decreasing (*b*) substrate concentration value. (See text for details.)

ratus adding NaOH. At each point, an additional amount of substrate was added, verifying zero order kinetics. Because of the high rate of BAEE hydrolysis catalysed by hydroxyl ions at *p*H values higher than 10.5, compared with the low reaction rate, excess substrate was also verified at the end of the experiment. This was done by reducing the *p*H of the solution to 7, removing the electrode, adding papain to the solution and monitoring the amount of base added. The external *p*H was first lowered and then raised to eliminate an apparent effect due to possible loss of activity.

The uricase membrane was produced on a plane glass surface. A cross-linking reaction occurred for 2 h in a solution containing 30 mg ml^{-1} albumin, 2 mg ml^{-1} glutaraldehyde and 50 mg ml^{-1} uric acid in phosphate buffer 0.02 M, *p*H 6.8. Before spreading on the glass surface, 0.5 mg ml^{-1} of uricase was added to the solution[12]. To stir and to reduce the mass transfer problems of oxygen, the measurements were performed with several pieces from the same membrane. The pieces were immersed in a buffered solution of substrate (glycine-NaOH buffer, *p*H 3.3, 0.05 M) and put in continuous air bubbling. A time gradient of urate is established by a controlled pumping (chemical stat) and the rate of substrate consumption was recorded spectrophotometrically at 293 nm. Though the oxygen concentration in the system is much lower than the urate concentration, zero order kinetics for oxygen exists because the oxidation of urate to allantoin is the rate limiting step in the reaction chain[13].

The kinetic behaviour of both enzymes, in solution, was extensively studied[14,15] and no metastable states were found. It is clear that the memory which had been introduced into the above systems by diffusion limitations is more probable than a memory mechanism based on molecular hysteresis. It demonstrates once again the importance of taking structural influence into account, especially when dealing with complex biological systems bearing enzymes.

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1 Changeux, J. P., and Thiery, J., in *Regulatory Function of Biological Membranes* (edit. by Järnefelt, J.), **11** (Elsevier Publishing Co., Holland, 1968).
2 Katchalsky, A., and Oplatka, A., *Neurosci. Res. Symp.*, **1**, 352 (1966).
3 Katchalsky, A., and Spangler, R., *Q. Rev. Biophys.*, **1**, 127 (1968).
4 Lehninger, A. L., *Proc. natn. Acad. Sci., U.S.A.*, **60**, 1069 (1968).
5 Katchalsky, A., and Neumann, E., *Int. J. Neurosci.*, **3**, 175 (1972).
6 Thomas, D., and Caplan, S. R., in *Membrane Separation Processes*, (edit. by Meares, P.) (Elsevier Publishing Co., Holland, in the press).
7 Denbigh, K. G., and Turner, J. C. R., in *Chemical Reactor Theory*, (University Press, Cambridge, 1971).
8 Degn, H., *Nature*, **217**, 1047 (1968).
9 Caplan, S. R., Naparstek, A., and Zabusky, N., *Nature*, **245**, 364 (1973).
10 Naparstek, A., Thomas, D., and Caplan, S. R., *Biochim. biophys. Acta* (in the press).
11 Goldman, R., Silman, H. I., Caplan, S. R., Kedem, O., and Katchalsky, E., *Science*, **150**, 758 (1965).
12 Thomas, D., Broun, G., and Selegny, E., *Biochimie*, **54**, 229 (1972).
13 Malandain, H., and Broun, G., *Clin. chim. Acta* (in the press).
14 Whitaker, J. R., and Bender, M. L., *J. Am. chem. Soc.*, **87**, 2728 (1965).
15 Mahler, H. R., in *The Enzymes*, **8**, 285 (1963).

Correlation between speed of pollen tube growth and seedling height in *Zea mays* L.

EVIDENCE of a correlation between gametophytic and sporophytic qualities could be useful because gametophytic competition is both frequent and severe in natural populations[1]. A statistically significant correlation has been found in *Zea mays* between the relative speeds with which pollen tubes penetrate the lengths of styles and the relative competitive abilities of the resultant zygotes[2]. This correlation was found in a study of mixtures of two pollen types when the sporophytic competition was limited to interactions between zygotes developing within a common inflorescence. It is therefore possible that faster growing pollen tubes allow a relatively early start in postfertilisation growth which accounts for the competitive superiority of the resultant zygotes, although there is a conflicting report[3].

The present study was designed to test for correlations between relative speed of pollen tube growth and relative seedling weight, avoiding the possible influence of differences in time of fertilisation. Pollen types, of known tube growth rates, were each applied to separate inflorescences on plants of known genotypes. Five replications of each cross were made. All genotypes used were highly inbred and none of the crosses was a self pollination. The relative speeds of pollen tube growth were determined by the certation technique[4], modified to allow quantitative expression of the results[2].

From each of the 35 well-filled ears produced by the unmixed pollinations of the present study, 36 seeds were removed from the middle segment, avoiding the extreme sizes of apical or basal seeds. Seeds were weighed individually and planted singly in sand-filled pots 2 inches square in size. The environment was controlled at 22±1° C, 2,500 f.c., and daylength 18 h. Fourteen days after planting, seedlings were cut at ground level and weighed.

The data were subjected to partial correlation analysis with three variables: the relative speed of faster growing tubes[2], relative seedling weight and relative seed weight (see legend to Table 1). Partial correlation analysis tests for correlations between any two factors and, in effect,

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Table 1 Crosses made and the results observed.

Pair No.	Inbred lines used and their genotypes*	Number of well-filled ears obtained	Relative speed of faster pollen	Relative seedling weight† from faster pollen	Relative seed weight‡ from faster pollen
1	BB = yy × 16 = YY	2	0.0017	0.928	0.988
	BB = yy × 8 = yy§	2			
2	3A = yy × 1c = yy	1	0.0057	1.088	0.993
	3A = yy × 6A = YY§	3			
3	BB = yy × 1c = yy	3	0.0088	0.938	0.988
	BB = yy × 6A = YY§	2			
4	BB = yy × 16 = YY	2	0.0154	1.209	0.988
	BB = yy × 3B = yy§	3			
5	BB = yy × 16 = YY	2	0.0108	0.996	0.978
	BB = yy × BB = yy§	2			
6	BB = aa × 9c = aa	1	0.0198	1.183	1.049
	BB = aa × BB = AA§	3			
7	BB = aa × 9A = aa	2	0.0300	1.697	1.008
	BB = aa × BB = AA§	3			
8	16 = YYaa × 12 = AA	2	0.0281	1.148	1.021
	16 = YYaa × 9 = laa§	2			

*Lines designated as BB were obtained from Dr Robert Briggs, now of Funk Seed International, Inc., Bloomington, Illinois. All others were supplied by the Maize Genetics Cooperative.
†Average weight of seedlings resulting from fertilisations by gametes of the faster growing tubes divided by that from fertilisations by gametes of the slower growing tubes.
‡Average weight of seeds from fertilisations by gametes of faster growing tubes divided by that from fertilisations by gametes of the slower growing tubes.
§Faster pollen of the pair.

removes the influence of additional factors. Thus, in the present study, the value $r_{12.3}$ refers to the correlation between the first two factors, relative pollen tube speed and relative seedling weight, when the influence of the third factor is removed (Table 2).

The partial correlation between relative pollen tube speed and relative seed weight, $r_{13.2}=0.566$, is not statistically significant, nor is that between relative seedling weight and relative seed weight ($r_{23.1}=-0.241$). The latter result does not indicate that relative seedling weight and relative seed weight are generally independent of each other, it just means that in this study, no statistically significant portion of the variation in one factor could be attributed to variation in the other factor. This is understandable in that a separate analysis of variance (not presented here) indicated that if each inflorescence receives pollen from only one plant, weights of seeds resulting from fertilisations by gametes of the relatively fast pollen tubes do not differ significantly from weights of seeds produced by gametes of relatively slow pollen tubes, a conclusion in agreement with that of Jones[4].

Table 2 Correlation and partial correlation analyses for relative pollen tube speed, relative seedling weight, and relative seed weight.

r_{12}	= 0.781*
r_{13}	= 0.608
r_{23}	= 0.355
$r_{12.3}$	= 0.761*
$r_{13.2}$	= 0.566
$r_{23.1}$	= −0.241

*Significant at the 5% level.
r_{12}, correlation coefficient between the first and second factors.
$r_{12.3}$, partial correlation coefficient between the first two factors with the influence of the third removed.

The partial correlation coefficient between the first factor, relative pollen tube speed, and the second, relative seedling weight, with the influence of the third, relative seed weight, removed is $r_{12.3}=0.761$, which is significant at the 5% level (d.f.=5 with two variables) (ref. 5). This demonstrates that a significant number of genetic factors which are expressed in the gametophyte are also expressed in the sporophyte.

This statistically significant correlation between relative pollen tube speed and relative weight of the resultant seedlings cannot be due to differences in time of fertilisation because there were no interactions between the two types of zygotes. It also suggests that seedling vigour, a selectively important quality[6], can be influenced by gametophytic competition.

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[1] Hartl, D. L., *Evolution*, **24**, 415 (1970).
[2] Mulcahy, D. L., *Science*, **171**, 1155 (1971).
[3] Collins, G. N., and Kempton, J. H., *US Department of Agriculture Bureau of Plant Industries Circ.*, 124 (1913).
[4] Jones, D. F., *Selective Fertilization* (Univ. Chicago Press, Chicago, Illinois, 1928).
[5] Crow, E. L., Davis, F. A., and Maxfield, M. W., *Statistics Manual* (Dover Publications Inc. New York, N.Y., 1960).
[6] Black, J. N., *Aust. J. agric. Res.*, **9**, 299 (1959).

Heterocyst formation in a blue-green alga, *Cylindrospermum*

THE blue-green alga *Cylindrospermum*, unlike a similar alga, *Anabaena*, where intercalary heterocysts are present, contains only two terminal heterocysts (Fig. 1*A*). When either or both the heterocysts are detached, new heterocysts regenerate in time. In *Anabaena cylindrica*, where the heterocysts occur at regular intervals, the spacing pattern is determined by intercellular interactions, probably involving some inhibitory substance produced by the heterocyst itself. No two heterocysts are produced side by side either in *Anabaena* or *Cylindrospermum*. The inhibitory substance could be ammonia or a substance derived from ammonia[1]. Here we report observations which indicate that an inhibitor controls the spacing of heterocysts.

Clonal cultures of *Cylindrospermum trichotosporum* were grown on Allen and Arnon's medium[2], both on solid and liquid media. In some experiments it was also cultivated on media containing combined nitrogen, ammonium chloride (3 mg ml^{-1}) or ammonium phosphate (3 mg ml^{-1}). The growth and formation of heterocysts was followed by the methods described by Kale *et al.*[3]. Algal material, generally 15 d old, was suspended in fresh medium and blended in a modified kenmix blender for 0.5—1 min and after dilution, drops of the suspension were placed on agar plates. Selected filaments were focused under the microscope and observations made.

The number of vegetative cells present between the two terminal heterocysts is a fixed characteristic of an algal population for a particular set of culture conditions in *Cylindrospermum*, the average length of the filament between the two heterocysts is 418 μm (107 cells). On liquid media with constant shaking, filaments tend to be longer than the average and while on agar, the spreading filaments break into two, usually each fragment having a single heterocyst, generally 111–150 μm (21-28 cells) long. When the filaments are broken into fragments by blending at high speed, the fragments so formed also have a single heterocyst and are of a similar range in length (111–130 μm). This seems to indicate the presence of naturally occurring weak points of breakage in the filaments of *Cylindrospermum*.

Table 1 Growth needed before the formation of first heterocyst in fragments of *Cylindrospermum* grown on various media.

Control (Allen and Arnon's medium)	36*	54†
Medium with ammonium chloride, 3 mg ml^{-1}	55*	41†
Medium with ammonium phosphate, 3 mg ml^{-1}	51*	18†

* % increase in length.
† No. of observations.

Fragments of filaments with one heterocyst still attached, produced by natural breaking or by high speed blending, regenerate the missing heterocyst at the broken end when placed on agar (Fig. 1*B* and *C*). This is always preceded by growth, however, which is found to be inversely related to the initial length of the fragment. A fragment with single heterocyst and initial length of 42.8 μm, increased to 142.8 μm (2.38 times), and another with an initial length of 100 μm grew to 157 μm (1.75 times) before the second heterocyst developed. The interheterocyst distance in 52 cases (total 100) was 141–150 μm and in 74 cases 131–160 μm, with an average value of 145 μm as the minimum length needed for the regeneration of the second heterocyst.

When fragments with no heterocysts attached to them were plated on agar plates they invariably regenerated one heterocyst first before further growth and the formation of the second heterocyst. The amount of growth preceding the formation of first heterocyst is variable and is not always related to their initial length but seems to depend largely on their location on the intact filament from which they were derived. The second heterocyst, however, generally formed when the average length of the fragment attained a range of 141–150 μm.

When the alga was grown on combined nitrogen, ammonium chloride or ammonium phosphate, the filaments were free of heterocysts because, as in *Anabaena*, combined nitrogen inhibited their formation. If the filament fragments were taken from such cultures, the first heterocyst appeared in them after a prolonged growth on nitrogen-free medium, more than what was needed for a fragment previously grown on medium free of combined nitrogen (Table 1).

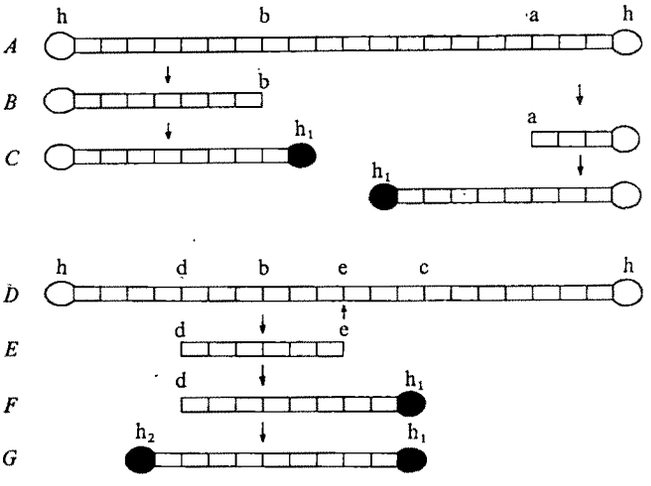


Fig. 1 Diagrammatic representation of a filament of *Cylindrospermum* showing the position of heterocysts (h) and the regeneration of new heterocysts (h_1,h_2) in the fragments. *A–G*, explanation in text.

If heterocysts do produce an inhibitor that controls the formation of other heterocysts, its concentration in a vegetative cell would be proportional to its distance from the existing heterocyst. Accordingly, the end of the longer fragment which is farther from the heterocyst develops another heterocyst more quickly (after growth) than the one in a shorter fragment. The fragment must grow before the formation of a second heterocyst to reduce the level of the inhibitor.

Heterocyst formation in fragments which do not already contain them (Fig. 1*E*), is very different, however. Such fragments can arise from any portion of the intact filament (Fig. 1*D*), either from the middle portions or eccentrically from one side (Fig. 1*D*, b-c, d-e). The fragment b-c may contain the inhibitor at equal concentrations at both ends and when heterocysts develop they may arise simultaneously after a limited growth. On the other hand, in the fragment d-e, the inhibitor level would be greater at d than at e (as d is nearer to a heterocyst) and as a result the fragment

develops a heterocyst first at e and later at d, only when growth has taken place to reduce the inhibitor to a sufficiently low level.

During our work, we found very few cases where the heterocysts formed simultaneously in a fragment. In general, it seems that in *Cylindrospermum*, filaments do not easily break from the middle region and that almost all fragments arise eccentrically on either side of a mid point between the two terminal heterocysts. The successive formation of heterocysts in a fragment containing no heterocysts is also an indication of the existence of polarity in the filament of the alga, which is itself a reflection of a gradient in the level of the inhibitor. A normal filament may have a decreasing gradient of the inhibitor starting from the terminal heterocyst to the mid point of the filament, the other half of the filament being a mirror image of this.

When fragments of *Cylindrospermum* were taken from the alga previously grown in a medium containing combined nitrogen and plated on agar medium free of ammonia, they were found to regenerate their first heterocyst only after a prolonged time, during which greater growth has taken place in them compared with the fragments grown earlier on molecular nitrogen. Presumably, by growing on combined nitrogen, the internal level of the inhibitor remained at a high level and as growth took place decreasing its concentration, the formation of heterocysts was initiated. The heterocysts appeared sequentially, however, first at one end and later at the other. This shows that the filaments whether grown on molecular nitrogen or combined nitrogen, still show polarity, that is their terminals are predetermined with regard to regeneration.

The nature of the inhibitor produced by heterocysts to control their spacing pattern is still debatable[1-5] but recent findings indicate that heterocysts are the site of nitrogen fixation[5]. Fogg's suggestion[6,7] that ammonia concentration in the filament is responsible for such an inhibition gains further ground, but it may not be the only deciding factor[1].

We have already discussed in some detail[8] how the spacing of heterocysts could be controlled in *Anabaena* in terms of the known metabolic activities of the heterocysts as well as the vegetative cells (see also, Wahal *et al.*[9] for the role of ascorbic acid). Although filament polarity is found in many blue-green algae, such as *Rivularia*, nothing is known of its nature and maintenance. In *Anabaena*, it has been shown[10] that cell division is asymmetrical and heterocyst spacing is controlled by the interaction between developing cells[11] but it is yet to be seen whether a similar situation exists in *Cylindrospermum* also.

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1 Carr, N. G., and Bradley, S. C., *Symp. Soc. gen. Microbiol.*, **23**, 161 (1973).
2 Allen, M. B., and Arnon, D. I., *Pl. Physiol.*, **30**, 366 (1955).
3 Kale, S. R., Bahal, M., and Talpasayi, E. R. S., *Indian Biologist*, **2**, 30 (1970).
4 Wolk, C. P., *Proc. natn. Acad. Sci., U.S.A.*, **57**, 1246 (1967).
5 Fay, P., in *The Biology of Blue-Green Algae* (edit. by Carr, N. G., and Whitton, B. A.), 238 (Blackwell, Oxford, 1973).
6 Fogg, G. E., *Ann. Bot.*, **13**, 241 (1949).
7 Fogg, G. E., Stewart, W. D. P., Fay, P. and Walsby, A. E., *The Blue-Green Algae*, 235 (Academic Press, London, New York, 1973).
8 Bahal, M., and Talpasayi, E. R. S., in *Taxonomy and Biology of Blue-Green Algae* (edit. by Desikachary, T. V.), 197 (University of Madras, Madras, 1972).
9 Wahal, C. K., Bhattacharya, N. C., and Talpasayi, E. R. S., *Physiologia Pl.*, **28**, 424 (1973).
10 Mitchison, G. J., and Wilcox, M., *Nature*, **239**, 110 (1972).
11 Wilcox, M., Mitchison, G. J., and Smith, R. J., *J. Cell Sci.*, **12**, 707 (1973).

GENERAL

Commercial radionuclide dispensing

Commercial suppliers of radionuclides operate under stringent regulations. Most companies give specific assurances of nuclide identity and purity. Examples are the plots of γ spectra, radiochromatograms and, most important, the data sheet that specifies such things as quality, purity and radiation intensity of the specific nuclide. Unfortunately, however, the controls used by suppliers are inadequate and the technical data sheet cannot be relied upon implicity.

In support of other reports[1-3] I shall relate an experience which involved a shipment of radioiron. On arrival, the package was monitored and the γ activity was about 10% of that expected. The supplier confirmed this and explained that a technician had calculated the stock to be 28.7 mCi ml^{-1} instead of 2.87 mCi and ml^{-1} had therefore dispensed only 10% of the required volume. Despite the small volume, however, the radiation value show, on the technical data sheet was that expected for 2 mCi of ^{59}Fe (about 70 mr h^{-1}), which suggested that the number was either recalled from memory or copied from an old sheet. I stated this in a letter and the company responded with a written acknowledgment of error. The compounded errors, they explained, were because of rapid expansion and shortage of personnel. They believed that safeguards and routine controls, (which were not specified), would be effective in the future.

Eliminating the monitoring step and entering an assumed value for radiation were serious errors. Companies should enforce separate duties, so that a person who dispenses radionuclides cannot also perform assays, complete data sheets, package, monitor and authorise final release for shipment.

It seems reasonable, as a partial answer to this problem, that if readout plots of γ spectra or chromatograms of various nuclides can be supplied to customers, then automatic recordings of radiation could also be provided. Such records could be inspected by supervisors to detect nuclide deficiency or excess and then be duplicated to provide a customer copy. The production of an authentic radiation report for the sample would be of the greatest importance. This should help eliminate the temptation to take short cuts and to falsify reports with assumed radiation values.

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1 Von Korff, R. W., *Nature*, **238**, 347–348 (1973).
2 Goldman, I., *Science*, **167**, 237 (1970).
3 Gordon, B. E., *Chem. Engng. News*, **49**, 3 (1971).

book reviews

Lobotomy

Psychophysiology of the Frontal Lobes. Edited by K. H. Pribam and A. R. Luria. Pp. xii+332. (Academic: London, August, 1973.) $19.95.

THE starting point of interest in the frontal lobes was the celebrated "Crowbar Case" reported by Harlow in 1848. One Phineas Gage, a quarry worker, had a large iron tamping rod blown by a stick of dynamite into his jaw and and out through the top of his head, producing serious injury to both frontal lobes. As a result, Gage underwent a disastrous change of personality. From having been an efficient and capable foreman, he became fitful, irreverent and obstinate, and is said to have "indulged in profanity that would have embarrased an 18th century British Sea Captain". Thus was born the "frontal lobe syndrome", the study of which in modern times owes much to the Russian neuropsychologist Alexander Luria, himself a coeditor of this volume.

Although a subject of fascination to psychiatrists and others, the frontal lobes have hitherto excited comparatively little interest among physiologists, largely because they are in the main electrically inexcitable and their excision does not occasion gross defects in sensation or movement. Indeed it was not until systematic behaviour experiments were begun in the thirties that any appreciable progress was made. These experiments claimed to reveal some fairly constant behaviour deficits in subhuman primates following bilateral frontal excisions and also—incidentally—led Moniz to inaugurate the era of psychosurgery. Dr Karl Pribram, likewise a coeditor, has been active over many years in charting the behavioural deficits produced in monkeys by circumscribed cerebral excisions. Their underlying physiology, however, has remained largely unexplored.

This volume represents an attempt, if a somewhat diffuse and ill-coordinated one, to fill this gap. Based upon a symposium on frontal lobe function held during the 18th International Congress of Psychology in Moscow some years ago, it brings together fourteen papers concerned with the neurophysiology of the frontal lobes and the effects of frontal lesions on the electrical activity of the human brain. Of the contributors, fourteen are American (mostly based on the West Coast), eight are Russian (all based on Moscow) and one is English (based on Bristol). The history of the enterprise and its relation to other recent symposia on closely related topics is outlined in a brief preface.

Taken as a whole, this volume reveals a distinct difference in approach as between the Russian and American contributors, though there is, of course, a good deal of common ground. By and large, the Russian work is almost exclusively limited to experiments with human subjects, mainly evoked potential and EEG studies, whereas the Americans give far more attention to the data of animal experiments and to explanation of behavioural deficits in physiological terms. Although much of the work is pretty specialised, it does serve to give an overview of current physiological work on the frontal lobes, though alas there is nowhere any real sign of a breakthrough.

In his concluding chapter, Pribram discusses frontal lobe functions in terms of spatial and temporal organisation and, more interestingly, the inhibitory mechanisms that, when deranged, produce the distractibility so marked in the 'frontal' animal or patient. He also places stress on the frontal cortex as the "executive" of the brain in the overall control of behaviour. In this latter claim, at least, he and Luria see eye to eye.

O. L. ZANGWILL

Breaking bones

Mechanical Properties of Bone. By F. Gaynor Evans. Pp. xiii+322. (Thomas: Springfield, Illinois, June 1973.) $25.75.

THIS interesting and well illustrated compendium of data on the mechanical properties of bone is a necessary reference work for all biologists and engineers who are concerned with the mechanical properties of materials. For the biologist, and this includes the orthopaedic surgeon, neurosurgeon, orthodontist and others who must be particularly interested in the effects of force either applied to, or generated within the body, an understanding of the mechanical properties of bone, which forms some 18% of the body, is essential for the prevention and treatment of a variety of conditions afflicting the musculo-skeletal system. An assessment of the change in mechanical properties of biopsy specimens of bone in the course of disease and its treatment may before long become a diagnostic and prognostic procedure.

For the engineer an appreciation of the design of the skeleton, the structure of bone and its mechanical properties is of considerable interest. In particular the materials scientists and structural engineers who are concerned with the design of passenger-carrying vehicles, which are increasingly involved in accidents, need the information concerning the mechanical properties of the living item contained within the vehicle. This book deals with, in the main, the mechanical properties of bone as a material. The thoughts of those who are responsible for the development of new materials may well be stimulated by the section dealing with the effects of the microscopic structure of bone when considered as a structural material.

For those who are interested in the mechanical properties of the whole bone this work should be read in conjunction with the author's other publication *Stress and Strain in Bones.*

JOHN T. SCALES

Water crossing membranes

Water Transport in Cells and Tissues. By C. R. House. Pp. vi+562. (Monographs of the Physiological Society, No. 24 Arnold: London, January 17 1974) £10.50.

THIS book is a mine of information and the fact that more than 900 references are included in the bibliography is a measure of the thoroughness with which the relevant literature has been surveyed. Of the total of ten chapters, seven make unexceptionable reading and the accounts of transcapillary and transepithelial water transport are outstandingly clear and comprehensive. The permeability characteristics of cell membranes in general and of artificial membranes also receive detailed and informative consideration as do the physical and chemical properties of water, which are dealt with at the beginning of the book.

The account of fluid dynamics in the embryo is less impressive, although this seems to be due more to the shortage of precise information than to any fault of the author, who is led to the conclusion that "our ignorance of the processes that trigger, enhance and finally block the formation of extra-embryonic cavities is enormous". One may question the wisdom of devoting nearly 25 pages to the exposition of this topic, except perhaps as a challenge to future investigators, to whom the message could have been given rather more

succinctly. On the other hand, the treatment of water movement in plants is somewhat skimped and includes virtually no discussion of transport in the xylem. I was particularly disappointed to find no reference to the extraordinary ability of the giant Redwood trees to raise water.

The author in his preface refers to the dilemma which faced him in deciding upon the place of mathematical theory in his book, a dilemma which he resolved by introducing a short "practical preamble" at the beginning of his chapter entitled "Phenomenological Description of the Transport Process", and then following this with a fairly lengthy excursion into irreversible thermodynamics. For the *cognoscente* this excursion is probably unnecessary; the presentation is too compressed to be really helpful to the novice and does not bring out at all clearly the thinking behind the development of irreversible thermodynamics from its more familiar progenitor, equilibrium thermodynamics, sometimes dubbed "thermostatics". These are contrasted in terms of entropy changes rather than of the notion of moving from states to rates. But the mathematics used is relatively straightforward and furthermore, the succeeding parts of the book can be read profitably without any particular mathematical expertise.

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Dr House's approach to his subject is commendably judicial and the evidence for and against the ideas which he discusses is carefully evaluated and, where appropriate, he has interposed a a 'summing up'. Occasionally he abandons his detachment and confesses, with engaging candour, to personal bias. For example, this bias is freely admitted following a spirited and critical examination of the widely accepted dogma that active transport of water is inadmissible on energetic grounds. A plausible, if slightly confusing case, based as it is on a mixture of reversible and irreversible thermodynamics, is put forward for bringing in a verdict of "not proven" on this highly controversial issue.

This monograph forms a worthy addition to the series published for the Physiological Society. To predict that it will successfully stand comparison with some of the best of its predecessors implies both an established criterion for judgement and at the same time a deserved compliment to Dr House.

R. V. Coxon

Warm blooded

Temperature Regulation in Mammals and other Vertebrates. By John Bligh. Pp. xix+436. (North-Holland: Amsterdam and London; American Elsevier, New York, 1973.) $30.20.

Dr Bligh is to be congratulated on writing a book which is stimulating and concise, covering the whole field of thermoregulation in vertebrates—an area in which he himself is a distinguished worker. The book is written from the physiological standpoint and it will prove unsatisfying to physiological ecologists, since, for instance, many of the adaptations shown by mammals living in unusual environments are mentioned only briefly or not at all. It includes chapters on the traditional areas of thermoregulation (central control, sensing systems, effector mechanisms, neuronal influences, central transmitter substances and so on).

There are very stimulating chapters on the various models of the thermoregulation system which have been proposed, and there are useful sections on unorthodox theories, fever and exercise. In the section on comparative thermoregulation there seems to be no mention of the recent work on thermoregulation in certain large fish. This, however, does not take away from the overall high standard of the book which is highly recommended. It is a well produced book but perhaps rather expensive.

J. N. R. Grainger

Scientific literature

Understanding Scientific Literature: a Bibliometric Approach. By Joseph C. Donohue. Pp. xiii+101. (MIT: Cambridge, Mass. and London, 1973.) $10.

Twenty years ago, the American librarian Jesse Shera was advocating the study of a 'social epistemology' defined as "the analysis of the production, distribution and utilisation of intellectual products in much the same fashion as that in which the production, distribution and utilisation of material products have long been investigated". He pointed out that all the requisite data are to be found in the books and journals that fill the shelves of our libraries. By analogy with econometrics the term bibliometrics was later coined to cover the essentially data-collecting and analytical phase of a more theoretical study, which would be concerned with the organisation of mankind's exosomatic stores of knowledge for more effective social use.

In an uncoordinated way the statistical analyses Shera called for have been developed in recent years and the slim volume under review is the first monograph wholly devoted to these matters. One would expect the author of such a work to offer a critical review and constructive coordination of the widely scattered contributions and thus present the first consolidation of the emergent study. The need for such a work is now more urgent than it was twenty years ago because during that time large-scale computerised documentary information systems have been developed and the feasibility of even larger world systems is now being discussed. But all systems so far designed have been based on the very questionable assumptions that all published scientific papers have equal scientific weight, that 100% coverage is a desirable goal and that all papers retain their scientific interest *sine die*. These systems can only become more effective, more frequently used and less costly when their designers pay attention to the use made of scientific information by its scientific users—a use which is fully documented within the scientific journals themselves.

There are several techniques which bear on these matters and which one would expect to find described in a 10-dollar monograph. Two empirical laws have been known for some time. In 1925, Lotka noted that the number of authors who contribute r papers to any specified subject literature over any specified period is proportional to $1/r^2$ (though the exponent is 1.89 rather than 2). And in 1934, Bradford noted that, when bibliographical sources are ranked in order of decreasing productivity, the number of items contributed

by the source of rank r is proportional to $1/r$ if r is greater than a certain limit—an empirical law which applies to many other social contexts. More recently, Goffman has shown that the dissemination of new ideas is closely analogous to the spread of infectious diseases with endemic outbreaks preceding the take-off to epidemic growth. He has considered the conditions that have to be satisfied before take-off can occur and also the conditions under which scientific communication begins. to break down under the weight of its own documentation.

It has also been noted that scientific literatures not only grow at different exponential rates but decay exponentially at comparable rates, with half-lives of four to 16 years, so that the 'information explosion' worries only those who wish to keep everything—alive or dead. Work has also been done, mainly in the United States, on 'bibliographic coupling'—a technique of tracing who cites whom to identify networks of scientific interaction. There is therefore a varied and comprehensive task to be done by the author of the first monograph describing these techniques and their applications to information system economy.

Unfortunately, a great opportunity has been missed. The author makes a personal selection from the techniques. He inadequately and uncritically discusses only the Bradford law, the epidemiological model and some cross-citation techniques. He makes idiosyncratic selections of illustrative material drawn wholly from American sources and bibliographically ill defined. The numerical conclusions and graphs are firmly expressed though they are based on modest samples with no intimation that sampling errors might arise. The 100-page monograph reduces to 70 pages of text and 30 pages of singularly uninformative graphs. Thus the price of this modest introductory essay of 20,000 words is highly inflationary.

B. C. Brookes

Neutron generators

Activation Analysis with Neutron Generators. By Sam S. Nargolwalla and Edwin P. Przybylowicz. Pp.xvii+662. (Chemical Analysis: A Series of Monographs on Analytical Chemistry and Its Applications.) (Wiley Interscience: New York and London, January 1974.) £16.25.

It is often very important to be able to take one's microanalytical kit with one around the Universe. This has not been easy with neutron activation analysis, since fission reactors are heavy and one does not take them easily down a mine or to the moons of Jupiter. But small generators of fusion neutrons (say using the D-T reaction) can be made, although they have some problems such as the stability of their neutron yield.

This book attempts to summarise the uses of generators and can be described as "nearly a handbook" on the subject. A handbook normally leads you through mazes of research material easily, smoothing out the little wrinkles in the patchwork of data. In parts of this book, I feel the authors have indeed essayed such a task of inspection of pedigree and smoothing of data. In others, the reader is not sure whether a table of figures on, say, a type of neutron reaction is raw from one man's research or represents the sum of knowledge. The intent of many of these tabulations, which could have been properly signalled in only a few words, is sometimes left unclear. Thus, this book is perhaps best described as a research review which nearly achieves handbook status.

The authors certainly attempt completeness of coverage; they have used the prodigious documentation services of the US National Bureau of Standards (NBS) at which institution they once worked together, to compile the knowledge of the bureau and others on the use of neutron generators and the course of nuclear reactions activated by fusion neutrons.

The techniques for the generation of neutrons and the exposure of samples are lovingly described, with detailed engineering drawings and dosimetric maps. A section follows on the treatment of the diverse types of systematic error which can interfere with the analytical accuracy of the method and this section certainly convinces that, for certain important elements, neutron activation can surpass X-ray fluorescence or atomic absorption methods. It is, however, this first half of the book, which is somewhat littered with tables of doubtful intent, that will neither help the student nor satisfy the man in search of a handbook; by contrast, the second half of the book consists of a 300-page chapter that takes in turn each element which will succumb to activation analysis. The authors tabulate the nuclear reactions which work with fast neutrons, and then give for each a set of comments and data useful to the analyst, including a uniform set of computer-generated curves for buildup of radioisotope against time, decay against time and so on. Here, the reader's confidence in completeness and uniformity can be high. Finally, there are several appendices giving tabulations, by other workers, of those nuclear reactions which are useful in analysis. These are doubtless useful to have to hand but are, again, insufficiently clear in intent.

The impression left is that neutron activation analysis is a formidable subject to venture into unless armed with much knowledge and time. This is not the expressed intention of the authors, who wish to stimulate the broader use of the method and fairly state the advantages and disadvantages of the method compared with those of the rival techniques. They have certainly done a great service in dragging out and starting to organise the files of the NBS on this subject: however, it will take a firmer hand to encourage the hesitant to embark on this technique or to construct a true handbook out of all that information. On the other hand, a scientist already committed will learn all about the special techniques needed to maintain analytical accuracy and all the rest of the practical issues in this undoubtedly useful field of work.

A. G. Holmes-Siedle

Stars for students

Introduction to Stellar Atmospheres and Interiors. By Eva Novotny. Pp. xii+543. (Oxford University: London and New York, September 1973.) £7.00.

This book is intended as a textbook for senior undergraduates and commencing graduate students. It is a generally well written and well produced book but in my opinion the balance of the book is wrong for a textbook. The basic concepts of the subject tend to be described very briefly and important fundamental results are often quoted without proof, whereas many pages of tables and diagrams are devoted to the detailed results of particular solutions of the equations of stellar structure and evolution.

Two examples will serve to illustrate what I mean. About fifteen pages are devoted to tables of the monochromatic absorption coefficient in stellar atmospheres and a further ten pages to tables of the properties of model stellar atmospheres, but there is no detailed discussion of the form of the equations which must be solved to obtain a model stellar atmosphere, except in the case of a grey atmosphere in local thermodynamic equilibrium. It is mentioned that there is a maximum mass for white dwarf stars and a reference is given to where a physical explanation of the existence of a maximum mass can be found. The book would have been much improved if one page of tables had been sacrificed so that a brief explanation of the existence of the maximum masses of cold bodies of various types and of the near inevitability of the existence of black holes could have been included.

One danger of including too many

Plant Cell Structure and Metabolism

J L Hall, T J Flowers and R M Roberts.

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E M Lind and M E S Morrison

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detailed results in a first textbook is that they may be regarded as being more reliable than they really are. Although in some cases it is made clear that the results quoted only represent one point of view, this is not always true. In the discussion of the evolution of stars approaching the main sequence, the Hayashi theory is fully tabulated but no mention is made of the later work by Larson which casts considerable doubt on the previous results.

More detailed criticisms of the book include the feeling that far too much space is devoted to the Vogt Russell theorem which is certainly not generally true and that the concepts of thermodynamic equilibrium and local thermodynamic equilibrium should have been described much more clearly. It is also a pity that the discussion of numerical methods does not include the Henyey technique, which has been most widely used in recent years.

Now that I possess the book I may well find it very useful to have in one volume so many tables and figures illustrating recent studies of stellar structure and evolution and students will similarly be able to use it as a reference book. But it seems very unlikely that for students it will replace such texts as that by Schwarzschild, which is considerably cheaper and which gives a much deeper physical understanding of the subject.

R. J. TAYLER

Magnetic rocks

The Physical Principle of Rock Magnetism. By Frank D. Stacey and Subir K. Banerjee. Pp. ix+195. (Developments in Solid Earth Geophysics, Vol. 5.) (Elsevier: Amsterdam, London and New York, 1974.) Dfl.60; $2.10.

THIS book was advertised for over a year before its appearance, but it has been worth waiting. It is a slim volume deliberately planned as a physical supplement to Irving's *Palaeomagnetism and its Application to Geological and Geophysical Problems* (Wiley, 1964). It must also be considered to effectively replace Nagata's excellent, but outdated book, *Rock Magnetism* (Maruzen, 1961) although it does not contain any instrumental discussion that formed a significant part of Nagata's work.

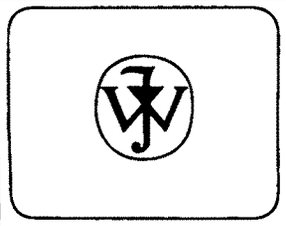
The organisation is logical, starting with the basic magnetic properties of solids and then turning to the common ferromagnetic minerals, magnetite and haematite and their solid solutions with ilmenite and ulvospinel. This chapter certainly brings the reader up to date on the current view of the changes in parameters for synthetic solid solutions and pure minerals, but more information on the effects of impurities would have been useful. Only the two pages on pyrrhotite are not really adequate for a mineral that is extremely complex and not all that rare. Single and multi-domain theories are considered next, first in relation to grains of magnetite and then haematite. The outline and discussion tends to follow closely Stacey's ideas, originally published in 1963, as does the subsequent treatment of the effects of time and temperature.

The next chapter is devoted to the remanence of sediments and is followed by a discussion of chemical remanence that is largely confined to exsolution behaviour as titanomagnetite solid solutions are oxidised. The effect of anhysteritic magnetisation and stress fields then receive considerable, warranted attention. The latter is, however, concerned almost entirely with stress effects on the direction of remanence and does not consider the converse, that is using magnetic anisotropy as a means of determining strain and hence evaluating past stress fields. The evidence for reversals of the geomagnetic field and possible self-reversal mechanisms are considered in rather a generalised way; the correlation between petrology and polarity that has been established in some regions is dismissed as being "paradoxical" in only four lines.

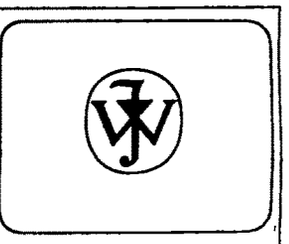
Finally extraterrestrial magnetisation is discussed and the remanence of meteorites is attributed to strong solar fields when the Sun passed through a T-Tauri stage. Strangely no mention is made of the magnetic properties of lunar rocks—presumably to avoid being overtaken by more recent findings.

The book is well written, adequately illustrated and its production is up to the usual high Elsevier standard. The only significant weakness is really in terms of the magnetisation of sedimentary rocks which are discussed in less than 5% of the total book, yet more and more sedimentary rock types are now being investigated and the magnetic effects during cementation and the diagenetic changes of detrital grains are much more important than simple detrital magnetisation. A little more on rotational hysteresis and physical techniques for identifying both the composition and physical parameters of ferromagnetic particles in rocks would have been beneficial. The bibliography is really a reference list that effectively only goes up to 1971 (only six references for 1972 with one "in press" and one "in preparation" for 1973). The authors defend their continued use of c.g.s. units, although the use of MKS can only come about when the textbooks adopt the system. These, however, are essentially quibbles about a book which must form part of any geophysics library.

D. H. TARLING



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1974, 254 pages, Dfl. 80.00 (about US$30.80)
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Cover picture

A three-spiral figure in the rear recess of the central burial chamber at Newgrange, a passage grave in a Neolithic cemetery in County Meath, Ireland. The design of the roof of the grave suggests that those who built it, in about 3100 BC, had studied the cycles of the Sun. See page 517.

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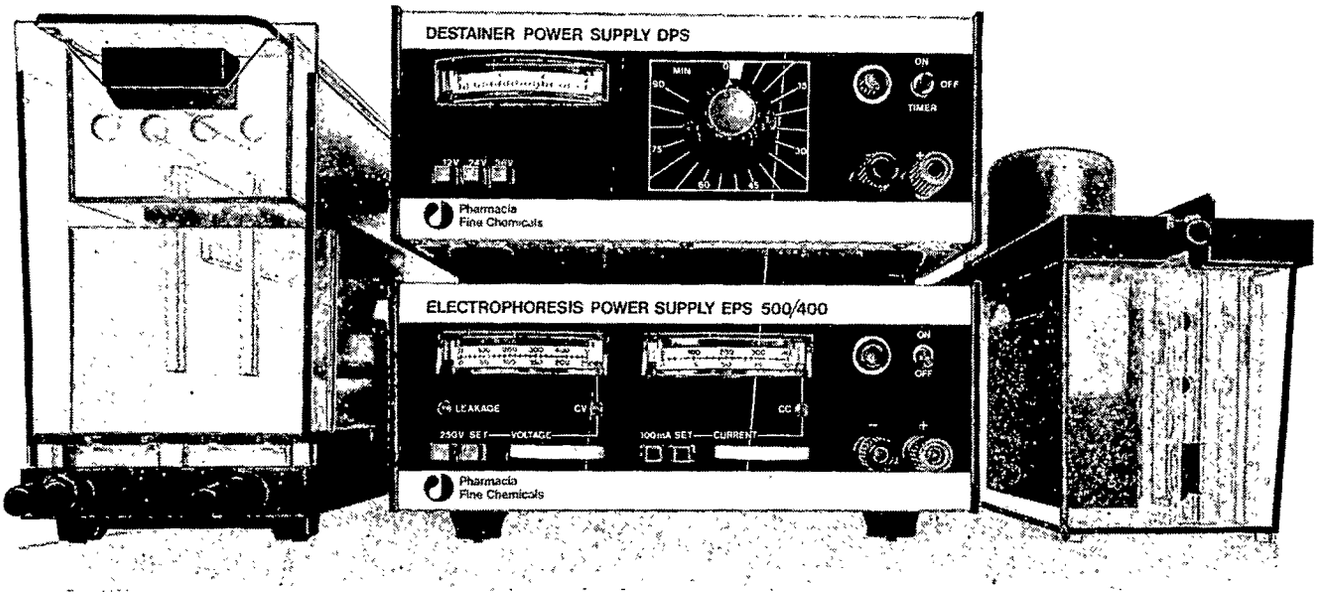
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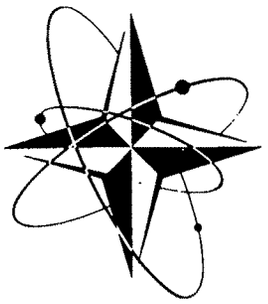
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Volume 249 June 7, 1974

Problem-solving PhDs

ALTHOUGH the disillusionment of industry with at least some aspects of the present PhD system has been highlighted this year by the report of the House of Commons Expenditure Committee on postgraduate education, declarations on the need for change were made by academics and industrialists on the Swann Committee as long ago as 1968. That committee looked into the whole question of the flow of qualified personnel into employment and roundly stated that the universities should "experiment boldly" with the PhD and with the regulations governing its award.

Academics protest, quite rightly, that the PhD is not only a training in methods of research but also a means of preserving the continuity of university research—the aim of which is to generate new knowledge. That said, the question is whether too many 'conventional' PhDs are being produced. The fact is that of some 4,600 scientists graduating with higher degrees in 1971, some 1,200 took up employment outside teaching and lecturing, and although many of these had master's degrees a substantial number had doctorates. It seems logical therefore that some PhD students should carry out postgraduate work that will fit them for their eventual career in industry.

Changes along these lines have already been made by the Science Research Council (SRC). First, it has been running the so-called CASE scheme (Co-operative Awards in Science and Engineering). In 1972, 191 of these awards were made, out of a total of 2,220 new SRC research studentships. The SRC also offers postgraduate studentships to people who wish to do a postgraduate course after a spell in, for example, industry or teaching; 313 of these were taken up in 1972, for both research leading to a PhD and for advanced courses.

Like all collaborative schemes CASE has not been without its problems. The scheme requires joint supervision of the student by a member of the staff of the university concerned and a person from the collaborating institution, which following a relaxation in the rules may be a body like a hospital board or a local authority rather than specifically an industrial company. Trouble has arisen because the projects to be tackled have in some cases been badly or vaguely specified and often because of a lack of mutual understanding between the industrialist and the academic.

Yet another kind of doctorate that has emerged in the past few years is the interdisciplinary PhD. The idea arose from the discussions following the publication of the Swann report and involves a graduate, often, though not necessarily, a scientist, getting to grips with a real industrial problem which calls for an appreciation of economics, industrial relations and the like. The SRC and the Social Science Research Council jointly fund almost 100 interdisciplinary research studentships, about 30 of which are held at the University of Aston.

The University of Aston is also in the throes of preparing for the advent of "total technology" PhD courses. These are specifically for engineers and have many of the characteristics of the interdisciplinary higher degree scheme but with more formal course work. What the research student is required to do is to solve real engineering problems in a real company. The University of Aston is well placed to take advantage of this because it already has the central organisation that is required if projects for students are to be thought out properly before the student arrives. The SRC awarded the first six total technology studentships last year.

That is all very well, but what scope is there for the pure scientist who wants to do a 'nonconventional' PhD yet wants something different from the CASE scheme. One answer is that there should be more scope for research done in an industrial environment to form the basis of a university PhD thesis. Unfortunately some universities take the view that they are not in the business of externally examining for a PhD. They might do well to re-examine such an isolationist attitude.

Some may say that to extend the PhD concept too much is to reduce the degree to a certificate of ability to solve problems. But does this matter? Provided that those who are really cut out to do research in the true sense of the word still have a reasonable chance to do it the appearance of more doctors of problem solving (which is all some 'conventional' PhDs are at the moment anyway) seems to be much more of a benefit than a threat.

100 years ago

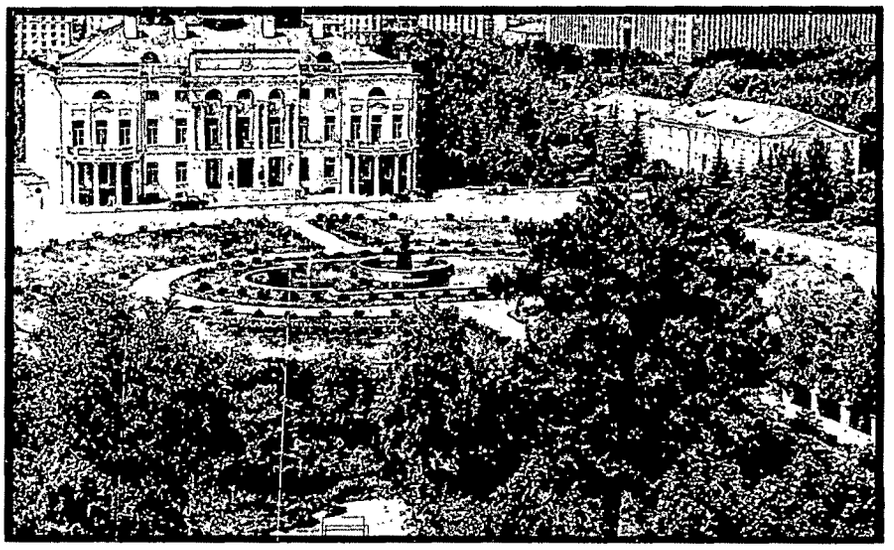


AN extract from a letter by Mr. Dunn, the geologist, now on a special exploring expedition to the Transvaal, published in the *Cape Argus* of May 5, gives a description of a thunder and hail-storm which he experienced at Pietermaritzburg, on April 17:—"Hail-stones, liberally mingled with great masses of ice of very irregular forms, poured down with great violence. The hail-stones were seldom less than 1 in. in diameter; the average was from 1½in. to 2 in. in diameter. These were of very regular spherical form, and consisted of a nucleus of white snow, with an envelope of hard transparent ice. Sometimes they presented, when broken through, a concentric arrangement of zones, alternately white and opaque and transparent. The irregular masses were formed of a nucleus generally longer in one direction than the others, from 2 in. to 4 in. in diameter; projecting all over were stalactites, each one about the thickness of a little finger, and presenting, when broken across, an agate-like structure, as though segregation had built them up. Of these masses I weighed a few with the following results:—Three weighed over 8 oz., two over 6 oz., and one over 4 oz. The mischief done will not be covered by 2,000*l.* or anything like that sum."

From *Nature*, 10, 115, June 11, 1874.

Russia's curtained window on the west

In the 250th anniversary year of the Russian Academy of Sciences, Vera Rich examines the rôle the Academy has played in international science.



The Academy's Moscow headquarters.

It can hardly be doubted that, in founding his Imperial Academy of Sciences, Peter the Great intended it to form one of the cornerstones of his Westernising policy—a consummation at the highest intellectual level of the European outlook that had driven him, earlier in his reign, to cut off his subjects' beards and to impose the death penalty on courtiers found sleeping with their boots on.

Yet Peter, who was personally curious about all branches of science and technology from the theory of navigation to do-it-yourself dentistry, having, after more than 20 years' cogitation, finally signed the decree "establishing" the Academy, on January 28, 1764, did not live to attend its opening. Exactly a year later, he died, succumbing to a chill caught, not in the pursuit of his innovations, but as a result of attending the traditional Epiphany Eve blessing of the waters! It was left to his widow and successor, the erstwhile illiterate Livonian peasant-girl, who reigned as Catherine I, to bring into being Peter's Academy, and it was on her nameday, December 27, 1725, that the inaugural meeting was held.

Although he is reputed never to have opened a book (and it must be said in passing that the improbably tall stories surrounding his name are more than legion), Peter seems to have been giving serious thought to the foundation of some eminent scientific body to grace his projected new capital and "window on the west" as early as the 1690s. During his visit to England in 1698, he visited the Royal Society (January 27), Greenwich Observatory (March 9) and Oxford (April 8) where he was presented with a number of mathematical books.

Back in Russia, he founded a Naval Academy, Artillery, Engineering and Medical Schools, and Russia's first national museum, the *Kunstkamera*, in his new capital of St Petersburg, together with several printing presses using the simplified typography, which he had introduced in 1708. He also began a prolonged correspondence with Leibniz, who had organised the Berlin Academy of Sciences in 1700, on the possibility of establishing a similar institution in St Petersburg. Although neither Peter nor Leibniz were to see the Academy come to fruition, nevertheless, in 1725 the first meeting finally occurred, and with a membership mainly of foreign, especially German savants.

This foreign, or rather, international, membership, persisted through the first difficult years of the Academy's existence (it had no official constitution until 1747, nor any established source of income). Prominent Academicians of that time include such eminent names as Nicholas (II) and Daniel Bernouilli (elected 1725), Leonard Euler (1727) and Georg Wilhelm Steller (of 'sea-cow' fame) (1737). It was from Euler's time at the Academy that the famous problem of the 'seven bridges'—one of the early landmarks of topology—dates. In spite of this source of inspiration, Euler found the atmosphere of St Petersburg unconducive to the free exchange of ideas, and in 1741 he accepted an invitation from Frederick the Great to join the Berlin Academy. (Asked by the Dowager Queen why he could only, apparently, speak in monosyllables, Euler is reported to have replied that "I've just come from a country where, if you speak, you're hanged!") In 1785, however, on the invitation of Catherine II, Euler returned to St Petersburg, where, in the intervals of research, he was expected to produce such diversions for the court as a formal mathematical refutation of the 'atheistic' views of the visiting French philosopher Diderot. (Euler's tongue-in-cheek "proof": "Sir, $(a+b^n)/n=x$, hence God exists; reply!", astounded the court, discomfited the unmathematical Diderot—and is reported to have found a place in current theological treatise!)

In spite of such Imperial digressions, the Academy at last settled down to producing its own scientists and scholars. Among numerous lesser luminaries, one finds names such as Lomonosov (elected 1741), Lenz (1828), Ostrogradskii (1828) and Krylov (of the *Fables* (1841).

There was still, however, a strong international atmosphere, reflected specially in the honorary members, who included Réaumur (1737), Voltaire (1778), Linné (1754), Boscovitch (1759), d'Alembert (1764), Diderot (a reward, maybe for valour?) (1773), Lagrange (1776), Bode (1794) and Kant (1794). British honorary members included such well-known figures as Maskelyne (1776), Priestley (1780), Sir Joseph Banks (1780), Malthus (1826), Sir William Parry (1826), Sir Humphrey Davy (1826) and Faraday (1830). In the eighteenth century, the United States was represented by honorary members Benjamin Franklin (1789) and John Churchman of Philadelphia (1795).

Yet in spite of its international beginnings, its use of Latin, French or German as its medium of publication (the *Comptes Rendus* of the Academy was still appearing in French in the 1940s), and the continuing election of foreign scientists as honorary members, the international contacts of the Academy did not proceed smoothly and unbrokenly. The early years of the nine-



Peter the Great: intended the Academy to form one of the cornerstones of his Westernising policy—a consummation at the highest intellectual level of the European outlook that had driven him to cut off his subjects' beards and to impose the death penalty on courtiers found sleeping with their boots on.

teenth century showed the Academy fallen on hard times. This was partly as a result of the foundation of new universities, so that the Academy lost a certain amount of prestige, being no longer the *ions et origo* of all learning in Russia, partly this was due to the conditions of the Napoleonic wars. Further, under Paul I (1796–1801), the receipt of literature from abroad was forbidden and international contacts were broken off. The membership of the Academy was reduced to half its original strength—and a large proportion of the remaining members were superannuated veterans, no longer able to attend meetings!

Paul's successor, Alexander I, was keenly interested in education, founding Universities (or reconstituting those, Vilna and Dorpat, which he had gained in recent territorial acquisitions). But although the Academy gradually regained its old strength and vigour, the vexed question of censorship remained. Knowledge might, and did, flow out of Russia—it was considerably more difficult for it to flow, or even to trickle in. The nineteenth century witnessed a running battle between the Academy and Universities on the one hand and the Government on the other, on the right of learned bodies to import foreign literature, especially periodical literature, without inspection by the censors. (It should be noted that censorship was, at times, ludicrously rigid; on one notable occasion a mathematical treatise was refused publication since it was feared that the " . . . " of the formulae for infinite series denoted a lacuna into which some revolutionary or otherwise treasonable matter would be inserted by dissident minds.) Although from the beginning, the Academy and Universities had been able to import scientific works (indeed, without them, no learned institution would have ever been able to begin functioning), the dispute arose out of the definition of "scientific". The new constitution of Vilna University (1803) implies that the Universities are to censor themselves. The decree on censorship of 1828 permitted the Universities and the Academy to "receive books without inspection by the censorship", but they are obliged "every time to inform the censorship committee for foreign books what the books are, how many, and when and by what route they were sent".

Although even the natural sciences (with their suspect symbolism) were not exempt from import difficulties, the problem was particularly pressing with the 'philosophers', who needed to read current political journals. The restrictions seem to have been imposed somewhat intermittently, according to the prevailing political atmosphere. Thus in the 1840s for example, the Academy was able to receive the *Allgemeine Zeitung* and *Berliner Nachrichten* fairly freely. A decree of 1860, however, imposes somewhat narrow limitations: "The Imperial Academy of Sciences, the Pulkova Observatory and the Universities may enjoy the right to obtain through the post from abroad books and periodical literature of academic content in an open wrapper, without extension of this right to members of the Academy and Universities." And since the opinion of the censorship on what was, and was not academic, was extremely arbitrary, one may find in these restrictions at least a partial explanation of the many claims that Russian scientists have discovered or elaborated some principle "independently" of work done abroad. Although most of the more fantastic claims disappeared with the excesses of the personality cult, one still finds from time to time references, for example, *Pravda*, June 12, 1973, to Popov as the inventor of radio. Such parallelism may be attributable, at least in part, to these censorship difficulties—a Russian working in a given field might well have been completely unaware of what his opposite numbers abroad were doing. Yet, conversely, certain eminent nineteenth century Russian scholars were never to become Academicians, Lobachevskii spent his life in the backwater of Kazan' and Mendeleev was refused membership on account of his dangerously 'liberal' views. One way and another, the nineteenth century Academy lost much by governmental intervention.

From the leisured days of the beginnings of the Academy (one eighteenth century silhouette shows an open air meeting reminiscent of a *fête champêtre* rather than a learned discourse) to the earnest days of post-Revolutionary Petrograd seems a far cry, yet, according to an official report of 1927, one of the first thing that the new régime did was to restore the old international contacts. "In the last ten years, the international relations of the Academy have taken the following forms: (1) participation of the Academy in permanent international unions and associations of general scientific interest or of special disciplines; (2) joint scientific enterprises, such as expeditions, publications, etc.; (3) participation of the Academy in International and other meetings and congresses convened

abroad, and in the anniversaries of learned societies, scientific institutions or individual scholars; (4) scientific missions abroad, including the permanent work of our scholars in laboratories of other countries; (5) rational and consistent exchange of scientific publications; (6) exchange of collections and the dispatch abroad, on a basis of reciprocity, of collections, manuscripts, and rare editions; (7) awarding of prizes and honorary titles to foreign scholars and mutual invitations to lecture at higher education institutions or to take part in conferences on particular occasions and, finally, rational organisation of the acquisition abroad of publications, instruments, apparatus and all other scientific material, necessary for the Academy and its institutions".

To what extent this excellent programme was implemented it is difficult to judge—official reports of this kind always have a tendency to overstate achievements. In any case, the picture was due to change. With the Stalinist purges of the 1930s, all contact with the West became not only discouraged—they were positively dangerous for those involved. Scientists who had trained abroad (under the plans so recently praised), were particularly vulnerable. In the Academies of the Union Republics (now virtually satellites of the All-Union Academy) where the accusation of 'nationalism' or 'separatism' was always possible, the situation was particularly tense. The Kiev Academy, for example, had no less than 13 Secretaries between 1921 and 1938, all of whom were, in turn, arrested. The election of foreign honorary members and corresponding members to the Academy came to a sudden halt. In the immediate post-revolutionary period, no foreign members were elected: then one member was elected in 1922, 18 in 1924 (including Frederick Soddy), one in 1925, one in 1926, five in 1927, four in 1928, nine in 1929, three in 1930, two in 1931, six in 1932, one in 1933, and in 1934, the year in which the Academy formally moved its headquarters (with 250 wagonloads of equipment and more than 300 scientists) to Moscow, time was still found to elect five new foreign members (of whom Max Born was one).

Then comes a complete gap. No further foreign honorary members or corresponding members were elected until 1942, when five new names (including J. B. S. Haldane) appear in the records, presumably as a symbol of Soviet friendship towards the Allies.

As for the work produced by the Academy during the 1930s, in spite of the new site, increased subsidies, the foundation of new institutes, and all other material government help that a learned institution might well desire, the growth of what was later to be known as the 'personality cult', was not conducive to sustained and concentrated research. As Robert Conquest has observed, although the man in the street could, in conditions of peril, stop talking, the professors and academicians had to go on lecturing! With the constant attrition due to the disappearance of those whose views failed to coincide with the current official line, and the occasional endorsement of a new theory from Party headquarters, Soviet science was not noted for the free dissemination of ideas. It became inward-looking, and self-congratulatory. When the 220th anniversary of the Academy was celebrated in 1945 (the 'Catherine' date being chosen, since on the 'Peter' date, the Soviet Union was fully involved with her war effort) we find in the proceedings of the occasion that the history of science has been considerably revised!

In aerodynamics, Mendeleev anticipated Prandtl's views on hydrodynamic friction by some 30 years, and a certain Rykachev in 1871 forestalled Eiffel's experiments on the lift of an airscrew by 40 years. The Danish Bering was no longer the discoverer of the Strait which bears his name—he merely confirmed the the discovery made by "the Cossack Deinev" in 1648. The forces of "chemical constitution" (the nature of the chemical bond) were investigated by Butlerov in 1862—although it is somewhat naively observed that as with many other Russian scientists, "his work was not always duly appreciated abroad!"

In the 1950s the Academy made a determined effort to free itself from the tight controls which demanded an immediate economic outcome of all projects, and, in spite of conventional lip-service to successive five-year plans, does manage to achieve a considerable amount of basic research that has little immediate connection with the needs of the economy.

On the international scene, however, all is not entirely well. International co-operation does take place—but with an increased emphasis on co-operation within the Comecon block or on aid to the developing science of the third world. Specific agreements for co-operation between the Soviet Union and a specific Western country on a specific project or set of projects only underline the lack of a general atmosphere of free exchange. Soviet scientific journals flow out freely to the West (and agreements have been made for the production abroad of cover-to-cover translations which appear within a few months of the Russian originals). But western journals entering the Soviet Union do so in single copies, which are then photocopied and distributed to academic institutions with any doubtful matter replaced by back-material of a more innocuous nature (for example, advertisements). The campaign of September 1973 against Academician Sakharov stated that "To be a Soviet scientist means to be a patriot"—one must not bite the Party hand that subsidises one's research, by advocating too close a co-operation with the west. The position of Jewish scientists who wish to emigrate to Israel who are refused a visa to emigrate, but at the same time dismissed from their posts so that they can no longer exercise the talents which the state is supposedly conserving for its own use has raised new and bitter feelings abroad.

The inaugural address of 1725 spoke of bringing Russia into the world scientific community. But, no reference was made to the fact that the Royal Society, so admired by Peter, specifically excluded all considerations of the religious or political views of its members—nor, would it seem, has the Academy yet learned, in the 250 years of its existence, to view a scholar's scientific achievements apart from his personal philosophy. The cancellation of the international celebrations of the anniversary of the Academy, and its replacements by a programme of popular scientific lectures to the grass-roots of Soviet Society—the workers and peasants, is more, therefore, than a gesture of pique over recent criticisms abroad of the restrictions and lack of academic freedom imposed on certain intellectuals in the Soviet Union—rather is it a repetition once more, of a pattern of isolationism which runs through the history of the Academy, a firm closing, yet again, of what was to have been one of the most prominent casements of Peter's "window on the west".

Charter of the Academy.

PRAEFATIO.

LEges, quibus Academiam ſuam AVGVSTA PROTECTRIX regit, inter cetera ſalutariter ſtatuta, etiam id officii Academicis injungunt, ut, pro excolendis communi conſilio & cura ſcientiis, bina ſingulis hebdomadis vice omnes conveniant; alteri, ut ſive deſtinationes, ſive commentationes ſuas Sociorum judiciis exponant; alteri, ut communicatis invicem ſententiis, ſi quid de argumento diſquiſitioni expoſito utile vel

international news

Canada's reply to Indian nuclear explosion

David Spurgeon, Ottawa

CANADA has responded quickly and firmly to India's explosion of an underground nuclear device. External Affairs Minister Mitchell Sharp announced four days after the weekend's test that Canada has suspended all nuclear assistance and cooperation and recalled Atomic Energy of Canada Limited's resident representative.

Mr Sharp said that the Canadian government is very disturbed by the tests, in which a device of approximately 15 kilotons yield was reported to have been exploded at a depth of 100 metres. "For all intents and purposes", he told a press conference, "India now has developed the capability of producing a nuclear weapon".

Canada's concern has resulted from the likelihood that the plutonium used in the explosive device came from a reactor named CIRRUS that was a gift from Canada. Canada has also designed and helped to build and equip two electric power reactors at Rajasthan (Rapp I and Rapp II), and most of India's nuclear personnel have been trained in Canada.

Canada has long been fearful of India's nuclear intentions. Copies of correspondence between Prime Minister Pierre Trudeau and India's Prime Minister Indira Gandhi in October 1971, which were distributed at the press conference, reveal Canada's concern at that time for the prevention of proliferation of nuclear weapons.

"The use of Canadian supplied material, equipment and facilities in India, that is, at CIRRUS, Rapp I or Rapp II or fissile material from these reactors, for the development of a nuclear explosive device would inevitably call on our part for a reassessment of our nuclear cooperation arrangements with India, a position we would take with any other non-nuclear weapons state with which we had cooperation arrangements in the nuclear field", Mr Trudeau warned.

"My government", replied Mrs Gandhi, "reiterates its commitment to the provisions contained in the nuclear cooperation agreements between India and Canada to which your government is also committed. Our two governments have acted in conformity with these arrangements for the past several years. The obligations undertaken by our two governments are mutual and they cannot be unilaterally varied".

What appears to have let the Indians off the hook, at least in their own minds, is their interpretation of the underground device they exploded as a "peaceful use" of nuclear energy. This interpretation has never been shared by Canada, but it seems to have been India's all along. Canada signed the Nonproliferation Treaty but India refused on the grounds that it was "discriminatory".

Canada did win inspection provisions for the two Rapp power reactors by the International Atomic Energy Authority but there were no such provisions for the CIRRUS reactor. CIRRUS seems the most likely source of plutonium for the device. For other reasons: AECL officials say that the one Rapp reactor now running has not been operating long enough to produce sufficient plutonium for an explosive device. The Rapp reactors are of the Candu type, fuelled with natural uranium and they are not ideal for producing weapons grade material in any case.

The CIRRUS was initially fuelled by Canada but subsequent fuel was manufactured by the Indians themselves, according to Canadian officials. The Indian fuel was not subject to inspection. Thus the plutonium for the device could have come from the Indian fuel and have been separated out in India's reprocessing plant, which has been operating since 1964. In fact, press reports in India were said by a spokesman in Canada's Department of External Affairs to have quoted Indian officials as saying that this was the case.

Asked for his personal reaction to news of the explosion, J. L. Gray, President of AECL, said quite frankly "I was really quite surprised and very disappointed". Gray suggested that halting Canada's nuclear assistance at this point will have little effect on India's nuclear power programme. "It might delay them but it won't stop their programme," he said. One of the Canadian-built Rapp reactors is already running, the other is nearly finished and India is building four more of its own.

In future Canada will try to assure that there is no ambiguity about the meaning of the phrase "peaceful uses" in agreements with other countries. But it is not clear how that will be accomplished for Mr Trudeau's 1971 letter to Mrs Gandhi seemed clear enough. Mr Sharp said that Canada "made it clear in international discussions and in bilateral exchanges with India that the creation of a nuclear explosion for so-called peaceful purposes could not be considered as a peaceful purpose within the meaning of our cooperative arrangements".

India's action has also brought up the question of Canada's aid programme to that country in general. Mr Sharp said that Canada is conscious of the very large costs involved in the development of nuclear energy for peaceful purposes and appreciates the substantial resources needed for the development of explosive devices. "Canada does not intend to share the burden of relieving such costs" he said. Thus the Canadian government is not prepared to agree to any roll over of India's commercial debt to Canada which is largely related to her nuclear energy programme.

Apart from the danger of nuclear weapons proliferation, many Canadian officials have found it hard to understand the rationale of putting a high priority on an expensive nuclear device which has a questionable utility while such vital national problems as assuring an adequate food supply continue to go unresolved. Indian spokesmen said the device was needed to aid in developing new energy sources, but understood nuclear devices have not been notably successful in such uses, even in the United States.

Oil from Russia?

from our Soviet Correspondent

SPEAKING in London recently, Academician Vladimir A. Kirilin, Deputy Chairman of the Council of Ministers of the USSR and Chairman of its State Committee for Science and Technology, called for cooperation between Britain and the USSR over energy matters—both in nuclear energy and oil production.

The occasion was the third meeting of the Permanent UK/USSR Intergovernmental Commission for Cooperation in the fields of Applied Science, Technology, Trade and Economic Relations. Mr Peter Shore, Secretary of State for Trade led the United Kingdom delegation and pre-

sided; Academician Kirilin headed the Soviet delegation.

The meeting paid considerable attention to ways of implementing the recently signed (May 6, 1974) Ten Year Agreement on the Development of Economic, Scientific, Technological and Industrial Cooperation, studying, in particular, the cooperation in major technological projects on a compensation basis and the necessity for flexibility in planning individual cooperative ventures.

It was recommended that further exchanges should take place in the fields of crystal structure and macromolecules, astronomy, phase synchrotron radiation, plasma physics, hydrostatic extrusion, corrosion and tribology.

At the press conference after the meeting, Academician Kirilin said that cooperation in the field of breeder reactors "in which both countries have had notable achievements" could considerably reduce the cost of energy production.

As to oil production, he said that talks have begun with British Petroleum, to be continued in Moscow next month, to outline a possible scheme by which the United Kingdom would provide the plant and that in return the Soviet Union would deliver oil for BP "for we do not expect at he moment, the UK will deliver oil to the Soviet Union!". This, however, he stressed, is still in the planning stage, and a number of possible options for cooperation in this field lie open.

Speaking generally, Academician Kirilin stressed that the current round of talks and, indeed, the 10-year agreement could only bear their full fruit in an atmosphere of detente and cooperation, which, he averred was a fundamental tenet of Soviet foreign policy. This raised a number of questions about the rights of scientists to emigrate, and what proportion of the goods to be exchanged had originated in Labour Camps (the questioner was doubtless thinking of *The First Circle*). Academician Kirilin replied that there were no labour camps in the Soviet Union, and that because of semantic difficulties, this question could not be profitably discussed, that "almost all" scientists who wished to emigrate could do so—the exceptions being those concerned with national security, and that as for the Panovs (who as dancers could hardly fall into that category), he personally had never heard of them until he came to London, but he was sure their fate "would be dealt with fairly". At which point, in the cause detente and cooperation, Mr Shore intervened and brought the discussion back to the safer channels of mutual economic benefits and exchange.

After the conclusion of the official talks, the Soviet delegation began a programme of visits to laboratories and research establishments with which possible cooperation was envisaged. The first visit on the programme was to the fusion laboratories at Culham, where Academician Kirilin presented a paper read on his behalf by Dr John Chubb, on the present and future energy situation in the Soviet Union. His data seemed perhaps, a little on the optimistic side—fast-neutron reactors a practical possibility by 1985, and it was interesting to note that in spite of the importance which he places on cooperation in thermonuclear research as a source of cheap power in the future, he stressed the immediate emphasis in the Soviet Union on the coal industry, and recommended a rethinking of the advantages of coal to the West!

Archaeology in crisis

from a Correspondent

A BASIC reorganisation of archaeological excavation in Britain is heralded in a recent press release from the Department of the Environment. It scarcely hints, however, at the underlying problems created by the increase in rescue archaeology, and by the apparent failure of the Department of Education and Science to respond to the current archaeological crisis with the awareness shown by its sister Department.

Mr Crosland's announcement promises an increased grant of £1,063,000 for rescue excavation and post-excavation work in Great Britain during 1974–5. When compared with the grant of £440,000 for 1972–3, and £813,000 for 1973–74, this shows a continuing awareness of the widespread destruction which urban development and mechanised farming are wreaking upon what is left of Britain's past. For the evidence can be rescued only now, before it is gone for ever.

The accelerated growth of rescue archaeology in this country has created its own practical problems, however. There is a shortage of skilled archaeological fieldworkers for the senior positions, and especially of trained diggers—the equivalent, in a sense, of laboratory technicians in the natural sciences. Even so, there are now many hundreds of these diggers, working all the year round, replacing at least in part the amateur archaeologists who until a decade ago made up the entire work force on any summer dig. The emergence of what is in effect a new profession—of full-time digging archaeologists—requires some coordination in terms and conditions of employment, and trade union membership is now under active discussion (*The Times*, May 25, 1974). The formation of some sort of Association of Professional Archaeologists has now been proposed, which could propose and promote some unified career structure, serving the varied interests of archaeologists in museums, the universities, the civil service and the local authorities as well as the full-time diggers.

These logistic questions are less important, however, than the crucial one of using the annual rescue budget of £1 million to good effect. Hitherto this has been entirely the responsibility of the Ancient Monuments Inspectorate of the Department of the Environment —and its very title recalls that until a decade ago the Inspectorate's principal task was the maintenance of the ancient buildings and archaeological sites under its guardianship, and the protection from destruction of other sites listed as scheduled monuments. The explosive growth of rescue excavation, financed by the Department of the Environment, has put heavy new responsibilities upon its archaeological personnel.

The second, and ultimately more important part of the Minister's announcement takes account of this by setting up a number of advisory committees. The first is a new national advisory committee (a sub-committee of the Ancient Monuments Board, which advises the Secretary of State on the scheduling of Ancient Monuments) to advise on the priorities of archaeological excavation in England. In addition 13 advisory committees are to be set up on a regional basis to advise on local priorities for rescue excavation and archaeological survey where sites are threatened.

It remains to be seen how this structure of regional committees and a national committee will operate (and there has been immediate criticism that the regional committees are not to have direct representation on the national committee). But at least it goes some way to meet a fundamental point, which the urgency of rescue excavation and the expenditure of public money upon it both at national and local level, has sometimes served to obscure. The only purpose of rescue excavation, where the site itself is by definition doomed, is the provision of information about the past. All rescue excavation is research, aimed at providing new information to enlarge our knowledge and understanding of the post. Yet hitherto, as both the Council for British Archaeology, and Rescue, The Trust for British Archaeology have pointed out, there has been no coherent research strategy of any kind in this country. Sites have been dug simply because they were threatened with destruction. But while this is a necessary condition for the expenditure of public funds, it is certainly not a sufficient one, for even the increased funds allow for the complete excavation of only a tiny fraction of the sites threatened. In general, in-

deed, there is too much hasty digging and not enough thinking, resulting in the production of large quantities of data without any clear idea of just what prolems it is supposed to solve. Moreover the total failure of publication to keep up with the increase in excavation has recently been stressed (Addyman, *Rescue News*, **6**, 1; 1974), nor can all the publications which appear be rated as significant contributions to our knowledge of the past. These are problems which the new advisory committees must now tackle urgently if the increase in rescue excavation is to be in any way effective in counteracting the loss of evidence by development and destruction. The task now is to make rescue research more effective in terms of significant results and their rapid publication.

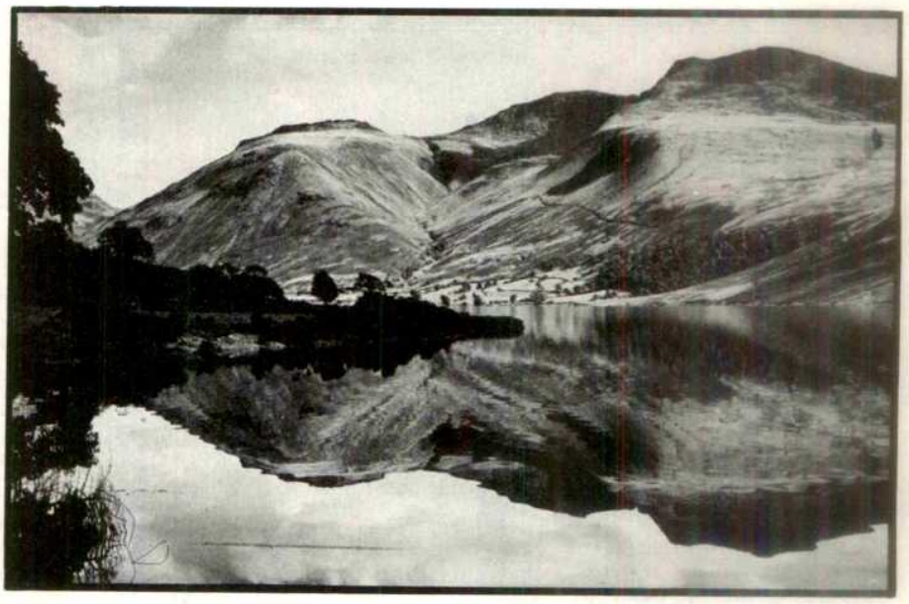
And it is here that the Department of Education and Science has so far failed for, unlike the Department of the Environment, it actually has a formal public commitment to scientific research. Now, archaeological excavation under the often hasty conditions implied in rescue work, does not offer the ideal laboratory for the development of new techniques and the investigation of entirely new approaches to the past which are essential for real progress. Yet archaeological research is considered as outside the field of interest of each of the three research councils (SRC, SSRC and NERC) which might be expected to support it, to the extent that the only way an archaeological research project can find support from them is to masquerade as something else and lodge itself in a department of physics or geology. The British Academy, to be sure, makes a number of small grants, but these are distributed over the whole field of the humanities. Some time ago the British Academy and the Royal Society held joint discussions on this curious state of affairs. It is believed that they resolved jointly to approach the Department of Education and Science with a view to the institution of some body, analogous to the SRC or to NERC, by which scientific research in archaeology could be supported. But so far nothing more has been heard. So we have the paradox that rescue excavation is supported by the government on an increased scale, reflecting real awareness of the archaeological crisis, while the further scientific research which should exploit this great opportunity by analysing existing data and by developing new techniques, is simply not taking place. Archaeological research in Britain is thus in a state of considerable confusion. Mr Crosland's advisory committees will have to work long and think clearly if the funds for rescue excavation are to yield commensurate scientific and historical rewards.

The future of National Parks

Peter J. Smith

ACCORDING to the late John Dower, a National Park in the British context was to be "an extensive area of beautiful and relatively wild country in which . . . . (a) the characteristic landscape beauty is strictly preserved, (b) access and facilities for public open-air enjoyment are amply provided, (c) wildlife and buildings and places of architectural and historic interest are suitably protected, while (d) established farming use is effectively maintained". The precise wording of the National Parks and Access to the Countryside Act 1949, under which the ten National Parks of England and Wales were later created, was slightly different that they were to be designated areas in which an attempt would be made by careful control to avoid radical change in either natural or man-made characteristics whilst encouraging greater recreational use.

Twenty-five years later the result, now examined by a committee under Lord Sandford (*Report of the National Park Policies Review Committee*, HMSO, London, 1974), can be seen to be mixed. There is no doubt that the National Parks have managed to maintain some distinction and are generally held in high public regard, but at the same time they have been eroded to a much greater extent than their creators envisaged or would have desired. Part of the conflict between the ideal and the reality has arisen because, rightly or wrongly, the 1949 Act made no attempt to change the ownership of the land within the designated areas, and so total responsibility for each park has never been in the hands of a single administration. This lack of complete control by park authorities has been particularly unfortunate where it relates to the use of land previously established for farming.



The Scafells across Wastwater : inviolate?

As Sandford points out, the scenic quality of the man-made part of the environment in National Parks is due primarily to the grazing of sheep and cattle, which maintains the characteristic open landscapes. But large scale afforestation of open land with alien conifers has taken place as a result of national economic decisions—a development which park authorities have been largely powerless to prevent, and heather moorland and other rough grazing have been converted to more productive pasture altering the open character of the country and impeding access. The political difficulty is that in the last resort the park authorities have little power over the activities of private landlords. Sandford therefore recommends that where mutual agreement on land use proves impossible, the authorities should have the power of compulsory purchase, and that afforestation in particular should be subject to planning controls.

As for development, parks have generally been able to avoid excessive residential and small-scale commercial development but there has been what many people take to be a deplorable amount of large-scale development, generally under pressure from central government and supposedly "in the national interest". Sandford's view is that because the National Parks account for 9% (over 3.3 million acres) of the area of England and Wales, and contain important mineral and water resources, it would be unrealistic to hold them inviolate against such development. At the same time, the

report acknowledges that "in the past amenity values appear to have been set aside too easily", and suggests that in future there should be a strong presumption against development which conflicts with park purposes.

Some of the parks' more obvious difficulties have arisen from the inherent conflict between preservation, on the one hand, and public enjoyment of the preserved facilities, on the other; the ever-increasing number of visitors has begun to threaten the very characteristics which attract the visitors in the first place. Road alterations have been carried out with no overall scheme in mind and in ways more appropriate to the urban than the rural environment; cars, coaches and caravans have been allowed to penetrate 'sensitive' areas; and roadside walls and verges have suffered from over-heavy vehicles.

The general problem here as Sandford sees it is that both park authorities and the government have been slow to react to changing circumstances; they have failed to come to terms with the fact that, although the walkers and climbers in the forefront of thinking during the 1940s are now more numerous than ever, the drivers have multiplied at an even higher rate. What, specifically, should be done? The Sandford recipe includes the routing of new trunk roads around the National Parks, the abandonment of any attempt to maintain uncongested traffic flow within the parks by means of new road development, refusal to permit alterations in existing road width or alignment, encouragement of peripheral parking linked to public transport inside the parks, and the resiting and screening of prominent caravan sites. Of course, it is one thing to identify sensible solutions, but quite another to carry them out—and the Sandford committee goes little into detailed implementation. On the other hand, there can be no doubt that the basic principle is sound and worth restating—that where the preservation and enhancement of beauty conflict acutely with the promotion of public enjoyment, the former must prevail.

Finally, the report suggests that there should be designated "national heritage areas" in which "the conservation of environmental qualities would be the supreme objective . . . . taking precedence over all others". There would be no settlement larger than a hamlet, no industrial development, no quarrying or mining, no reservoirs or defence works, no main roads or railways, no facilities for visitors, and visitor access would be limited to foot, bicycle and horse. This is the one genuinely radical suggestion in the whole report, although it is actually a minority proposal from four members of the committee. It deserves the widest debate, for this may be the last available opportunity for conserving "areas of the highest environmental quality".

correspondence

Chilean symposium

SIR,—We are scientists who firmly hold political opinions which differ from one to another. We are not concerned to try and understand these differences. We are, however, convinced that every scientist (like every nonscientist, indeed) has the right to hold what ideas he likes and to maintain them within his own country in the manner which he deems best.

We are similariy convinced that all political matters should be excluded from international scientific associations. Officialdom, no more than the organisers of, or the participants in international meetings, should neither impose nor allow any mixing of science and politics.

This is why we object to the following phrase "the re-establishment of social peace and internal order guarantees and secures to carry out this scientific event" in the invitation emanating from the Chilean section of the International Association of Volcanology and Chemistry of the Earth's Interior to attend a symposium in Santiago.

We cannot accept that a regime which has covered its large and unhappy country with blood, and which still continues to permit torture and political assassinations, should be permitted to include such a phrase in its circular of invitation to foreign scientists.

Needless to say, we do not propose to participate in this symposium and are of the opinion that a similar attitude should be adopted by any volcanologist sensible of the terrible Chilean tragedy, since his very presence there might tacitly imply tolerance from the human point of view.

Yours faithfully,

FR. BARBERI

Pisa

E. LOCARDI

Rome

G. MARINELLI

Pisa

M. MITTEMPERGER

Rome

A. RITTMANN

Catania

H. TAZIEFF

Paris

J. VARET

Paris

Spilt milk

SIR, — John Hall rightly adduces several arguments against the change from breast feeding to bottle feeding which is apparent in less developed countries. But his description of the character of the "Baby Killer", bottle feeding, is incomplete as it does not mention an effect of bottle feeding that will probably kill even more babies than the associated infections, malnutrition and poverty do. Lactation postpones postpartum ovulation and therefore contributes to birth spacing. It has been shown that prolonged lactation is indeed an important factor in controlling population growth. Populations in less developed countries that are changing from breast feeding to bottle feeding are likely to be insufficiently familiar with other means of family planning to compensate for this growth mechanism. I know of a case where all the female patients in a rural East African hospital were given a bra as a Christmas present, though family planning programmes in that area had not yet yielded any obvious result and the women concerned had hardly ever shown interest in bras. Whether due to well-intentioned grafting of our breast-centred culture into less developed cultures or due to deliberate promotion of dairy and bra industries, such behaviour is likely to add to baby killing population pressures. We should be aware of such incipient trends disturbing the balance between mortality and birth rates in less developed countries in addition to those already caused by Western medicine and technology.

Yours faithfully,

J. WIND

Institute of Human Genetics, Free University of Amsterdam.

news and views

Gene expression in development

DIFFERENTIAL gene transcription is often put forward as the major source of control for protein synthesis during differentiation and development. But there are other possibilities such as nuclear processing and selection of messenger RNA for transport as well as various forms of translational control. It is clear that gene expression has a quantitative and a qualitative aspect since highly specialised cells such as reticulocytes, lymphocytes, silk gland and lens cells all produce a large amount of a few polypeptide chains and a small amount of a much larger number of proteins. Greater rates of transcription from genes appropriate to the end cell line may account for the quantitative differences in protein synthesis observed in these cells, although other possibilities such as differential mRNA stability, for which there is some evidence (Kafatos, *Fifth Karolinska Symposium*, 319; 1972) are not rigorously excluded.

Presumably gene expression also has a qualitative aspect but examples are harder to find and interpret since the answers obtained depend on the level of discrimination set by the techniques used in the analysis. Nucleic acid hybridisation procedures have recently provided evidence in a variety of systems for qualitative differences between RNAs extracted from different adult organs of the mouse (Brown and Church, *Devl Biol.*, **29,** 73; 1972); Grouse, Chilton and McCarthy, *Biochemistry*, **11,** 798; 1972) and different developmental stages of the slime mould *Dictyostelium* (Firtel, *J. molec. Biol.*, **66,** 363; 1972). These workers have used purified labelled unique DNA sequences in nucleic acid hybridisation reactions with RNA in excess to investigate the proportion of unique DNA sequences represented by RNA transcripts in any given RNA preparation. Either total cell or total nuclear RNA preparations have been used to drive the hybridisation reactions. To compare two RNA preparations they are hybridised separately and together under identical conditions and the amount of labelled unique DNA found in RNA/DNA hybrids can then be determined. From the results it is possible to compute the sequences common to both and also those present in one but not the other. For instance, in *Dictyostelium*, Firtel has shown that about 20% of the genome is represented in RNA at all stages of development and about 37% consists of sequences expressed at particular developmental stages. These studies provide evidence for qualitative changes in gene transcription during developmental and differentiation, but do not throw light on the question of qualitative differences between nuclear and cytoplasmic unique gene transcripts.

Two recent articles (Smith, Hough, Chamberlin and Davidson, *J. molec. Biol.*, **85,** 103; 1974; Galau, Britten and Davidson, *Cell*, **2,** 41; 1974) suggest in a developmental situation that the total number of diverse unique DNA sequence transcripts in nuclear RNA is ten times greater than in mRNA from polysomes. Using sea urchin embryos at the gastrula stage of development Smith *et al.* isolated heterogeneous nuclear RNA (hnRNA) labelled by a 10 min pulse of ^{3}H-uridine. The hnRNA was fractionated in non-denaturing sucrose gradients to give three fractions sedimenting less than 23S, between 23 and 40S and greater than 40S. The greater than 40S fraction was used for hybridisation experiments with unlabelled sheared total DNA in excess under various conditions.

First the amount of hnRNA hybridising to DNA by C_0t 40 was determined at two DNA/RNA ratios. These conditions represent an assay for repetitive sequence transcripts since by C_0t 40 75% of repetitive DNA sequences but less than 5% of single copy DNA should have reannealed. About 25% of total ^{3}H-labelled hnRNA is recovered in hybrids separated from non-hybridised RNA on urea hydroxyapatite columns. Treatment with ribonuclease before recovery of hybrids reduces this value to 5–8% of total ^{3}H-hnRNA, suggesting that one-fifth to one-third of the hnRNA hybridised by C_0t 40 is in DNA/RNA duplex and the rest is in unhybridised RNA tails which are removable by ribonuclease treatment. RNA size was determined before (3,000 nucleotides) and after (1,100 nucleotides) hybridisation suggesting that during hybridisation and subsequent isolation of hybrids the RNA is cut on average once or twice per molecule. Assuming the cuts are random and that there is no more than one repetitive element per bound fragment, then about 70% of the hnRNA molecules entering the reaction should contain one such sequence. If on the other hand some bound fragments were composed wholly of repetitive sequences and others contained smaller than average sized repetitive sequences this value will be lower but was calculated to be not less than 50%. Other interpretations, such as 75% of molecules composed of non-repetitive sequences and 25% composed of intermingled repetitive and non-repetitive elements, do not, however, seem to have been excluded.

Another point of interest is that the average size of repetitive and non-repetitive sequences in the hnRNA C_0t 40 hybrids can be computed from the average length of hybridised molecules (1,100 nucleotides) and the proportion of hybrid which is resistant to ribonuclease (20–35%). Repetitive sequences in sea urchin gastrula hnRNA are therefore about 300 nucleotides long, a figure which is in good agreement with the average length of interspersed repetitive sequences found in sea urchin (Graham *et al.*, *Cell*, **1,** 127; 1974) and *Xenopus* DNA (Davidson *et al.*, *J. molec. Biol.*, **77,** 1; 1973).

Smith *et al.* next investigated the hybridisation of hnRNA to non-repetitive DNA sequences by allowing the hybridisation reaction to proceed beyond C_0t 40 and up to C_0t 4,000 at which point about 47% of the ^{3}H-labelled hnRNA is in hybrid; this value can be increased to 65–70% at very high DNA to RNA ratios. It is clear from the shape of the C_0t curve that a large proportion of hnRNA molecules contain sequences transcribed from non-repetitive DNA. Although the detailed interpretation of these results suffers from some uncertainties—mainly to do with possible variation in specific activity of the reacting species—the suggestion is that a few unique sequences are present in hnRNA in much greater amounts than others, since only at very high DNA/RNA ratios are there enough sites provided for most of the RNA to hybridise. Most unique sequences in hnRNA are present in relatively few copies since there is essentially no increase in hybridisation at C_0t 4,000 from a DNA:RNA ratio of 30 up to 15,000. This means that enough single copy DNA sequence is present at the lower ratio to hybridise the bulk of the sequence complexity in hnRNA. Although it is not possible directly to calculate non-repetitive sequence

complexity from these results (RNA-driven reactions are required for this), a rough minimum estimate of 10% of the unique sequence of the total genome is obtained. Preliminary results using RNA-driven hybridisation reactions indicate a value two or three times greater.

In order to compare the genetic information in hnRNA with that in polysome-bound mRNA it is necessary to estimate the proportion of unique DNA sequence complexity present in cytoplasmic mRNA. Galau *et al.* have done this for sea urchin gastrulae using RNA-driven hybridisation reactions with ^{3}H-labelled purified non-repetitive DNA as the probe. First they isolated polysome-bound RNA by procedures previously shown to extract most of the cytoplasmic mRNA essentially free of hnRNA although containing substantial amounts of ribosomal RNA (Goldberg, Galau, Britten and Davidson, *Proc. natn. Acad. Sci. U.S.A.*, **70,** 3516; 1973). This was hybridised with ^{3}H-labelled non-repetitive DNA in 3–40 fold excess RNA for various lengths of time to give RNA C_0ts up to 60,000. Since DNA renaturation is not prevented subsequent analysis distinguished between DNA/DNA and DNA/RNA duplex. It was found with four different RNA preparations that a maximum of 1% of the total ^{3}H-DNA enters DNA/RNA hybrids. Proof that termination of the hybridisation reaction is due to exhaustion of DNA sequences complementary to mRNA was provided by reisolating unreacted ^{3}H-DNA and rehybridising it to a further sample of RNA. Only 0.02% formed new hybrid although when annealed with excess cold DNA most of it would still enter DNA/DNA duplex. The non-repetitive DNA probe contained as contamination a small amount of repetitive sequence and proof that the DNA in the RNA/DNA hybrid is non-repetitive DNA was given by reisolating the DNA from the hybrids and reacting it with an excess of sheared total DNA, when it renatured with the kinetics expected of unique sequence DNA. From these data in a calculation which allows for asymmetric transcription and the measured proportion of DNA unavailable for hybridisation, Galau, Britten and Davidson conclude that 2.7% of the total single copy DNA sequence is represented by RNA transcripts in gastrula polysomes. Assuming a number-average length for mRNA of 1,200 nucleotides, this means that about 14,000 different mRNAs are present at this stage of development. It was calculated that only about 8% of the mRNA (called the complex class) drives the hybridisation reaction and contains 90–95% of the total sequence complexity. The rest (called the prevalent class) contains only 5–10% of the sequence complexity since no early low RNA C_0t transition was seen during the course of the reaction. From these figures, together with estimates for the total amount of mRNA in gastrula polysomes, the average number of complex class mRNA molecules per gene at any one time was calculated at 340; for the prevalent class of mRNA this figure must be two orders of magnitude greater.

It is clear from the results of these two papers that the proportion of the unique DNA transcribed into hnRNA is at least four times and maybe ten times greater than the proportion in active polysomes of sea urchin gastrulae. There is considerable debate at present over the possible role of hnRNA as a nuclear precursor to mRNA. If it is, these results suggest that 75–90% of diverse unique sequence transcripts present in nuclear RNA do not reach the cytoplasm, that is processing restricts qualitatively with respect to sequence the mRNA appearing in the cytoplasm.

This result is particularly interesting as it provides evidence for control of gene expression at a level beyond transcription. But before this interpretation can be accepted it is important to ascertain what proportion of the unique DNA sequences code for protein, and also that the unique sequence transcripts present in hnRNA but absent from the cytoplasm are representative of sequences which do code for proteins and do not have some other function.

PETER J. FORD

Einstein in crisis?

IN spite of the great aesthetic and philosophical appeal of Einstein's general theory of relativity, it is still, after 50 years of widespread acceptance, one of the least well-founded theories in physics as far as experimental confirmation is concerned. Of the three classic tests of the theory, the most impressive concerns the motion of the planet Mercury. In the nineteenth century it was known to Leverrier that the perihelian of Mercury's orbit precessed around the Sun at a calculated rate of 43 arc seconds a century. Such a deviation from a perfect elliptical orbit, although very small, was none the less a significant enough departure from Newton's theory of gravitation to lead to the search for an undiscovered planet (which even had a name—Vulcan) which supposedly lay within the orbit of Mercury, and was responsible for perturbing its motion.

Vulcan was not found, and in 1915 Einstein published his theory of relativity which beautifully accounted for the discrepancy in Mercury's motion. This success must be regarded as the backbone of observational support for the general theory of relativity, so that any questioning of the result must be regarded with the gravest concern. To question the result is just what Dicke of Princeton University has been doing for some years, by directing his attention to the shape of the Sun. As a result of a long series of observations, Dicke claims to have detected a solar oblateness (a difference between the polar and equatorial radii) of $(4.51 \pm 0.34) \times 10^{-3}$%. The details of the observations, together with a tentative theory of the solar interior, are published in a recent issue of *Science* (**184,** 419–429; 1974).

Dicke interprets the solar oblateness in terms of an uneven distribution of mass in the Sun's interior, leading to a small but significant quadrupole moment of $(2.47 \pm 0.23) \times 10^{-5}$. The presence of such a quadrupole moment would have a profound effect on the motion of Mercury; it would in fact reduce the perihelian precession by three arc seconds a century—a discrepancy of 7% from the relativity result.

Under these circumstances there are always a number of alternative theories of gravity waiting in the wings, and it is no coincidence that one of the more serious contenders also comes from Dicke. Known as a scalar–tensor theory, it is based on the idea that as well as the usual gravitational field which surrounds a massive object, there is an additional (scalar) field which adds a fraction, for example, s, to a body's weight. The value of s is not determined by the theory, but is generally held to be about 15–20%. Calculations using the scalar–tensor theory show that Mercury's perihelian would still precess, but at a somewhat reduced rate. If s is about 15%, the reduction is around four seconds of arc a century. Not surprisingly, Dicke believes that the scalar–tensor theory is in better agreement with the observations than is Einstein's theory, provided that the solar oblateness interpretation is accepted as well.

It is unlikely that a large oblateness would be believed by astronomers unless there were a fairly good theory of stellar structure to go with it, and Dicke outlines such a theory in his *Science* article. One likely explanation for a quadrupole moment is the possibility that the interior of the Sun might be rotating faster than the surface. To obtain an effect of the right magnitude, it is necessary to have a solar core rotating at 20 times the surface value.

It is thus necessary to account for the relatively slow rotation of the surface layers. Interestingly, there is a ready made explanation available, provided by the 'solar wind'. This consists of streams of high velocity charged particles continually shooting out from the surface of the Sun. Such particles would couple strongly to the Sun itself through any magnetic field present, and would have the effect of 'braking' the relatively tenuous outer surface of the Sun as the field twists up because of rotation.

Needless to say, the presence in the solar interior of a high differential rate of rotation raises deep problems concerning stability. Nevertheless, observations of the rotation rates of other stars slightly more massive than the Sun indicate that young stars do indeed rotate about 20 times faster than the surface of the Sun, but that this rate falls off progressively as the star ages, presumably as a result of magnetic braking. The crucial question though, is whether the interiors of the older stars (such as the Sun) are still spinning at a very high rate. Extraordinary evidence that this might be so is provided from an unlikely source.

In order that the solar surface continues to rotate under the breaking action of the solar wind, there must be a coupling between the rapidly spinning core and the surface, to provide a mechanism for the transfer of angular momentum. One such mechanism, involving turbulent diffusion of the solar fluid, carries angular momentum upwards, but also carries lithium downwards. Lithium is produced in certain quantities in all stars, but in the Sun there is apparently a depletion by a factor of 200 from the expected abundance near the surface. Dicke advances this as evidence of a rapidly rotating core.

These provocative ideas of Dicke have caused a great deal of controversy in recent years. Nevertheless, at this time, he feels that criticisms have been well met, and that Einstein's theory faces a very real crisis. Naturally, the case cannot be decided on the basis of solar oblateness arguments alone. The complexities of stellar structure, and our ignorance concerning the condition of the Sun below its visible surface layers, preclude such sweeping conclusions. One other possibility of discriminating between the scalar–tensor theory and general relativity concerns the value of the Newtonian gravitational constant G, which in the former theory is actually a time-dependent quantity. The extremely slow variation of G predicted by Dicke may soon be accessible to observation using radar-ranging techniques to measure the motions of the nearby planets. The importance of the issue at stake should lead to observations like these being vigourously pursued in the next few years.

P. C. W. DAVIES

Does the geomagnetic field affect climate?

ALTHOUGH the suggestion that there may be a relationship between climatic changes and variations in the Earth's magnetic field was first made more than 20 years ago, convincing correlations between the two have been discovered only much more recently. The surprise is not that it took so long but that it ever happened at all. For what possible connection could there be between phenomena so obviously disparate? Yet the correlations exist; and although a direct causal relationship may be genuinely doubted, the fact that climate and geomagnetic field are linked at all raises the need to identify the intermediate factors by which the correlations come about.

Apparently for historical reasons, some workers have drawn significance from the fact that both climate and magnetism seem to be related to fluctuations in atmospheric radiocarbon activity. De Vries (*Proc. K. ned. Akad. Wet.*, **B61**, 94; 1958) seems to have been the first to propose a correlation between radiocarbon activity and climatic changes, in which he was followed ten years later by Damon (*Met. Monogr.*, **8**, 151; 1968); but by Damon's time Elsasser *et al.* (*Nature*, **178**, 1226; 1956) had already pointed out that radiocarbon activity was likely to be influenced by changes in the magnetic field. The argument here is that the cosmic radiation entering the atmosphere to generate the radiocarbon will be reduced if the strength of the geomagnetic field is increased, for the field has a shielding effect—and this phenomenon has received support from many subsequent workers. So variations in the intensity of the Earth's magnetic field fluctuations in atmospheric radiocarbon activity and climatic changes are apparently correlated for the past 7,000 yr, and there is a clearly understood relationship between the magnetic field and radiocarbon activity. Unfortunately, however, all this does nothing to explain just how and why climate comes to be involved.

More recently, important, albeit phenomenological, light has been thrown on the climate connection by the work of Wollin and his colleagues at the Lamont-Doherty Geological Observatory. Wollin *et al.* (*Earth planet. Sci. Lett.*, **12**, 175; 1971), for example, examined deep sea sedimentary cores for the past 470,000 yr and found that changes in the intensity of magnetisation and the palaeomagnetic inclination of the sediments could be correlated with the climatic changes in the same sediments as represented by variations in, for example, the abundance of *Globorotalia menardii* and the coiling direction of *G. truncatulinoides*. As a result, they tentatively concluded that a "cause and effect relationship links changes of the Earth's magnetic field and climate" (for example, that higher magnetic intensity produces, or is produced by, colder climate). They also suggested that "it is possible that magnetism may modulate climate to some degree by the shielding effect against solar corpuscular radiation provided by the Earth's magnetic field"—thus invoking a clear, if perhaps forced, analogy with the phenomenon known to affect cosmic rays. Later, Wollin *et al.* (*Nature*, **232**, 549; 1971) used other deep sea cores to extend the correlation between magnetisation variations and climatic changes back to 1.2 Myr.

The best way of summarising the work of Wollin and his colleagues on deep sea sediments would probably be to say that the correlations themselves are convincing whereas the explanation is not, which is why Amerigian (*Earth planet. Sci. Lett.*, **21**, 321; 1974) has sought to produce something "simple and less astonishing". Amerigian's basic point is that at least equally feasible causes of the observed correlations may be invoked, based on seafloor dynamic processes already known and known to be climatically variable. For example, one of the principal factors affecting the intensity of magnetisation of sediments is the intensity of magnetisation of the particles which settle to form the sediments in the first place, and this in turn depends on composition. Most of the cores examined by Wollin *et al.* were high in organic calcium carbonate and, as Amerigian shows in a scatter plot, the greater the proportion of essentially non-magnetic calcium carbonate present in a sediment the lower (statistically) is the sediment's intensity of magnetisation. In other words, calcium carbonate production, which is climatically controlled, can affect intensity of magnetisation in a way which is completely unrelated to the strength of the geomagnetic field. Under such circumstances it would clearly be unwise to suppose that a correlation between intensity of magnetisation and (other) climatic indicators necessarily implies one between geomagnetic field intensity and climate.

The second important factor influencing the intensity of magnetisation of sediments is the efficiency of the process by which the settling particles are aligned into the prevailing magnetic field direction, and this in turn is related not only to the strength of the field in which the deposition takes place but also to the grain size distribution of the magnetic material involved. Materials with different grain size distributions may settle out into the ambient field direction with different efficiencies (that is, the proportion of material attaining a magnetically controlled orientation will vary) depending on the current velocity. Thus if the bottom current velocity is related to climate, the intensity of magnetisation of the sediment will be partly related to climate. Again, then, it does not necessarily follow that a

correlation between intensity of magnetisation and climatic indicators means that the latter correlate with geomagnetic field strength.

Amerigian then goes on to show that the reported correlations between palaeomagnetic inclination and climatic indicators can result from variable inclination errors introduced by bottom-current velocity changes which are also climatically controlled. He does recognise, however, that his various mechanisms are only valid if the magnetisations of sediments are depositional rather than post-depositional, for climatically controlled depositional processes can obviously only be relevant to the observed correlations as long as sediment magnetisations arise at the time the sediment is formed. There are known to be several ways in which post-depositional magnetisations can be, and probably are, produced, although it is by no means clear that such processes are universal. In any case, Amerigian's aim seems to be less to push particular explanations or combinations of explanations, for the observed correlations than simply to demonstrate that the correlations need not be direct.

Where Amerigian may be on less certain ground is in his treatment of correlations between directly observed geomagnetic field changes and climatic variations. Since their work on sedimentary cores, for example, Wollin *et al.* (*Nature*, **242**, 34; 1973) have reported just such correlations, but Amerigian dismisses them because of what he takes to be "unsatisfactory" data selection. Whether or not he is justified in this particular instance, the fact is that other workers are also finding relationships between magnetism and climate. It is by no means clear that these will be so easily refuted.

PETER J. SMITH

Lymphocytes at war

IT is a precept of many immunologists that the primary role of the vertebrate immune response is defensive, enabling the responding organism to resist attack by such foreign agencies as bacteria and viruses. Although there are objections to this notion—for example that invertebrates do not have such specific immune responses and yet they seem to defend themselves well—attack and defence have become so firmly rooted in the thinking of immunologists that many of their present-day analytical methods could be described as war games. One of the games with the most immediate impact is that which is fought *in vitro* between two genetically and thus antigenically distinct cell populations. It is supposed that the outcome of this battle in some way reflects the manner in which foreign mammalian cells are dealt with in the living animal after tissue or organ transplantation. There is also the idea that tumours are antigenically distinctive and thus the various kinds of stimulation which can be shown *in vitro* are a manifestation of an anti-tumour surveillance mechanism. Like many war games the moves of those played by immunologists are complex and irritatingly abstruse to the uninitiated. Three recent communications in *Nature* illustrate the genre.

Lafferty and his colleagues Misko and Cooley (*Nature*, **249**, 275–276; 1974) lay down the basic rule of their game in their first sentence. In translation it says that the response to a particular set of transplantation antigens *in vitro* is sometimes enhanced if there is a second and different set of antigens available to respond: two adversaries are better than one. Lafferty *et al.* cultured the cells from the lymph nodes of C57BL/6J mice with γ-irradiated BALB/c spleen cells. This is said to be a one-way mixed lymphocyte reaction in which the outcome is simplified by inactivating the capacity of one of cell populations to respond but leaving its stimulatory property apparently intact. The severity of the reaction can be measured either by incorporation of tritiated thymidine into the cultures at an appropriate time or by subculture of the responder cells onto a ^{51}Cr-labelled tumour cell population which has some of the same surface antigens as the BALB/c stimulator cells (H-2^d).

Lafferty *et al.* go on to show that although γ-irradiated cells are adequate stimulators, ultraviolet-irradiated cells are not. It seems that ultraviolet-irradiated cells still have the relevant antigens as they are effective absorbers of the appropriate anti-H-2 antisera. If, however, to cultures containing responder C57BL/6J lymph node cells (H-2) and (poor) stimulator ultraviolet-irradiated BALB/c spleen cells (H-2^k) is added a γ-irradiated CBA spleen cell population (H-2^k), then the C57BL cells respond vigorously and generate anti-H-2^d cytotoxic cells. Lafferty and his colleagues think that their experiments raise the possibility that "the antigenic differences between donor and host may not constitute a major barrier to all transplantation" but that if the graft contains more effectively antigenic donor lymphocytes then a more vigorous response against the antigens of the major portion of the graft will ensue.

Dennert's experiments (*Nature*, **249**, 358–360; 1974) are like those of Lafferty *et al.* in that the DBA/2J mastocytoma (H-2^d) is again a target cell for attacking lymphocytes. Dennert injected the tumour into C57BL/6J mice and then assayed for cytotoxic cells in their spleens 9 days later. He found that if he used fresh tumour cells to immunise, then cytotoxicity developed but if he used cells fixed with glutaraldehyde or formaldehyde little cytotoxicity was discovered. Dennert then went on to estimate the immunological capacity of the spleens of the variously immunised C57BL mice for their 'helper' capacity by incubating them *in vitro* with trinitrophenyl (TNP)-coupled tumour cells and subsequently measuring the anti-TNP response by a plaque assay. If the spleen cells are immune anti the tumour cells then, because the tumour cells are acting as the carrier for the TNP hapten in the culture, an anti-hapten response should follow. Paradoxically the spleen from mice immunised with fresh tumour cells, which had in the other tests shown such a vigorous cytotoxic response, had little helper activity whereas those from mice immunised with fixed cells had helper activity but little cytotoxicity.

Dennert goes on to show that spleen cells with high cytotoxic capacity are also able to suppress the expression of helper function. These experiments serve to exemplify the intricacy of contemporary cellular immunology and Dennert feels that they suggest strongly that cytotoxic effector cells and helper cells are different 'subsets' of T (thymus-derived) cells in contradiction to the earlier suggestion of Kreth and Williamson (*Nature*, **234**, 454; 1971) that they were the same cells. Categorisation of heterogeneity of T cells is presently popular but it will remain to be seen whether the various putative classes are related to some basic diversity or represent alternative physiological conditions of initially the same cell populations. Thus Dennert's article raises the larger question of whether lymphocytes are heterogeneous before antigenic stimulation or even as a result of it.

The third of the trilogy by Plata and Levy (*Nature*, **249**, 271–274; 1974) draws attention to the fact that although all the effector cells in chromium release assays of cell-mediated cytotoxicity against murine sarcoma virus, induced tumours in C57BL/6 mice seem to be T cells, both T and non-T cells can be detected in the microcytotoxicity assay (Takasugi and Klein, *Transplantation*, **9**, 219; 1970). Furthermore, it proved possible to show that the capacity of T cells to kill as detected by chromium release was not blocked by serum from tumour-bearing animals whereas in the microcytotoxicity assay blocking occurred. Like Dennert, Plata and Levy wish to conclude that they are dealing with two populations of T cells distinguished by their functional capacities *in vitro*.

A. J. S. DAVIES

Pioneering Jupiter meeting at RAS

by John Gribbin

On Friday May 24 the Royal Astronomical Society broke new ground with their first meeting devoted entirely to discussion of the planet Jupiter. The stimulus to hold such a meeting at present came, of course, from the historic data gathered by Pioneer 10 a few months ago, and which are now being analysed. The process of analysis and interpretation is a long one, and although some of the speakers at the meeting are, it seems, regular acquaintances from similar meetings held at various institutions every few weeks, they have yet to hear each other covering entirely familiar ground. So what emerged from the recent RAS meeting was not in any sense a definitive statement about the nature of Jupiter—rather, a progress report of the state of the art, which raises one or two extremely puzzling questions.

The meeting began with a resume from K. Runcorn (University of Newcastle upon Tyne) of the pre-Pioneer picture of Jupiter and its radioactivity. It is now some 52 years since Jeffreys produced the first model of Jupiter, Runcorn recalled, and 37 years since advances in quantum mechanics showed that metallic hydrogen can be formed at pressures comparable to those found in the interior of that giant planet. For some two decades the presence of radio emission from Jupiter has been known—indeed it is the brightest object in the radio sky—and this ties in well with the idea of a fluid metallic core in which dynamo processes operate.

No discussion of Jupiter is complete without mention of the Great Red Spot, which rotates with a period close to 9 h 55 min 40 s, some 10 s more than the rotation period of radio features. This can be explained, broadly speaking, by Hide's model which Runcorn called "the most physically satisfying explanation of the Great Red Spot". In this model, the spot is seen as the visible part of a Taylor column produced by an irregularity (mountain or crater) in the underlying solid surface. Then, the spot period represents the rotation period of the solid mantle, and the radio period the period of the weakly coupled core.

If that is the case, it is to be expected that slight variations in the two periods—a few milliseconds or so—could be correlated in much the same way that variations in the rotation of the Earth's magnetosphere can be correlated with changes in the length of day. Tantalisingly, the available radio observations can neither confirm nor deny this hypothesis, although to my eye the curves presented by Runcorn seem to fit this proposal better than the alternative hypothesis, that the radio period is strictly constant. One thing the Earth-bound radio observations do imply is that the Jovian magnetic field is, if dipolar, inclined at about 11° to the rotation axis—and this provides a neat tie in with the Pioneer 10 data.

E. J. Smith (Jet Propulsion Laboratory, CalTech) described the Pioneer observations of the Jovian magnetic field. The data are not sufficient to define a unique model, but the simplest model which fits them is of a dipolar field, inclined at 11° and offset from the planet's centre, with a strength of 4 gauss at the equator. Closest approach of the vehicle to Jupiter was 2.8 Jovian radii, and the field showed a steady cube law dependance on distance for most of the trajectory, as expected for a dipolar field. The offset of the dipole from the centre of Jupiter seems to be about one tenth of the planet's radius (R_J), much more than the equivalent offset of the Earth's field.

The magnetosphere beyond some 20 R_J is strongly deformed from the dipolar form which is characteristically closer to the planet, and seems to contain a thin current sheet. This has an important effect on the distribution of energetic particles around Jupiter, as J. Simpson (Enrico Fermi Laboratory, University of Chicago) and R. W. Fillius (University of California, San Diego) reported.

Much of the data concerning the particle distributions and the sweeping up effect of some of the Jovian satellites is already familiar from published work, and the latest developments add little to the earlier analyses. But two intriguing features remain to be explained. First, the distribution of both protons and non-relativistic electrons peaks slightly further out from Jupiter than the Pioneer 10 periapsis. Extrapolation of the measurements suggests that there is a 'particle hole' close to the planet, and this can hardly be attributed to the influence of Amalthea since high energy (35 MeV) electrons are not affected. Pioneer 11 is now targeted to fly through this hole, so its reality should soon be confirmed. If the distribution of protons does, however, peak again close in to Jupiter then Pioneer 11 is unlikely to survive.

But the most intriguing discovery mentioned at the RAS meeting concerns the bursts of relativistic electrons apparently ejected by Jupiter. These bursts were first detected by Pioneer 10 half a year before encounter, and got bigger as the spacecraft approached the planet. Clearly they are not solar particles, since they come from the wrong direction; the idea that they might represent interstellar particles leaking in to the Solar System from outside held some appeal at one time, but now it seems clear that they do originate from Jupiter.

The crucial evidence is that once Pioneer 10 had made its dive through the Jovian magnetosphere it was possible to extrapolate back from observations of the roughly 10 h periodic variations produced by the rotation of the planet. This extrapolation showed that all the bursts seen on the way to Jupiter showed structure on a timescale of 10 h exactly in phase with the variations inside the Jovian magnetosphere. Clearly, unless Pioneer 10 had travelled in a trajectory specially favoured for particle ejection (which seems most unlikely), Jupiter must be radiating waves of particles outwards over a wide range of latitudes. This is very disturbing for established theories which suggest that particles should be nicely contained in the equatorial plane, and shows how much remains to be discovered about Jupiter. At the same time, this source of non-solar particles provides an invaluable new tool for investigating propagation of particles in interplanetary space. With Pioneer 11 still to make its (perhaps suicidal) close dive past Jupiter, it looks as if this will not be the last RAS meeting devoted exclusively to the giant of the Solar System.

East African grasses



The second part of the Graminae section of the *Flora of Tropical East Africa* (edit. by Clayton, Phillips and Renvoize; Crown Agents of Oversea Governments and Administrations, London, April 1974, £2.75) contains this drawing of *Odyssea paucinervis* which is found south of the Congo River on saline soils between 1,800 and 1,900 m.

Ambiguous gravity

from Peter J. Smith
Geomagnetism Correspondent

THE chief difficulty in gravity interpretation is that gravity anomalities by themselves can never be used to determine subsurface structure uniquely. There is always a very large, and in theory infinite, number of structural solutions consistent with any observed anomaly, although in practice the quantity of possible structures may often be reduced to more manageable proportions by a combination of common sense, experience of comparable environments, and complementary geological, geophysical or geochemical data. In the end, however, the decision about the 'most reasonable' structure model is likely to depend as much on the quality and amount of the support data as on the gravity anomaly itself.

Disagreements over the interpretation of gravity are therefore common, especially where associated non-gravity data are poor; and this seems to be the situation in the Galapagos Islands. Last year, Case *et al.* (*Science*, **181**, 1040; 1973) reported that over the islands there is a residual negative anomaly of at least 80 mgal superimposed on a broader positive anomaly of unknown amplitude and extent; and they interpreted this in the light of geological evidence in terms of a low density subsurface region related to a hot spot or mantle plume.

But those earth scientists (and there are many) who do not believe in plumes may breathe again, for Watts and Cochran (*Science*, **184**, 808; 1974) have now come up with a model involving simply downwarping of the lithosphere under the weight of the islands—an alternative actually considered and not entirely discounted by Case and his colleagues themselves. Watts and Cochran do not claim their structure is necessarily the right one, nor that a plume does not necessarily underlie the Galapagos; their main point is to re-emphasise the inherent ambiguity of gravity data. Everybody agrees that the matter will not be resolved until other, perferably seismic, data become available.

Hedgerow removal and bird life

from Peter D. Moore
Plant Ecology Correspondent

IN 1962 it was estimated that there were 616,000 miles of hedges in Great Britain (Locke, *Q. Jl For.*, **56**, 137; 1962). This was calculated by Moore, Hooper and Davis (*J. appl. Ecol.*, **4**, 201; 1967) to represent an area of 448,000 acres, which was more than twice the area of the British National Nature Reserves of that time. It was further suggested in these reports that the number of breeding bird species in agricultural land was about twice as great in regions with hedges than in areas where hedges had been removed. Very naturally these authors regarded the extensive clearance of hedges in Britain as one of the principal detrimental events in land management in recent years and it had lead in particular to a reduction in the quantity and diversity of bird life in rural districts.

These findings have been generally accepted, but Murton and Westwood (*Br. Birds*, **67**, 41; 1974) now question their validity. These authors claim that the overall diversity of birds in a 200-acre sample area of agricultural land in Cambridgeshire has changed little, and may even have increased, since it was last censured in 1960–61. This is despite a reduction in hedgerows to one third of their former extent and the reclamation of half of the area then considered 'rough and waste'. These results are bound to cause confusion among conservationists.

One should first ask what is meant by diversity in this context. Murton and Westwood use an index of diversity devised by Fisher, Corbett and Williams (*J. anim. Ecol.*, **12**, 42; 1943) which is based on the assumption that the frequency distribution of species ranked according to the number of individuals by which they are represented, follows a logarithmic series. Although this frequency relationship has often been demonstrated in plant and animal communities, such theoretical indices can be misleading; an empirically derived diversity measure would have been preferable (see Hurlbert, *Ecology*, **52**, 577; 1971).

Whatever the relative merits of diversity indices, one is still faced with the fact that during the ten year period, the total number of species recorded in summer rose from 63 to 66 (although 19 of these species had decreased in numerical abundance) and in winter fell from 65 to 59; in effect there was little change in the total number of species recorded. Murton and Westwood account for this by considering the hedgerow to be a suboptimal habitat for most species which breed in them. Hedgerow removal therefore results in either a retreat of the species to optimal sites (such as woodland) or a modification in the bird's breeding behaviour (blackbirds may nest among the stumps of the cleared hedgerow and along ditch banks). Less adaptable species such as the song thrush may decrease, but are often replaced by other species such as the reed bunting.

These explanations are very acceptable but they do suggest that the area sampled is not typical of regions in which hedgerow clearance has been practised. It differs in the retention of woodland fragments, hedgerow stumps and ditches, all of which serve to maintain diversity. Their conclusions cannot, therefore, be construed as general principles relating to the widespread effects of hedgerow removal.

Lethal mutants in *Drosophila* development

from Benjamin Lewin
Molecular Genetics Correspondent

THE imaginal disks of *Drosophila* provide one of the most promising systems for the genetic analyses of development and the recent isolation of many lethal mutants in disk development is therefore an important step towards defining the components of the system. The disks are larval tissues which subsequently give rise to many of the adult tissues; they include wing, leg, antenna and eye disks. Each disk is thus determined to produce a particular adult structure, the differentiation of which is triggered on metamorphosis. The disks provide an interesting experimental system, for passage of disks through the abdomens of adult flies allows indefinite continued growth, without loss of the determined state (which can be tested by injected tissue into larvae and identifying the adult tissues formed from it after metamorphosis). Transdetermination occasionally takes place, when the state of determination of tissue changes from one disk type to another; and homoeotic mutants exist in which certain disks give rise to adult structures usually formed from other disks.

The independence of the programmes for larval and adult development in *Drosophila* is emphasised by the observation that mutants which prevent disk development may be lethal to the adult but need not affect the larval stage at all. The characterisation of lethal mutants which affect all the disks is reported by Shearn and Garen in the April issue of the *Proceedings of the National Academy of Sciences* (**71**, 1393–1397; 1974). The mutants fall into two classes: *diskless* mutants form no imaginal disks; whereas *small disk* mutants form some or all of the disks, but whichever are present are small, misshapen and cannot differentiate into adult structures.

Mutants were isolated by screening for lethals dying in late development after treatment with chemical mutagens. The 23 *diskless* and 34 *small disk* mutants fall into 52 complementation groups; 48 groups contained one

mutant, three contained two mutants each and one contained three *small disk* mutants. One of the groups with two mutants comprised two *diskless* phenotypes, one included two *small disk* types, and the third represented one of each phenotype.

Two types of developmental deficiency might influence all the disks: some feature of the larval environment essential for development of all disks might be lacking; or some autonomous function common to all disks might be defective. The first model can be tested by injecting disks of normal larvae into the *diskless* and *small disk* larvae to see whether normal growth is possible; the second model can be tested by injecting suspensions of cells from early embryos of the mutants into normal larval hosts to see whether adult structures derived from disks are present after metamorphosis.

Injection of cells from *diskless* early embryos into wild type hosts did not lead to development of any adult structures when the *diskless* mutant was homozygous; such structures can, of course, be formed by cells from *diskless*/+heterozygous embryos. Quite different results were obtained when cells were taken from *small disk* mutants—these could provide the complete range of adult structures specified by all disk types (whether the mutation was in homozygous or heterozygous state).

When this experiment was carried out in reverse, wild type disks injected into *diskless* mutant larvae appeared to grow normally; although this experiment cannot, of course, be taken to the desirable conclusion of following the differentiation of wild type disks in *diskless* larvae, when the injected disks were retrieved before larval death and then reimplanted into normal larvae, they developed normally during metamorphosis. But in contrast, wild type disks cannot grow in one *small disk* host, although they do develop normally on subsequent injection into a normal larva; and in another *small disk* mutant host, some growth (although apparently abnormal) takes place, and the disk then loses its ability to develop normally upon subsequent implantation in a normal host.

Small disk mutants therefore seem to possess competent imaginal disks at an early stage of embryogenesis, but the disks subsequently fail to develop normally because the environment is in some way defective. The developmental potential of the disks seems to be irretrievably lost by the third instar stage which immediately precedes metamorphosis. (But the phenotype of the *small disk* class seems to cover some heterogeneity; for example, the *small disk* and one *diskless* mutant behaves as a *diskless* host when wild type disks are injected into its larvae.) The *diskless* mutants seem to represent a different type of defect; some function involved in the (autonomous) development of all disks must be mutant.

The selective system used to isolate these mutants applied only to the third chromosome; by applying a Poission distribution to the number of complementation groups with one and two members, Shearn and Garen inferred that there may be almost 400 further (at present unidentified) groups on this chromosome. This would imply that the genome of *Drosophila in toto* may possess about 1,000 complementation groups implicated in disk development —about one fifth of the total estimated number of complementation groups. Although the sample of isolated mutants is not large enough to make this an accurate estimate, it clearly implies that a large proportion of the genes of *Drosophila* may be concerned with the development of imaginal disks, an important conclusion. Isolation and mapping of further mutants may therefore be profitable.

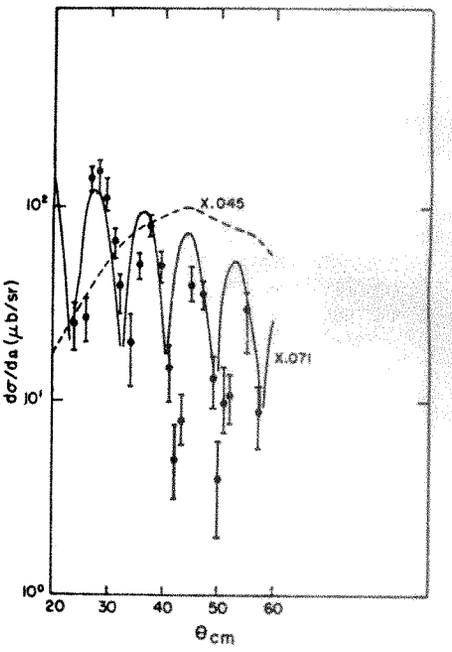
Most important, of course, will be experiments to implicate particular genes in defined pathways of disk development. And in addition to the many autonomous functions common to all disks, revealed by the *diskless* mutant class, there must be gene functions specific for each state of determination. Mutants defective in development of an individual disk type would therefore be useful, although difficult to obtain. And increasing success with genetic analysis must in turn emphasise the need for biochemical analyses of the disk system, a problem which at present looks difficult to overcome.

Transfer of excited alpha particles

from Peter E. Hodgson
Nuclear Theory Correspondent

MANY studies are now being made of the reactions in which an α particle is transferred from one nucleus to another, and these are providing important new information on nuclear structure. Some recent work by Charlton and Robson (*Phys. Rev. Lett.*, **32**, 946; 1974) has now provided evidence to suggest that the α particles can sometimes be transferred in an excited state and not only in its ground state as was previously assumed.

They analysed the differential cross section for the ^{32}S (^{16}O, ^{12}C) ^{36}Ar reaction obtained by Braun-Munzinger and colleagues in Heidelberg (*Phys. Rev. Lett.*, **31**, 1423; 1973). Some of these results are shown in the figure and it is notable that the cross section has a strongly oscillatory structure.



Differential cross section for the reaction ^{32}S (^{16}O, ^{12}C) ^{36}Ar at 30 MeV to the ground state of the residual nucleus compared with distorted wave calculations assuming the transferred α particle to be in its ground (– – –) and in its excited 2^+ state at 28.5 MeV (———). In – – –, $V=100$; $W=30$, $r^r_0=1.22$; $r^i_0=1.25$; $a^r=0.5$; $a^i=0.4$. In ———, $L_c=18$; △ $L=0.8$. The numbers on the curves are the spectroscopic factors obtained by normalising the calculated curves to the experimental data.

The cross sections for α-transfer reactions between nuclei can now be calculated quite well by the finite-range distorted-wave theory, provided the recoil effects are taken into account. Such calculations were made and gave a rather structureless distribution with a broad peak at about 45° as shown by the dashed line in the figure. This is in accord with semi classical calculations, which give a bell-shaped curve centred at an angle corresponding to the scattering of a particle whose orbit just grazes the nuclear surface. This behaviour is completely different from that found experimentally.

Further calculations were then made taking into account the possibility that the α particle is transferred in an excited state. The 0^+ level at 20.2 MeV and the 2^+ level at 28.5 MeV were considered, as these satisfy the required parity and isospin selection rules. Estimates using harmonic oscillator wavefunctions indicated that reactions with the α particle in the 2^+ state are about a hundred times stronger than those with the 0^+ state, so only the former was considered.

The excited states of the α particle were assumed to be made up of pairs of like nucleons with the smallest possible internal motion, and the quantum numbers for the relative motions of the pairs give the required total spin.

In the dissociation $^{36}Ar \rightarrow {}^{32}S + \alpha^*$, the four valence nucleons were assumed to be in the $0d_{3/2}$ orbit and the probability of the α particle being in an excited state was estimated using both the stretch scheme and the simple pairing model. Both calculations indicated that the α particle is more likely to be found in an excited state. The corresponding spectroscopic factors indicate that the transfer of an α particle in its 2^+ state is about five times more likely than the transfer in its ground state for the reaction considered.

The distorted-wave calculations for excited α-particle transfer were carried out in the standard way and used absorbing potentials depending on the orbital angular momentum in the way required by analyses of the elastic scattering of heavy ions by nuclei. The results are shown by the full line in the figure, and agree very well with the experimental data. Similar results are found for the corresponding reaction to the lowest 2^+ state of ^{36}Ar.

This is clearly a significant result that will stimulate many more investigations. In principle, the ground and all the excited states of the α particle should be added coherently to give the distorted-wave amplitude. More detailed analyses of similar reactions on other nuclei should provide further evidence of excited α transfer but the results of Charlton and Robson provide good evidence that such processes can take place.

Synchrotron radiation users meet

from G. V. Marr

THE electromagnetic radiation emitted from high energy electron synchrotrons, as a consequence of radially accelerating the electrons, is now well established as a means of studying the interaction of ultraviolet and X radiation with matter in its various forms. The first international symposium for synchrotron radiation users was held at the Daresbury Laboratory in January 1973 (see *Nature phys. Sci.*, **242**, 1; 1973) following the move of United Kingdom users from the Glasgow synchrotron to NINA in 1972. During the past year, demand for synchrotron radiation has increased and the conference held at the University of Reading from April 1 to 2, under the auspices of the spectroscopy group of the Institute of Physics, provided a means whereby the UK users could discuss the relevance of this source. The contributions covered present and future synchrotron radiation sources, applications to atomic, molecular, chemical and solid state physics, biological studies, chemistry, X-ray optics and radiometry. The conference concluded with an open meeting with Science Research Council representatives to discuss the SRC paper "A Plan for Future Research with Synchrotron Radiation based on a Dedicated Storage Ring".

The main energy loss from the electron synchrotron is in the form of electromagnetic radiation whose spectrum extends continuously from the infrared to the X-ray region. It provides an intense source of high energy photons, predominantly polarised in the plane of the electron orbit and, because of the bunching of the electrons, pulsed at the accelerator radio frequencies. The radiation is emitted under good vacuum conditions and with divergences from the orbit plane comparable with a laser beam (see *Endeavour*, **33** (119) 55–66, May 1974).

Facts and figures for existing synchrotron sources were provided by I. H. Munro (Daresbury and University of Manchester) who outlined criteria for the ideal synchrotron source based on design work for the dedicated electron storage ring now under consideration by the Science Research Council. Progress with existing synchrotrons in atomic and molecular physics was introduced by K. Codling (University of Reading). In addition to energy level analysis, accurate absolute cross-sectional data are being provided on photoionisation processes and new experiments which allow photo-electron analysis to be carried out with respect to the E vector of the ionising radiation are now in progress. Continuous variation of the photon energy is providing data which will extend knowledge of configuration interaction, molecular orbitals and vibronic coupling; a point which was taken up by S. Leach (University of Paris, Orsay) in his review of applications in chemical physics where excited state relaxation processes in molecules and solids can benefit from lifetime studies using the modulated nature of the ultraviolet radiation of the synchrotron.

In the field of solid state physics, A. J. Forty (University of Warwick) reported experiments on absorption and reflectivity measurements which are contributing significantly to knowledge of band structures. Photoemission from surfaces is also providing new possibilities in metallurgy. D. A. Urtch (Queen Mary College, London) said that chemists on the other hand were exploring the possible use of synchrotron radiation to study the atomic components of molecular orbitals by way of X-ray emission spectroscopy. The new ring would allow an extension of current work to the light elements where X-ray emission is less favoured. The polarised nature of the radiation offers a valuable new tool in studies of single crystal structures throughout the ultraviolet and X-ray regions. Of more immediate interest to molecular biologists, however, are the intensities of some $10–10^5$ over conventional continuum X-ray sources that could become available. J. C. Hazelgrove (Medical Research Council, Cambridge) discussed advantages of these radiations in studies of transient states where it is important to have detailed short exposure X-ray diffraction pictures of specimens whose decay under normal sources alters vital characteristics during the time of exposure.

On the instrumental side, P. J. Key (National Physical Laboratory) said that the synchrotron, as a standard of relative spectral distribution throughout the near ultraviolet to the X-ray region, is becoming well established and radiometry problems are being studied. A discussion on X-ray optics introduced by M. Hart (University of Bristol) showed that fluxes some 10^5 greater than obtainable with conventional sources can be expected when the polarised nature of the synchrotron radiation is exploited. Quarter wave plates, crystal spectrometers and the development of X-ray diffraction gratings were also discussed.

In spite of the progress made and the enthusiasm of the users, it was evident that NINA and the other synchrotrons, where the users are parasitic to the experiments of the high energy nuclear physicists, are not ideal sources. The parasitic mode of operation is extremely inefficient where users are unable to control the quality and duration of the synchrotron beam. The SRC plan would seem to meet the foreseeable needs of the Reading conference and it was with this in mind that they attended the SRC meeting. Quite properly questions were asked relating to costs, location and long term usefulness of the proposed storage ring. The scientists present were particularly concerned that delays at this stage could result in the United Kingdom being without a source of synchrotron radiation if present plans concerning NINA are adhered to. A preliminary estimate of between 12 and 18 months for the level of funding in the SRC document was given at the meeting. It was agreed that there is every need to impress upon the SRC a sense of urgency in proceeding with the plan beyond the design stage, in order to ensure that the period between the close down of NINA as a synchrotron source and the start up of the new storage ring is kept to an absolute minimum.

Midwinter sunrise at Newgrange

J. Patrick

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At Newgrange on the morning of the winter solstice the Sun's rays shine through a specially contrived slit in the roof, along a 19 m passage to illuminate the central burial chamber. This passage grave was built about 1,000 yr earlier than the third phase of Stonehenge, by a different culture. It supports the claim that prehistoric cultures had studied the cycles of the Sun and utilised them in orientating their monuments.

NEWGRANGE is a passage grave in a Neolithic cemetery in County Meath, Ireland, about 30 miles NNW of Dublin (0 301 703). Two large samples of charcoal which were collected from caulking in between the roof slabs, yielded dates of 2475±45 b.c. and 2465±40 b.c. respectively. Depending upon which correction curve is used, this gives an age of 3100±100 BC for the building of the tomb. It consists of a huge, artificially constructed cairn of water-rolled stones, which is approximately 80 m in diameter. A passage, 19 m long, runs into the mound to give access to a large chamber about 3 m square and 6 m high. There are three recesses that open into the chamber, so that the ground plan has a cruciform appearance (Fig. 1). In each of these recesses is a large stone basin which was used to hold the burnt remains of the interred occupants.

Carvings

The tomb has been open to visitors since 1699 and an unknown amount of the original burial deposit may have been removed since then. In 1967 excavation of the tomb floor in front of the basin stones revealed the burnt bone fragments of about five people. These were accompanied by some grave goods characteristic of this type of tomb in Ireland, including stone pendants and beads, stone 'marbles' and bone points.

At the base of the mound is a continuous kerb of large slabs. It is believed that dry-stone walling was built on top of the kerb to a height of 3 m and that this wall was made of quartz for about 30 m on each side of the entrance. The sun on the

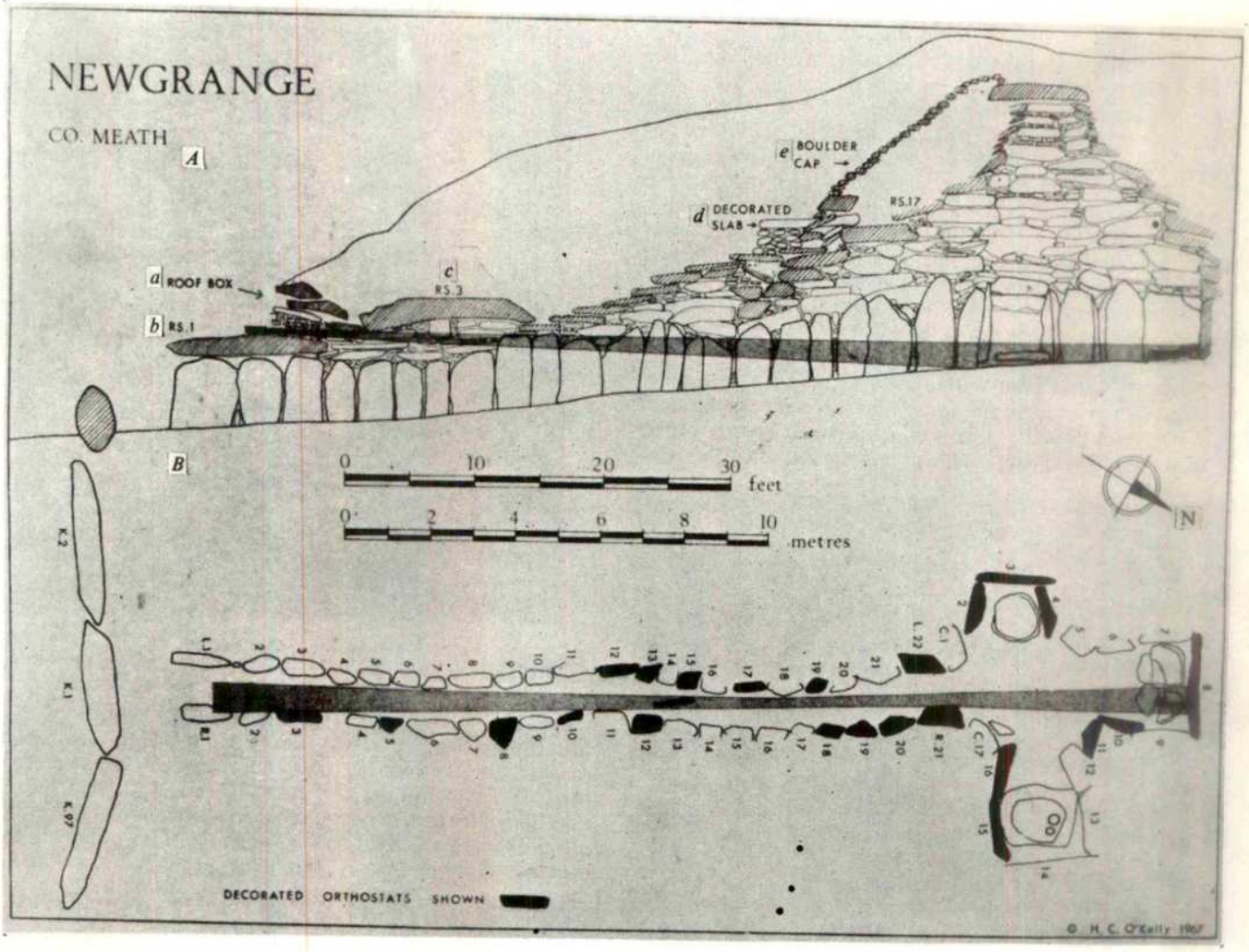


Fig. 1 The passage-grave at Newgrange, County Meath. *A*, Sectional elevation: *a*, roof box; *b*, roof slab 1; *c*, roof slab 3; *d*, decorated slabs; *e*, boulder cap. *B*, Plan view: wall orthostat stones are numbered; black shading, decorated orthostats; grey shading, range of path of Sun's rays during mid-winter solstice. (From ref. 1.)



Fig. 2 The decorated Entrance Stone in front of the burial chamber. The roof box can be seen, surmounted by the lozenge relief carving. (Green Studio.)

glistening white quartz would have presented a spectacular sight from the surrounding countryside. Most of this wall collapsed soon after completion of the monument, and the kerb stones became completely buried and remained hidden until uncovered again at the start of this century. Outside the kerb, there are 12 large standing stones that may form part of a circle, but their true relationship with the mound is not known for certain. They may be older than the passage-grave.

One of the most outstanding features of Newgrange is the decoration on the stones. The Entrance Stone, set in front of the passage, is regarded as one of the most impressive pieces of Megalithic art in Europe (Fig. 2). The artist who executed this piece of work has succeeded in using the shape of the stone to the utmost advantage in creating its aesthetic appeal. Another small but remarkable design is the three-spiral figure in the rear recess (Fig. 3). There are many other decorations within the passage and chamber, and on the kerbstones, virtually making Newgrange a Megalithic art gallery.

Astronomical alignment

Perhaps the most interesting feature of Newgrange is the 'roof box'. Dry stone walling has been constructed on top of the passage orthostats to support the second roofslab at a higher level than the first, leaving a gap about 20–25 cm high and 1 m wide (Figs 1 and 2). The gap is protected from the weather by a box like structure of slabs supported on dry walls. This is open to the front. For further protection against the weather, the rear edge of the first roof slab and the front edge of the second roof slab have water channels cut on them. The leading edge of the uppermost roof slab of the roof box has an excellent relief carving of lozenges (Fig. 2).

During the winter solstice in 1969 Professor M. O'Kelly observed that 4 min after sunrise the Sun's rays shone through the roof box, along the passage, and up to the rear recess of the burial chamber, which became fully illuminated (Fig. 4). The spectacle lasted for 17 min before the sun moved out of alignment. Later, Mrs O'Kelly recalled a tradition that at a certain time of the year the sun lit up the three-spiral figure in the end of the chamber[1].

In 1972 Professor O'Kelly asked me to make an accurate survey of the roof box to see if this phenomenon would have occurred when the burial chamber was first built. The passage



Fig. 3 The three-spiral figure in the rear recess of the chamber. (Green Studio.)

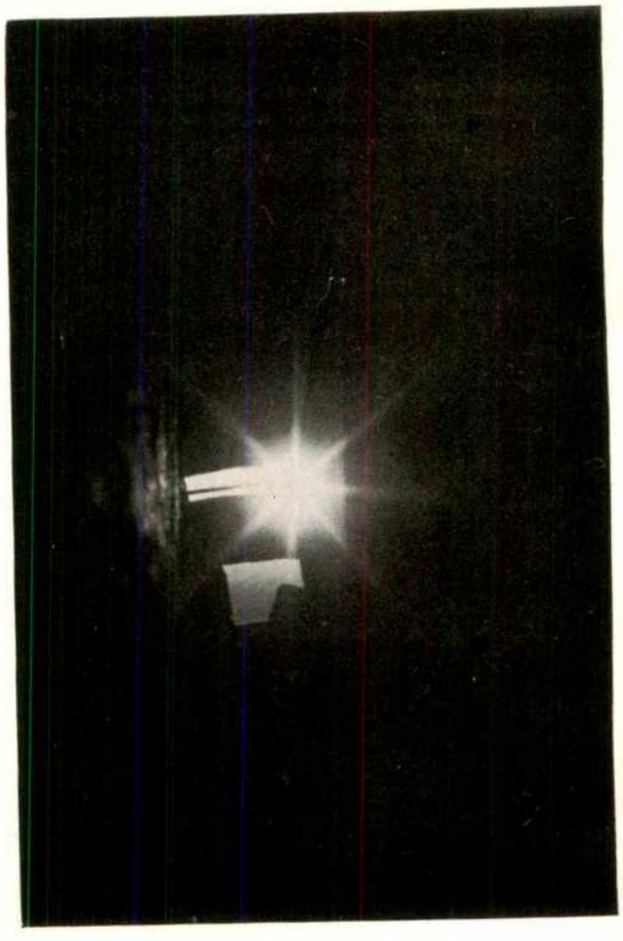


Fig. 4 Sunlight shining through the roof slit during mid-winter solstice. (M. J. O'Kelly.)

is in the form of two curves (Fig. 1), so that for a ray of light to travel directly from the roof box to the back wall of the rear recess it must be in the azimuth range 133° 42′–138° 24′. The elevation of the distant horizon (0° 51′) is the minimum elevation of which the Sun's direct rays can enter the slit. The floor of the chamber is about 15 cm lower than the roof box, so at the minimum elevation sunlight will extend across the floor and into the rear recess. Light rays will not enter the chamber when the elevation exceeds about 1° 40′. This range of azimuths and elevations, reliable to about 15′ and 5′ respectively means that the Sun's rays will shine directly into the chamber if its declination lies between −22° 58′ and −25° 53′. It therefore seems that

the Sun has shone down the passage to the chamber ever since the date of its construction and will probably continue to do so for ever, regardless of secular changes in the obliquity of the ecliptic. It also means that the spectacle occurs for a number of days before and after the winter solstice.

Unfortunately, the vagaries of time have had their effect on the passage and some of the stones are now leaning inwards, thus trimming down the width of the beam of light. At the time of construction the beam would have been about 40 cm wide whereas now it is only 17 cm. The two principal orthostats causing the obstruction are L18 and L20 (Fig. 1). The first 10 orthostats on either side of the passage have been straightened but there is no way of straightening the rest without dismantling the whole structure.

There are about 200 passage graves in Ireland with many more allied tombs in Scotland and Brittany, but to my knowledge none of them has a roof box like that at Newgrange. As this structure is unique, and as the whole monument is so grandiose, it seems likely that its orientation is deliberate.

The monument best known for its astronomical orientations is Stonehenge on the Salisbury Plain. After it had been built, it was remodelled several times, and the main astronomically orientated structures were erected in its third phase, which is dated at around 1800–2000 BC. This is about 1,000 years after the construction of Newgrange, and there is little connection between the two monuments or between the cultures which built them. The unambiguous definition of both direction and altitude at Newgrange is by far the most convincing evidence that some Megalithic structures were deliberately orientated on astronomical phenomena. This must lend greater credence to the theory that different cultures in the British Isles were investigating the basic solar cycles. It is quite possible that data passed between different cultures, so that Stonehenge eventually became an important repository of astronomical knowledge.

I would like to thank Professor and Mrs O'Kelly for their assistance and advice.

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[1] O'Kelly, C., *Illustrated guide to Newgrange*, second ed., 94–95 (John English, Wexford, 1971).

Structure and orientation of an RNA polymerase operon in *Escherichia coli*

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The genes for at least two Escherichia coli *RNA polymerase subunits are contained in a single operon.*

THE DNA-dependent RNA polymerase is a key enzyme in expression of the hereditary material. In *E. coli* it consists of at least four different subunits α, β, β', and σ. The structural gene for the β subunit (*rif*) has been precisely located on the genetic map because mutations conferring resistance to the antibiotic rifampicin also result in the sythesis of RNA polymerase molecules altered in this polypeptide[1]. Here we present evidence based on deletion studies, indicating that the structural gene for at least one other subunit, β', is linked to the *rif* gene, and that both share a common promoter (P). These elements map clockwise on the bacterial chromosome in the order *P–rif–*β'. Thus our results provide a clear genetic basis for the suggestion made by Matzura, Molin and Maaløe that β' and β' may be synthesised on a single polycistronic mRNA[2]. In addition, they show that the structural genes and the promoter for this mRNA map clockwise on the bacterial chromosome, in the order *P–rif–*β'.

Because and deletion affecting RNA polymerase would be lethal in haploid cells[3], we have constructed strains partially diploid for the *rif* region of the chromosome by introducing into the cell an F′ factor which carries extensive amounts of chromosomal DNA on both sides of this locus (Fig. 1). Thus deletions into *rif* do not affect cell viability. Moreover we have chosen to isolate such deletions on the F′ factor, because they can easily be characterised by transferring the episome into a variety of appropriately marked strains. To obtain information about the structure and orientation of the operon, we have isolated a specific episomal deletion ending within the *rif* gene, and which extends leftwards into the nearby *arg*CBH operon (Fig. 1). This deletion, which has removed all the DNA immediately to th left of *rif*, has nevertheless left the β' gene carried by the F′ completely intact. This result is expected if the β' gene were adjacent to *rif* and located to its right. Moreover, this deletion also places the synthesis of the β' subunit under the control of the *arg*CBH operon. Since *arg*CBH is read in a clockwise fashion[4], we conclude that the β' and β' genes are both read in that direction.

Isolation of deletions into *rif*

We have developed a simple method for selecting deletion mutations on an F′ factor which simultaneously affect the *rif* gene and its neighbour, *bfe* (ref. 5); mutations in these loci confer resistance to antibiotic rifampicin and the virulent bactriophage BF23 respectivly. Mutations to resistance in either gene are normally recessive to the wild-type allele (ref. 3 and L.E. and J.G.S. unpublished). Thus, a partial heterodiploid of the *bfe*^{s} *rif*^{r}/F′ *bfe*^{s} *rif*^{s} is sensitive to both BF23 and rifampicin. Mutants with deletions inactivating the wild-type *bfe* and *rif* genes carried by the F′ will, however, become resistant to both BF23 and the antibiotic since the only intact *bfe* and *rif* genes remaining in the cell are those conferring resistance. We can therefore readily select for such rare mutants by plating cultures of the partial heterodiploid on solid medium containing rifampicin,

Table 1 Bacterial strains

Name	Genotype	Source/reference
AS13	F⁻ *met*B *arg* (C–B) $_{sup102}$ *pur*D *his str*^{r} *spc*^{r} *gal mal lac*	A. Silverstone
AW1	F⁻ *met*B *rec*A56	S. Austin
HTA1	F⁻ *his*⁻*trp*A9761 (amber) *arg*E171	S. Baumberg
HTA19	F⁻ *his*⁻ *trp*A9761 (amber) *arg*B2	S. Baumberg
LE104	*met*B *arg* (C–B) $_{sup102}$*pur*D rif$^r_{A1}$ *bfe*^{r} *rec*A56 *str*-r *lac gal*	See below
LE109	*met*B *arg* Δ (C–B) $_{sup102}$*pur*D *rif*$^r_{A2}$ *bfe*^{r} *rec*A56 *str*^{r} *lac gal mal*A/KLF10 Δ18	Text
LE113	*met*B *rec*A56/KLF10$^+$	LE104 × AW1
LE114	*met*B *rec*A56/KLF10 Δ18	LE109 × AW1
LE115	*met*B *rec*A56 *arg*R	A spontaneous *can*^{r} derivative of AW1
LE116	*met*B *rec*A56 *arg*R/KLF10$^+$	LE104 × LE115
LE117	*met*B *rec*A56 *arg*R/KLF10 Δ18	LE109 × LE115
LE210	F$^+$ *ara* (amber) lac_{L125} (amber) $galK_{U42}$ (amber) *trp* (amber) *str*^{r} *met*B SuIII IA2(P) rif$^0_{amD12}$λ$^+$	Derived from MB204 (ref. 17)
LE213	F⁻ *ara* (amber) lac_{L125} (amber) *gal*KU42 (amber) *trp* (amber) *str*^{r} *met*B SuIIIA2(P)* rif$^0_{amIII/8}$λ$^+$	Derived from MB204 (ref. 17)
MN8A	Hfr P4X *met*B *arg*B2 λ$^+$	S. Baumberg
P4X C53	Hfr P4X *arg*C53λ$^+$	S. Baumberg

The genotypes of our bacterial strains are shown in Table 1. The strain LE104 was specially constructed for our deletion selection. It was made from AB2147 *ilv arg*H *met*B *thi his lac*Y *gal xyl mal*A *tna str*^{r} (Wechsler, personal communication). In a series of steps the following markers were introduced into AB2147 (Silverstone and Errington, unpublished): *pur*D (cross against a *pur*D derivative of Hfr AB311 (ref. 18); Δ*arg*(C–B)$_{sup102}$ (cross against Hfr P4X sup102 (ref. 6); *rif*-r (spontaneous mutation); *rec*A56 (cross against Hfr KL16 recA56 (ref. 19); *bfe-r* (spontaneous mutation); KLF10$^+$ (cross against AW2).

* The pattern of *ara* and *lac* suppression in this strain is slightly different from that seen in LE210. However its suppressor is still temperature sensitive, since the strain does not grow on nutrient plates at 42° C.

spread with phage BF23 (Table 2).

The starting strain, LE104 (Table 1), carries the KLF10 episome which spans the *arg*CBH-*met*B-*rif*-*pur*D region of the chromosome (Fig. 1). The strain is Rec⁻ to prevent recombination between the episome and the chromosome and, to ensure that the cells retain the KLF10, the chromosome contains mutations in both *met*B and *pur*D. We can also determine whether the *bfe-rif* deletions extend into the nearby *arg*CBH operon, because the chromosome of this strain carries an Arg⁻ mutation (*sup*102)[6]; deletions terminating before *arg*CBH leave the cells Arg$^+$, whereas deletions extending into the *arg* operon will make the cells Arg⁻.

We have found that mutants having the desired Bfe^r Rif^r phenotype arise spontaneously at a frequency of about 3×10^{-8}. The following observations show that these mutants are due to deletions on the F′ factor (Table 2). First, many of them (63%) are Arg⁻ and have therefore lost, in addition to *bfe* and *rif*, one or more of the *arg* genes from the episome. Moreover, these Arg⁻ mutants do not revert to Arg$^+$ ($<10^{-8}$). Secondly, crosses in which the mutant F′ factors are transferred to recipients carrying mutations in *arg*E, C and B generate a deletion map (Table 2) congruent with the known map of the *arg* operon. Three of the deletions, Δ7, 12 and 18, enable us to order two amber *rif*^{0} mutations, *rif*$^0_{amD12}$ and *rif*$^0_{amIII/8}$, which affect the β subunit of RNA polymerase (ref. 7 and R.S.H., S. J. Austin, and J. G. S., unpublished).

Table 2 Isolation and characterisation of *bfe-rif* deletions

Deletion on KLF10	Frequency*	Wild-type recombinants formed in crosses against recipients containing: *metB*	*arg*E171	*arg*C53	*arg*B2	*arg*H119	*rif*$^0_{III/8}$	*rif*$^0_{D12}$	*pur*D
Δ4	1	+	−	−	−	−	<0.001	<0.01	+
Δ7	1	+	−	−	−	−	<0.001	0.03	+
Δ12, Δ18	2	+	+	+	−	−	<0.001	0.20	+
	11	+	+†	+†	+†	nt	nt	nt	+
Partially	12	+	−	nt	nt	−	nt	nt	+
characterised	3	+	+	+	−	−	nt	nt	+
Wild type	+	+	+	+	+	+	100	100	+

Isolation of deletions affecting *rif* and *bfe*. Cultures (5 ml) of LE104 (Table 1), grown overnight in minimal salts[20] containing glucose (0.2%) thiamin (10 μg ml^{-1}) and arginine (20 μg ml^{-1}), were mixed with the virulent phage BF23 (multiplicity of infection = 10) and shaken at 37° C (3 h). They were then concentrated to 0.3 ml by centrifugation and spread in soft agar (2.5 ml) containing 10^{11} p.f.u. of BF23 on three minimal agar plates containing arginine (20 μg ml^{-1}), thiamin (10 μg ml^{-1}) and glucose. After 4 h at 37° C each plate was overlaid with soft agar (3 ml) containing rifampicin (1.2 mg ml^{-1}), giving a final concentration in the plate of 100 μg ml^{-1}. The plates were incubated at 37° C for 2 d.

Characterisation of deletions. Each mutant carrying a deletion on KLF10 was mated against appropriately marked recipient strains by cross-streaking on selective medium[7]. Some combinations were not tried (nt).

Crosses against Arg⁻ recipients. The genotypes of the recipient strains, HTA1, P4XC53, MN8A and HTA19 are listed in Table 1. Deletion strains able to give Arg$^+$ recombinants (+) give confluent growth in the cross streak. Those unable to give recombinants (−) show no growth at all. Note that both *arg*C53 and *arg*B2 are in Hfr strains which only permit the recovery of chromosomal recombinants[21].

Crosses against *rif*^{0} mutants. Each donor was crossed against rif^0 recipients (LE210, 213, Table 1) containing a temperature-sensitive amber suppressor SuIII (A2(P))[17]. Met$^+$ derivatives containing each mutant KLF10 were recovered at the permissive temperature (30° C) on selective medium. They were then tested for the ability to generate *rif*$^+$ recombinants able to grow at the restrictive temperature (42° C). In this table we show the percentage of ts$^+$ cells in a colony of each derivative. Control colonies of the recipient *rif*°$_{amIII/8}$ (LE213) and *rif*°$_{amD12}$ (LE210) gave no more than 0.001% and 0.01% ts$^+$ revertants respectively.

Crosses against a *met*B *pur*D recipient. Our initial selection required that our deleted KLF10 factors retain *met*B and *pur*D (see text). That they do retain these markers was confirmed in crosses against AS 13 *met*B *pur*D.

* Number found in a total of 30 mutants recovered from a single culture of LE104.

† Deduced from the Arg$^+$ phenotype of the parental deletion strains in this class.

Operon containing the *rif* and ß′ genes

One of the mutants selected from LE104 (Table 1), can give Arg⁺ recombinants on transfer of the F factors to *arg*C53, but not *arg*B2, recipients (Table 2). Consequently its deletion, △18, must have one end in the *arg*C-B region of the episome. The other is between rif^{0}_{amD12} and $rif^{0}_{amIII/8}$ (Table 2). If the β′ gene lies just to the right of *rif*, △18 should inactivate *rif* but still leave the β′ gene intact. Cells containing KLF10△18 would thus be expected to have two copies of the gene for β′ and only one for β (located on the chromosome).

We assay the β and β′ subunits of RNA polymerase by electrophoresis of pulse-labelled, whole cell lysates on polyacrylamide gels containing sodium dodecyl sulphate, which separates the large β and β′ subunits from other cell proteins (Fig. 2)[2]. We have studied a number of KLF10△18 diploids in this way. Their behaviour (see below) shows that KLF10△18 still determines β′ subunit synthesis, and implies that the β′ gene lies just to the right of *rif*.

Evidence has previously been obtained suggesting that the β supunit is synthesised before the β′ subunit[2]. Thus, if *rif* lies just to the left of the β′ gene, both could be in a single operon read left to right (clockwise) on the chromosome (Fig. 1). This conclusion leads us to predict that a nonpolar deletion which begins in the *arg*CBH operon and ends in the *rif* gene should place the synthesis of β′ under the control of *arg*CBH, which is read clockwise[4]. Expression of the *arg* genes is subject to negative control requiring at least arginine (or a derivative) and the product of the *arg*R repressor gene[8]. When bacteria are grown in the presence of arginine their *arg*CBH operon is fully repressed, but in its absence, the operon becomes induced and the genes in the pathway are expressed at a (ten-fold) higher rate. Still higher rates of *arg* expression (100-fold) result when cells are prevented from synthesising functional *arg* repressor by mutations in *arg*R. In many *arg*R mutants expression of the operon proceeds at the full constitutive level, unaffected by the presence of arginine in the growth medium[9].

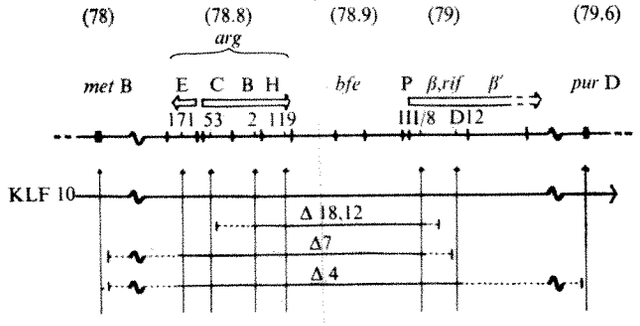


Fig. 1 The *metB-purD* segment of the *E. coli* chromosome. The figure shows the markers used in our genetic analysis, the wild-type KLF10 (omitting sex factor DNA) and the *bfe-rif* deletions isolated on this F′ factor. The arrows at the top of the figure indicate the direction of gene transcription and the numbers in brackets refer to the position of genes (in minutes) on the *E. coli* map[26].

In strains carrying KLF10△18 we are able to detect changes in the overall rate of β′ synthesis due to *arg* of the β′ gene carried by the episome. We find that an arginine-repressed KLF10△18 diploid (Table 3, line 4) synthesises nor more β′ than a normal haploid strain[27] and markedly less than a control KLF10 diploid (Table 3, line 2). In addition, the KLF10△18 diploid synthesises significantly more β′ when grown in the absence of arginine, although the rate of synthesis of β subunit is unaltered (Table 3, line 2). An isogenic control strain carrying wild-type KLF10 synthesises β and β′ at a rate not affected by the presence of arginine (Table 3, lines 1 and 2). More dramatically, we find (Fig. 2 and Table 3, lines 4 and 6) that the specific increase in β′ synthesis in KLF10△18 strains is enlarged about seven-fold as a result of an *arg*R mutation. This *arg*R mutation has an equivalent effect on the synthesis of ornithine transcarbamylase, one of the *arg* enzymes (T. Bretscher and S. Baumberg, personal communication)

Measurement of the amount of β′ synthesised by the KLF10△18 *arg*R diploid enables us to estimate the efficiency of the *arg*CBH promoter, if we assume

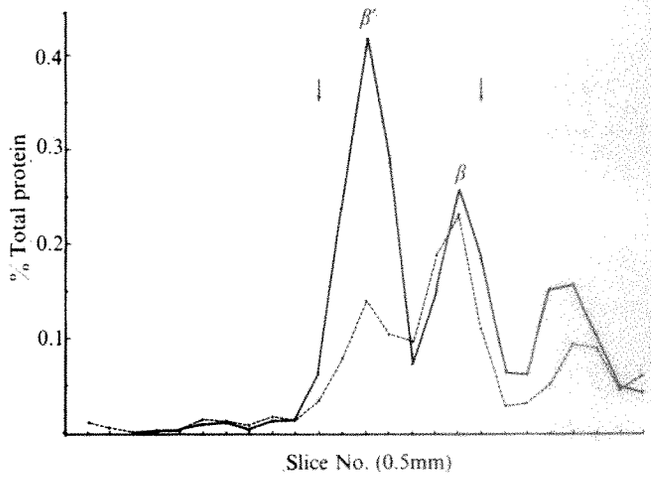


Fig. 2 Gel analysis of ³H-labelled protein in crude lysates of *arg*R⁺ (LE114) and *arg*R⁻ (LE117) strains carrying KLF10 △ 18. Both lysates were prepared from parallel cultures grown in excess arginine (100 μg ml⁻¹) and run with marker polymerase on separate SDS-polyacrylamide gels (Table 1). Only the top of each gel is shown. Fractions from the *arg*R⁺ *and arg*R⁻ lysates are shown by broken and solid lines respectively. The limits of the stained β′ and β subunits of the marker polymerase are indicated by the arrows. % Total protein = (³H per slice)/(³H in total protein applied per gel) × 100.

that △18 is not polar. We ascribe 0.95 (±0.37)% of the total leucine incorporation (pulse label) to arginine-controlled β′ synthesis (Table 3, lines 4 and 6). The mol% of leucine in the β′ subunit (molecular weight, 160,000) is about the same as that for total *E. coli* protein[10]. Thus, assuming that a single cell contains 3.3×10^{-13} g of protein[10], we calculate that the *arg*CBH promoter is set to give about 7×10^{3}–15×10^{3} protein molecules per cell. A comparable level of expression has ben reported for the typtophan operon[11].

Control of the RNA polymerase operon

We have characterised a deletion fusing the *arg*CBH operon to the middle of the *rif* gene, which puts synthesis of the β′ subunit under arginine control. Our finding shows that a gene determining synthesis of the β′ subunit lies to the right of *rif*. This gene, which is read clockwise, is presumably the structural gene for the β′ polypeptide. The episome-coded β′ synthesis we can detect in KLF10△18 diploids is entirely under arginine control. The deletion △18 has therefore removed the normal promoter for β′ synthesis without affecting the DNA sequence between *rif* and the β′ gene. Consequently the promoter for β′ synthesis

Table 3 Synthesis of the β and β′ subunits of RNA polymerase in KLF10 diploids

	Strain No.	*arg*R allele	KLF10	Arginine in culture (μg ml^{-1})	^{3}H in β′ subunit (% total labelled protein)*	P†	^{3}H in β subunit (% in total labelled protein)*	P†
1	LE113	+	wild-type	None	0.59 (± 0.05)	ns	0.63 (± 0.13)	ns
2	LE113	+	wild-type	100	0.68 (± 0.06)		0.69 (± 0.09)	
3	LE114	+	Δ18	None	0.51 (± 0.08)	<0.02	0.44 (± 0.13)	ns
4	LE114	+	Δ18	100	0.37 (± 0.06)		0.45 (± 0.11)	
5	LE116	−	wild-type	100	0.57 (± 0.05)		0.84 (± 0.06)	
6	LE117	−	Δ18	100	1.32 (± 0.28)		0.56 (± 0.06)	

*Data in lines 1–4 represent the means of four lysates; those in lines 5 and 6 are means of two lysates. The standard errors of these means are shown in bracket.s

†*P* values indicate the significance of the difference between cultures grown in the presence and absence of arginine computed by the method of paired comparisons[22]; ns indicates the difference was not significant.

The strains used in these experiments are isogenic (Table 1). They were grown in M9 medium[23] without methionine to guard against episome loss.

Preparation of labelled cell lysates. Young M9-minimal cultures (5×10^7 ml^{-1}) were pulse labelled for 1.5 min with 4, 5 ^{3}H-leucine, chased with cold leucine for 2 min and lysed as previously described[13]. We used minimal medium containing 100 μg ml^{-1} arginine to grow cultures repressed for the arginine biosynthetic enzymes.

SDS polyacrylamide gel electrophoresis. We used the buffer system of King and Lemmli[24]. The separating gel (60 mm) had 16% acrylamide, 0.094% NN′-methylene bisacrylamide (Bis), 0.025% ammonium persulphate and 0.025% (v/v), N, N, N′, N′-tetramethylethylene diamine (TEMED); the stacking-gel (10 mm) had 2.5% acrylamide, 0.6% Bis and 0.1% (v/v) TEMED. It was either chemically polymerised with 0.0167%) ammonium persulphate or photopolymerised with 0.0005% riboflavin. Each lysate sample (25 or 50 μl), reheated at 75° C for 5 min, was applied to the gel with bromophenol blue (final concentration 0.0002%) and marker RNA polymerase previously incubated in sample buffer[24]. Samples were stacked at a constant current of 0.375 m per gel at room temperature for approximately 3 h and electrophoresis was continued for a further 21 h at 1.1 m per gel. Gels were stained with 0.1% Coomassie Brilliant Blue R250 in methanol:acetic acid:H_2O (5:1:5) at 37 °for 2 h and destained in 7% acetic acid/5% methanol at 37° C O/N. The β, β′ region was sliced (0.5 mm) and placed in firmly capped scintillation vials containing 2 ml of Petri's permeabilising scintillation fluid[25] and POPOP (0.1 mg^2 ml^{-1}). The vials were incubated in the dark (18 h) at 37° C. The precooled samples were counted for ^{3}H in a Packard liquid scintillation counter. All labelled material was eluted and the efficiency of counting was constant and reproducible. Counts were computed as a percentage of the total protein determined by counting aliquots of whole lysate under identical conditions. This method of total protein estimation gave the same results as the method of Hayward *et al.*[13].

must lie to the left of *rif* and is presumably common to both genes.

Matzura *et al.*[10] find that, under widely different conditions of growth, *E. coli* synthesises the β and β′ subunits as a rather constant fraction of the total cell protein. In addition, no increase in their rate of synthesis is detected following *rif* and β′ gene replication[10] or in strains containing a λ *drif*[1] transducing phage[12]. Nevertheless the cell has the potential to increase in parallel the rates of β and β′ synthesis to a level considerably higher than normal[13]. These combined observations indicate that the cell coordinately regulates synthesis of at least these two subunits. On the basis of the results presented here we suggest that the expression of the *rif* and β′ genes is coordinately regulated at a site on the chromosome to the left of *rif*.

There are a number of candidates for a regulatory molecule acting at this site. For example, polymerase operon expression could be maintained at a constant level throughout the cell cycle if one or more of the polymerase subunits themselves acts as a repressor. An inbuilt self-regulatory function of this type has recently been attributed to operons in several organisms[14–16].

We are able to test whether the β′ subunit alone has the regulatory function suggested. In heterodiploid strains containing KLF10△18 β subunit synthesis is exclusively determined by the chromosomal *rif* gene, subject to normal polymerase operon regulation. A significant part of β′ subunit synthesis, on the other hand, is regulated by arginine rising to a considerable excess (about 2.5-fold) in the *arg*R strain. Thus, if the polymerase operon were self-regulated by the β′ subunit, *arg*R$^-$/KLF10△18 heterodiploids should show a concomitant reduction in the rate of β synthesis. We have been unable to find such a reduction (Table 3, lines 4 and 6) suggesting that repression of the polymerase operon is not achieved by the β′ subunit acting alone at the proposed regulatory site.

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1 Heil, A., and Zillig, W., *FEBS Lett.*, **11**, 165–168 (1970).
2 Matzura, H., Molin, S., and Maaløe, O., *J. molec. Biol.*, **59**, 17–25 (1971).
3 Austin, S., and Scaife, J. G., *J. molec. Biol.*, **49**, 263–267 (1970).
4 Elseviers, D., Cunin, R., Glansdorff, N., Baumberg, S., and Ashcroft, E., *Molec. gen. Genet.*, **117**, 349–366 (1972).
5 Jasper, P., Whitney, E., and Silver, S., *Genet. Res.*, **19**, 305–312 (1972).
6 Cunin, R., Elseviers, D., Sand, G., Freundlich, G., and Glansdorff, N., *Molec. gen. Genet.*, **106**, 32–47 (1969).
7 Austin, S. J., Tittawella, I. P. B., Hayward, R. S., and Scaife, J. G., *Nature new Biol.*, **232**, 133–136 (1971).
8 Jacoby, G., and Gorini. L., *J. molec. Biol.*, **39**, 73–87 (1969).
9 Jacoby, G. A., *Molec. gen. Genet.*, **117**, 337–348 (1972).
10 Matzura, H., Hansen, B. S., and Zeuthen, J., *J. molec. Biol.*, **74**, 9–20 (1973).
11 Morse, D. E., and Yanofsky, C., *J. molec. Biol.*, **44**, 185–193 (1969).
12 Kirschbaum, J. B., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1493–1497 (1971).
13 Hayward, R. S., Tittawella, I. P. B., and Scaife, J. G., *Nature new Biol.*, **243**, 6–9 (1973).
14 Smith, G. R., and Magasanik, B., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1493–1497 (1971).
15 Blasi, F., Bruni, C. B., Avitabile, A., Deeley, R. G., Goldberger, R. F., and Meyers, M. F., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2692–2696 (1973).
16 Kelly, L. E., and Brammar, W. J., *J. molec. Biol.*, **80**, 149–154 (1973).
17 Smith, J. D., Barnett, L., Brenner, S., and Russel, R. L., *J. molec. Biol.*, **54**, 1–14 (1970).
18 Taylor, A. L., and Adelberg, E. A., *Genetics*, **45**, 1233–1243 (1960).
19 Clark, A. J., *J. cell. Physiol.*, **70** (suppl. 1) 165–180 (1967).
20 Silverstone, A., Goman, M., and Scaife, J. G., *Molec. gen. Genet.*, **118**, 223–234 (1972).
21 Scaife, J. G., and Gross, J. D., *Biochem. biophys. Res Commun.*, **7**, 403–407 (1962).
22 Bailey, N. T. J., *Statistical Methods in Biology* (English Universities Press, London, 1968).
23 Anderson, E. H., *Proc. natn. Acad. Sci. U.S.A.*, **32**, 120–125 (1946).
24 King, J., and Laemmli, U. R., *J. molec. Biol.*, **62**, 465–477 (1971).
25 Petri, W. H., *Analyt. Biochem.*, **48**, 442 (1972).
26 Taylor, A. L., and Trotter, C. D., *Bact. Rev.*, **36**, 504–524 (1972).
27 Hayward, R. S., Austin, S. J., and Scaife, J. G., *Molec. gen. Genet.* (in the press).

Regulation of the expression of the histidine operon in *Salmonella typhimurium*

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A positive factor enhancing the expression of this operon binds at the promoter, but exerts its effect downstream on a DNA region which acts as an attenuator of transcription.

The histidine operon of *Salmonella typhimurium* consists of nine structural genes, transcribed into a polycistronic mRNA[1-3] (Fig. 1). There are five unlinked regulatory genes: *hisR, S, T, U* and *W*[1]. Products of these genes are thought to be required for the biosynthesis of a His-tRNA species involved in repression as a corepressor[1,2,4,5]. No gene coding for an aporepressor has been found.

Although most investigators have regarded the histidine operon as a model for negative control[1,2], some data suggestive of positive control have appeared recently[6]. All regulatory mutations tested, including *hisR, S,* and *T,* and promoter-operator mutants appear to carry transcriptional regulatory defects, suggesting that the major control mechanism of the *his* operon is at the level of transcription (unpublished data of myself and P. E. Hartman). Here I report evidence of a positive regulatory mechanism required for transcription of the *his* operon and indicate the possible general importance of such positive control mechanisms.

Transcription of wild-type operon

High frequency transducing phage (φ80 λ ^{imm}d*his*$^+$) carrying the *S. typhimurium* histidine operon was isolated by Voll[7]. The regulatory behaviour of a lysogenic strain indicates that the phage retains the intact regulatory segment (G. R. Smith, B. Tong and B. N. Ames, unpublished results) as well as all the structural genes of histidine biosynthesis (Fig. 2*a*).

Phage DNA containing the wild type histidine operon was transcribed by purified *E. coli* RNA polymerase holoenzyme and the product was fractionated through successive hybridisation steps. After the first-step hybridisation to nontransducing phage DNA (Fig. 2*a*), 60–80% of the remaining ^{3}H-mRNA had originated from the bacterial region of the transducing phage. The bacterial RNA was further hybridised to the separated strains of φ80 λ ^{imm}d*his*$^+$ DNA in the presence of excess cold RNA of various kinds (Fig. 2*b*). The cold competitor RNAs were from the following *Salmonella* strains: *his-515,* which is deleted for the entire bacterial region integrated into the phage genome (Fig. 2*b*); *his-712,* deleted for most of the histidine operon and a short distance beyond[3]; and *his01242,* a deletion of the distal portion of the operator region (Fig. 1) causing high constitutive levels of enzyme[8]. Pulse labelled *in vivo* RNA also was isolated from *his01242* cells and hybridised directly to the separated strains of φ80 λ ^{imm}d*his*$^+$ DNA as in the second step of the *in vitro* RNA fractionation (Table 1*b*). Results are expressed as the percentage of RNA transcribed from the bacterial segment of DNA. As Figure 2*b* shows the percentage of RNA derived from the histidine operon is given as the difference between the third and fourth rows for each DNA strand in Table 1*a* and *b*.

The coding strand of the *his* operon is shown to be the *r* strand of the transducing phage DNA (45% of counts in Table 1*b*). Genes outside the histidine operon, possibly including the gene for gluconate-6-phosphate dehydrogenase

Table 1 Hybridisation competiton *in vivo*

	DNA strand	Cold competitor RNA	Hybrid (^{3}H, c.p.m.)	%	
a	φ80λ^{imm}d*his*$^+$ RNA (^{3}H) hybridised the separate strands of φ80λ^{imm}d*his*$^+$ DNA				
		tRNA	2489		
		his-515	2258	100	
	r	*his-712*	1407	62	
					> 7
		his-01242	1247	55	
		tRNA	1561		
		his-515	1535	100	
		his-712	825	54	
					> 12
		his01242	684	42	
b	*his01242* RNA (^{3}H) hybridised to the separate strands of φ80λ^{imm}d*his*$^+$ DNA				
		tRNA	751		
		his-515	829	100	
	r	*his-712*	619	75	
					> 45
		his01242	246	30	
		tRNA	312		
		his-515	460	100	
	l	*his-712*	222	48	
					> 5
		his01242	198	43	

a, In vitro RNA was prepared as described by Nakanishi *et al.*[35] with some modifications. All incubations were done at 37° C. The transciption mixture contained Tris(hydroxymethyl) amino methane-HCl, *p*H 7.9, 20 mM, KCl 100 mM, dithiothreitol 0.1 mM, ATP, GTO, UTO, 0.15 mM each, ^{3}H-CTP 0.075 mM (specific activity 12.5 Ci mmol, New England Nuclear Corp.), EDTA (*p*H 8.0) 4 mM, $MgCl_2$ 15 mM, rifampicin (Sigma) 10 μg ml^{-1}, φ80λ^{imm}d*his*$^+$ DNA 25 μg ml^{-1} and RNA polymerase 10 μg ml^{-1}. DNA was first incubated for 2.5 min in the reaction mixture containing Tris-HCl, *p*H 7.9, KCl, dithiothreitol, nucleotide triphosphates and EDTA. RNA polymerase was then added and preincubation was followed for another 10 min. The transcription was started with the addition of excess $MgCl_2$ and was allowed to continue for 10 min. Rifampicin was added together with $MgCl_2$ to select for RNA chains initiated by the preinitiation complex. The total volume of the final reaction mixture was 125 μl. After the reaction was terminated by the addition of 0.5 ml of the stopping solution[35] containing DNase I, the reaction mixture was incubated at 0° C for 20 min. 0.5 ml of 0.1 M Tris-HCl, *p*H 7.2, containing 1% sodium dodecyl sulphate (SDS) and one drop of 20 mM acetic acid were added. RNA was isolated with *p*H 5.2 phenol and run through the double layered column of Dowex AG50W-X8 (K$^+$ form) and Sephadex G-25. (ref. 36). RNA polymerase was prepared from *E. coli* N7060 cells lacking RNase I and polynucleotide phosphorylase by the method of Burgess *et al.*[37]. The preparation had no detectable protein contamination on polyacrylamide gel electrophoresis with SDS. Cold *S. typhimurium* RNAs used in competition were isolated from exponentially growing cells. Hybridisations were carried out in two steps as described in Fig. 2. *b*, Each 155,000 c.p.m. (12.5 μg) of *in vivo his01242* ^{3}H RNA was hybridised to the separate strands as described in Fig. 2*b*. Preparation of *in vivo* ^{3}H RNA is described in Table 2.

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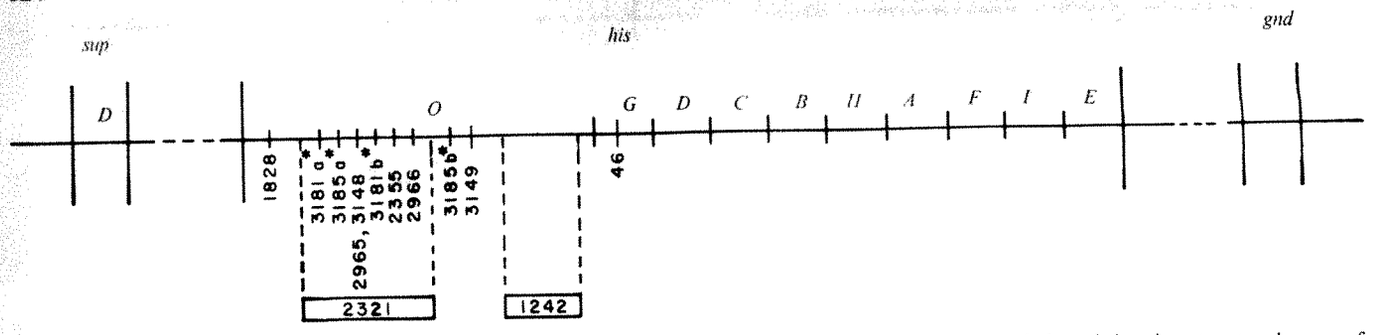


Fig. 1 The histidine operon of *Salmonella typhimurium*. The *his* operator-promoter region has been expanded, and the nine structural genes of the operon, *hisG* through *hisE*, are depicted[3]. Two outside markers, *supD* and *gnd*, are shown. Mutation 46 is a mis-sense mutation in the *hisG* gene; all other numbers refer to operator-promoter mutations. Genetic mapping of the operator-promoter region (*O*), approximately 300 nucleotide pairs long, was carried out by B. Ely, D. B. Fankhauser and P. E. Hartman (unpublished results).
* It is proposed that these mutants have more than one altered site.

(*gnd* in Fig. 2), may be preferentially transcribed from the *l* strand (52% of counts from *l* strand in Table 1*b*). *E. coli* tRNA gave competition no greater than *his-515* RNA, indicating that the entire transcribable region of the bacterial DNA carried by transducing phage is deleted in mutant *his-515*. Compared with the high level of *in vivo* *his01242* expression, the percentage of histidine mRNA henceforth called *his* mRNA) is very low when transcribed *in vitro* on φ80 λ ^{imm}d*his*$^{+}$ DNA as template (*r* strand in Table 1*a* compared with *r* strand in Table 1*b*). Since the level of *his* mRNA is compared with that of the neighbouring genes the results indicate that purified RNA polymerase, for some reason, does not support transcription of the wild type histidine operon to the same extent as transcription of the adjacent DNA regions.

Transcriptional barrier on DNA

Mutation *his01242* has been incorporated into the histidine transducing phage (G. R. Smith, B. Tong and B. N. Ames, unpublished results). When φ80 λ ^{imm}d*his01242* DNA is used as template for purified RNA polymerase, *in vitro* expression of the histidine operon is high relative to that from the external bacterial region and is essentially the same level as that found for *in vivo* expression of *his01242* (Fig. 3*a*). This high rate of transcription *in vitro* is not unique for a particular RNA polymerase or DNA preparation. These results agree with the observation that *his01242* DNA supports much more synthesis of L-histidinol dehydrogenase (encoded by the *hisD* gene) than does wild type DNA in coupled and uncoupled systems of *in vitro* protein synthesis (J. Broach, S. Artz and B. N. Ames, unpublished results). Such results indicate the presence of a DNA region which lowers the expression of the histidine operon *in vitro*. Removal of this region in the deletion mutant *his01242* allows purified RNA polymerase to transcribe the histidine operon at a greatly enhanced frequency.

DNA was isolated from phage carrying the promoter-type mutation, *his02355*. Although *his02355* expression is reduced about 50-fold *in vivo* (B. Ely and Z. Ciesla, unpublished results), φ80 λ ^{imm}d*his02355* DNA gives a level of *in vitro* transcription similar to wild type DNA (Fig. 3*a*). As expected in such experiments, the *l* strand shows low levels of hybridisation to ^{3}H-RNA within the histidine operon (Fig. 3*b*). The low levels found may be the result of read-through from preceding initiation sites or abnormal initiations.

The high level of transcription of *his01242* DNA by purified RNA polymerase indicates that it is neither binding of the RNA polymerase nor RNA chain initiation which is regulated in the DNA region deleted in *his02142*. The DNA region, therefore, must either block the elongation of RNA chains in transcription or expel RNA polymerase from the template in its travel towards the initiation site. If it is the elongation of the RNA chain that is regulated, the positive factor might act as an antiterminator.

Positive factor binds at promoter site

The genetic structure of the regulatory segment of the *his* operon has been determined (B. Ely, D. B. Fankhauser and P. E. Hartman, unpublished results) in conjunction with studies on enzyme levels (B. Ely, personal communication) and mRNA levels (unpublished data of myself and P. E. Hartman). I now focus on the most critical of these observations.

Most of the promoter-like mutations cluster upstream with respect to the *his01242* deletion (Fig. 1). The *in vivo* *his* mRNA levels of some typical promoter mutants with different genetic backgrounds are shown in Table 2. There are two kinds of promoter mutations: class I responds to the mutation *hisT1504* which produces a tRNA^{His} that is still active in protein synthesis but is not active as an effector of the *his* operon[4,5,9,10]. Class II has lost the response to *hisT1504* or retains a very low level of response. Even though they cannot respond well to *hisT1504*, three mutations (class II*B*) respond to essentially the full level of expression when they are combined as double mutants with *his01242* (last column of Table 2). It is extremely difficult to explain this phenomenon by a model exclusively involving

Table 2 Histidine mRNA levels* of promoter type mutants in different genetic backgrounds.

Strain	Type of promoter mutation	Second mutation		
		None	*hisT1504*	*his01242*
0+	Wild type	8	50	100
03181	I	8	33	44
03185	I	5	34	—
03148	IIB	10	4	98
02965	IIB	3	3	94
02355	IIA	4	5	5
02321	IIA	10	8	5
02966	IIB	8	4	92
03149	I	13	22	—

Strains were grown in the E medium of Vogel and Bonner[38] containing 0.1 mM L-histidine (repressed condition). Exponentially growing cells were pulse labelled for 3 min with ^{3}H-5-uridine (New England Nuclear Corp.), and lysed with dodecylamine and SDS (ref. 39). ^{3}H-RNA was isolated with phenol[36] and hybridised to denatured φ80λ^{imm}d*his*$^{+}$DNA and φ80λ^{imm}DNA retained on Millipore filters. The relative amount of mRNA was determined from the initial slope of the *his* operon-specific DNA-RNA hybridisation curve plotted against an increasing amount of *in vivo* ^{3}H-RNA. The methods will be described elsewhere in detail.
—, Not tested.
*The level of *his01242* was taken as 100.

negative control. The RNA polymerase binding site *per se* is not impaired in class II*B* mutants. And, if *his01242* is the site to which the repressor binds, why is it that inactivation of the corepressor in the *hisT* strain does not cause depression as does a deletion in the operator region?

The paradox is resolved by the following considerations. (1) The class II*B* promoter mutations reduce the binding of a positive factor. (2) The positive factor increases the expressibility of the *his* operon in the presence of the DNA region deleted in *his01242*. (3) Class II*B* mutants respond poorly to the *hisT1504* mutation simply because the positive factor is necessary to receive, either directly or indirectly, the altered information from the *hisT1504* mutation. Similarly, class II*B*/*hisG46* (Fig. 1) double mutants grown on histidinol fail to exhibit normal physiological derepression (B. Ely, personal communication).

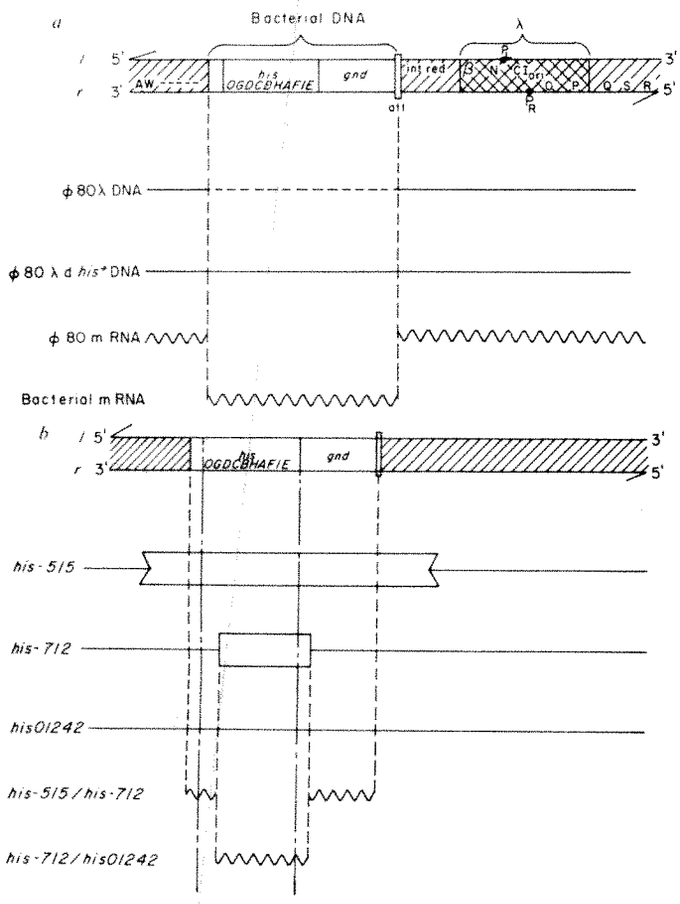


Fig. 2 Separation of histidine mRNA by two-step hybridisation. The strain used to grow the transducing phage was an *E. coli* *his* deletion mutant, doubly lysogenic for φ80λ^{imm}d*his*$^+$ and φ80λ^{imm} phages. φ80λ^{imm} carries the heat-sensitive mutation, CI857. The transduced segment of bacterial DNA carries the *gnd* gene in addition to the *his* operon[7]. This segment is tentatively located to the left of *att* since there is not enough phage chromosome between *att* and *ori* to accommodate the transduced segment. The *ori* function is required for the propagation of this phage[30] and the lower density of the φ80λ^{imm}d*his*$^+$ virion with respect to φ80λ^{imm} (my unpublished data) indicates that the transducing phage has lost more phage DNA than is replaced by the bacterial DNA segment. Phage from heat induced lysogens were purified by polyethylene glycol precipitation[31] and DNA was isolated from both types of phage after separation through CsCl (ref. 32). Strand separation of the transducing phage DNA was done by CsCl centrifugation in the presence of poly(U,G)[33]. Unseparated DNA strands were immobilised for DNA-RNA hydridisation on Millipore nitrocellulose filters (HAWP 047 00, Millipore) according to the method of Gillespie and Spiegelman[34]. Each filter of 6 mm diameter contained 6.7 μg DNA. *a*, in the first step hybridisation, 90,000 c.p.m. of *in vitro* ^{3}H-RNA was incubated with four filters which retain unseparated φ80λ^{imm} DNA strands in 150 μl of 4 × SSC (0.15 M NaCl, 15 mM sodium citrate) containing 2 mM CTP and 0.2 mg *E. coli* tRNA per ml (General Biochemicals Co.). Cold CTP was added to chase from the filter ^{3}H-CTP remaining in the *in vitro* ^{3}H-RNA preparation. Hybridisations were carried out for 15 h at 75° C in small glass vials with screw caps. To determine the proportion of bacterial RNA in this preparation, a 25 μl sample of the first hybridisation mixture was separately transferred to a vial containing two filters of φ80λ^{imm}d*his*$^+$ DNA and φ80λ^{imm}DNA, filled up to 50 μl with 4 × SSC and incubated for 8 h at 75° C. The filters were counted as described below and the purity (*P*) of the bacterial RNA was determined as follows:
P (%) = [(c.p.m. hybrid on φ80λ^{imm}d*his*$^+$DNA − c.p.m. hybrid on φ80λ^{imm}DNA)/c.p.m. hybrid on φ80λ^{imm}d*his*$^+$DNA] × 100
b, After quenching in an ice bath, a 25 μl sample of the first hybridisation mixture was transferred to a second vial with 50 μl of 4 × SSC containing 1.5–3.0 μg ml^{-1} *r* or *l* strand of φ80λ^{imm}d*his*$^+$DNA and 6 mg ml^{-1} of various cold competitor RNAs or *E. coli* tRNA. Hybridisation was continued at 74° C for and additional 7 h. At the end of the incubation, the reaction mixture was diluted with 1.5 ml of 2 × SSC and the DNA-RNA hybrid was collected on a Millipore filter. After treatment with 10 μg RNase A per ml (Worthington) in 2 × SSC at 37° C for 15 min, the filter was dried and counted in a toluene based scintillator.

Under this mechanism, it is clear why all three strains of the class II*B* type regain full expressibility when the barrier against RNA polymerase passage is removed by deletion of the *his01242* region (Table 2). Class II*A* mutations, on the other hand, may affect RNA polymerase binding or both RNA polymerase and positive factor binding. Our values for *in vivo* mRNA production in class II*A* mutants (3–10% in Table 2) are at near-background levels. Sensitive enzyme assays show, in fact, that class II*A* mutants do respond with an increase in enzyme on combination as double mutants with the *his01242* deletion, but they never attain full expression (B. Ely, personal communication).

Attenuator: a common phenomenon?

The above data seem to indicate the presence of a positive factor which binds at the promoter region and exerts its effect downstream on an 'attenuator' region of DNA deleted in *his01242*. The existence of a positive factor-binding mutation in strain *his02966* carrying a mutation located to the right of the presumed RNA polymerase binding site defined by the *his02355* mutation (Fig. 1) makes it likely that the positive factor travels together with RNA polymerase to exert its effect at the attenuator.

The existence of attenuator regions may be a general phenomenon. Extensive deletions in the *E. coli* tryptophan operon that include the region between the operator and the first cistron have a rate of transcription increased two to four-fold *in vivo* for the remaining genes, suggesting that the distal portion of the *trp* operator region limits operon expression[11]. A transcriptional barrier has been detected on λ phage DNA[12]. The *lac* operon might also contain an attenuator which is deleted or altered in UV-5 and similar mutants that exhibit, *in vivo*[13,14] and *in vitro*[15], a reduced requirement for a positive effector protein. In addition each constitutive gene has its own characteristic level of expression, and some constitutive genes are known to be susceptible to 'up-promoter' mutations[16–20]. The existence of an attenuator in certain operons suggests that the potential expressibility of constitutive genes may be much higher and the determination of the constitutive level of expression may be part of the function of the attenuator. A constitutive level of gene expression could either be determined by the base sequence of the attenuator, or by the level of the positive factor. If the DNA sequence of the attenuator solely determines the level of constitutive expression, the necessity of a specific regulatory protein is eliminated.

This model proposes that not only qualitative, but also quantitative information can be coded directly by the

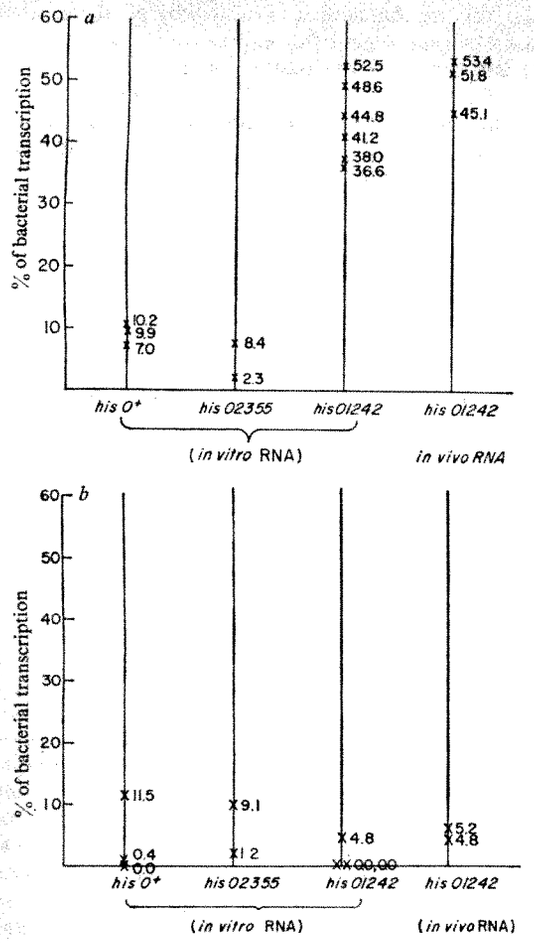


Fig. 3 Level of histidine operon transcription. Individual measurements of the fraction of *his* messenger (*a*) and *his* antimessenger (*b*) in the RNA corresponding to the transduced bacterial segment are plotted with various templates used (*in vitro*) and with the strain *his*01242 (*in vivo*). Methods are described in Tables 1 and 2. *a*, hybridisation to the *r* strand of φ80λ^{imm}-d*his*+DNA; *b*, hybridisation to the *l* strand of φ80λ^{imm}d*his*+DNA

sequence of DNA. Auxiliary protein factors in transcription, positive or negative, may be regarded as means to respond to external information that is superimposed on information built into the DNA polynucleotide sequence. An alternate possibility which is unlikely but is not rigorously excluded is the following. The attenuator could be an RNA chain initiator site (I_1) which requires the positive factor, and I_1 may be followed by a cryptic RNA chain initiation site (I_2) which does not require positive factor. If all RNA polymerase molecules for *his* operon transcription have to travel unidirectionally from the same promoter, the attenuation can be achieved at I_1 by blocking an RNA polymerase molecule which is not accompanied by the positive factor. I_2 may be available for the RNA polymerase only when I_1 is deleted by a deletion such as *his01242*.

Control of *his* operon

I propose that expression of the histidine operon is controlled as follows. Charged His-tRNA, possibly in conjunction with a protein factor, recognises the operon-specific control site on DNA and regulates the binding of a positive factor to the promoter region. This positive factor, in turn, reduces the efficiency of an attenuator which otherwise would limit the frequency of transcription of the operon.

The function of the positive factor as a component of the transcriptional machinery suggests that this molecule is shared by one or more classes of genes, rather than being specific to the *his* operon. Studies of regulatory mutants show that histidine enzyme levels[1] and *his* operon transcription (unpublished results of myself and P. E. Hartman) are regulated by the concentration of His-tRNA. The action of His-tRNA could be effected through interaction with some operon-specific protein such as His-tRNA synthetase[1,21,22] or phosporibosyl-ATP synthetase (EC 421C)[23–26], the target enzyme for feedback inhibition coded by the first cistron of the *his* operon[27–29].

A likely DNA site for the operon-specific control is the most proximal region of the regulatory segment where a large group of constitutive mutations, such as *his01828* (Fig. 1) are located (B. Ely, D. B. Fankhauser and P. E. Hartman, unpublished data). The fact that a single short deletion of the *his01242* region gives rise to full expression makes it unlikely that any dependent negative regulatory mechanism is involved. Thus, the negative control element on the proximal region of the *his* operon probably also exerts its effect through the attenuator. Consequently, it is likely that the binding or action of the positive factor is under the control of the level of His-tRNA. This idea is supported by the observation of a requirement for positive factor-binding site for the expression of a proximally located constitutive mutation, *his01828*. That is, the high enzyme levels elicited by *his01828* are considerably decreased when it is combined in *cis* as a double mutant with a positive factor binding mutation, *his03148* (B. Ely, personal communication).

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1 Brenner, M. and Ames, B. N., in *Metabolic regulation* (edit. by Vogel, H. J.), **5**, 350 (Academic Press, New York, 1971).
2 Goldberger, R. F., and Kovach, J. S., in *Current topics in cellular regulation* (edit. by Horecker, B. L., and Stadtman, E. R.) **5**, 285 (Academic Press, New York, 1972).
3 Hartman, P. E., Hartman, Z., Stahl, R. C., and Ames, B. N., in *Adv. Genet.* (edit. by Caspari, E. W.), **16**, 1 (Academic Press, New York, 1971).
4 Lewis, J. A., and Ames, B. N., *J. molec. Biol.*, **66**, 131 (1972).
5 Singer, C. E., Smith, G. R., Cortese, R., and Ames, B. N., *Nature new Biol.*, **238**, 72 (1972).
6 Wyche, J. H., Ely, B., Cebula, T. A., Snead, M. C., and Hartman, P. E., *J. Bact.*, **117**, 708 (1974).
7 Voll, M. J., *J. Bact.*, **109**, 741 (1972).
8 Roth, J. R., Antón, D. N., and Hartman, P. E., *J. molec. Biol.*, **22**, 305 (1966).
9 Brenner, M., and Ames, B. N., *J. biol. Chem.*, **247**, 1080 (1972).
10 Singer, C. E., and Smith, G. R., *J. biol. Chem.*, **247**, 2989 (1972).
11 Jackson, E. N., and Yanofsky, C., *J. molec. Biol.*, **76**, 89 (1973).
12 Dahlberg, J. E., and Blattner, F. R., in *Virus research* (edit. by Fox, C. F., and Robinson, W. S.), 533 (Academic Press, New York, 1973).
13 Beckwith, J., Grodzicker, T., and Arditti, R., *J. molec. Biol.*, **69**, 155 (1972).
14 Arditti, R., Grodzicker, T., and Beckwith, J., *J. Bact.*, **114**, 652 (1973).
15 Eron, L., and Block, R., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1828 (1971).
16 Müller-Hill, B., Crapo, L., and Gilbert, W., *Proc. natn. Acad. Sci. U.S.A.*, **59**, 1259 (1968).
17 Miller, J. H., Beckwith, J. R., and Müller-Hill, B., *Nature*, **220**, 1287 (1968).
18 Fraenkel, D. G., and Banerjee, S., *J. molec. Biol.*, **56**, 183 (1971).
19 Fraenkel, D. G., and Parola, A., *J. molec. Biol.*, **71**, 107 (1972).
20 Saier, M. H., Young, W. S., and Roseman, S., *J. biol. Chem.*, **246**, 5838 (1971).
21 DeLorenzo, F., and Ames., B. N., *J. biol. Chem.*, **245**, 1710 (1970).

[22] DeLorenzo, F., Straus, D. S., and Ames, B. N., *J. biol. Chem.*, **247**, 2302 (1972).
[23] Blasi, F., Barton, R. W., Kovach, J. S., and Goldberger, R. F., *J. Bacht.*, **106**, 508 (1971).
[24] Kovach, J. S., Berberich, M. A., Venetianer, P., and Goldberger, R. F., *J. Bact.*, **97**, 1283 (1969).
[25] Kovach, J. S., Phang, J. M., Blasi, F., Barton, R. W., Ballesteros-Olmo, A., and Goldberger, R. F., *J. Bact.*, **104**, 787 (1970).
[26] Kovach, J. S., Phang, J. M., Ference, M., and Goldberger, R. F., *Proc. natn. Acad. Sci. U.S.A.*, **63**, 481 (1969).
[27] Ames, B. N., Martin, R. G., and Garry, B., *J. biol. Chem.*, **236**, 2019 (1961).
[28] Martin, R. G., *J. biol. Chem.*, **238**, 257 (1963).
[29] Moyed, H. S., *J. biol. Chem.*, **236**, 2261 (1961).
[30] Stevens, W. F., Adhya, S., and Szybalski, W., in *The bacteriophage lambda* (edit. by Hershey, A. D.), 515 (Cold Spring Harbor Laboratory, New York, 1971).
[31] Yamamoto, K. R., and Alberts, B. M., *Virology*, **40**, 734 (1970).
[32] Matsushiro, A., *Virology*, **19**, 475 (1963).
[33] Hradecna, Z., and Szybalski, W., *Virology*, **32**, 633 (1967).
[34] Gillespie, D., and Spiegelman, S., *J. molec. Biol.*, **12**, 829 (1964).
[35] Nakanishi, S., Adhya, S., Gottesman, M., and Pastan, I., *J. biol. Chem.*, **248**, 5937 (1973).
[36] Kasai, T., and Bautz, E. K. F., *J. molec. Biol.*, **41**, 401 (1969).
[37] Burgess, R. R., *J. biol. Chem.*, **244**, 6160 (1969).
[38] Vogel, H. J., and Bonner, D. M., *J. biol. Chem.*, **218**, 97 (1956).
[39] Meynell, G. G., *Biochim. biophys. Acta*, **240**, 37 (1971).

UGA and non-triplet suppressor reading of the genetic code

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Mutants in a tRNA methylase gene, supK, of Salmonella typhimurium *give suppression of UGA and certain frameshift mutants. In addition suppressors which do not suppress UGA, but which suppress frameshift mutants of opposite (effective) signs are described.*

ADDITIONS or deletions of nucleotides in DNA in other than multiples of three are called frameshift mutations and give out-of-phase translation[1]. Analysis of such mutations has shown the triplet nature of the genetic code[2,3], and has helped in the elucidation of the translation termination signals (or barriers) either created by mutational events within genes[4] or naturally occurring at the ends of genes (W. P. Winter, quoted in ref. 5). Analysis of frameshift mutations has also shown the mutagenicity of various environmental chemicals[6]. We have been interested in seeing if single step mutations can give non-triplet reading at certain codons, because of the insight this may provide into the degree of fixation of the present genetic code in having three as the reading unit, and for the new types of alterations of the translation apparatus which may be revealed.

At an early stage it was found that frameshift mutations could be compensated for, or suppressed, by a second insertion/deletion change close to the site of the original mutation such that the wild type reading frame was restored[2,4]. Suppressors of this type are termed intragenic, or internal. More recently it was found that secondary mutations external to the gene in which a frameshift mutation occurs can also specifically suppress many frameshift mutations[7–11]. In three classes of external suppressor mutants there are alterations in the structural genes for tRNA species and another seems to have a defective tRNA-modifying enzyme[15]. In one mutant the frameshift suppressor tRNA has been shown to have an extra base in its anticodon[16]. Of four frameshift mutants which can be externally suppressed, three[12], and probably the fourth[13], have a single base insertion in a region of single base repeats, as shown by amino acid sequence analysis of the altered protein product in intragenic (+-type) revertants. The external suppressor of at least one of these frameshift mutants acts by reading four bases, including the frameshift site, as a codon[14]. Non-triplet reading can also be influenced by certain mutations in the genes for two ribosomal proteins[17].

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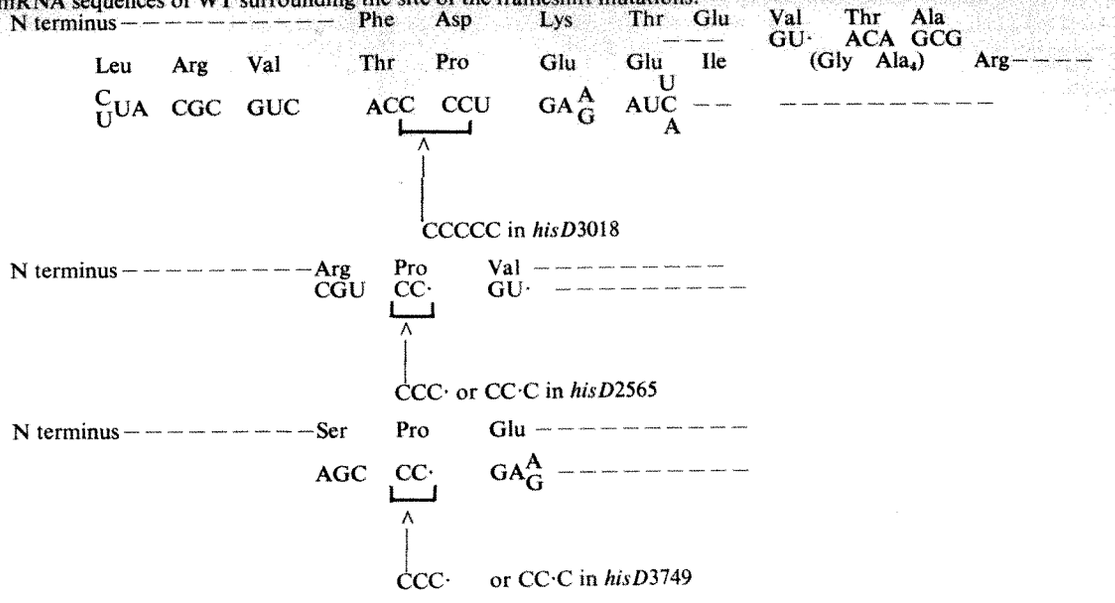
In addition to external suppressors which specifically compensate for frameshift mutations, one class of suppressors has been isolated which suppresses both a frameshift mutation, *trpA*91 of *Salmonella typhimurium*[7], and chain-terminating UGA mutations. We report that this class of suppressor mutations occurs in a gene, *supK*, which encodes a tRNA-modifying enzyme[18,11].

Selection of reversions

The prototype of extensively studied +1 frameshift mutants is *S. typhimurium hisD*3018 (ref. 19). It has a +c insertion type frameshift mutation in the region shown in Fig. 1 (refs. 14, 20). The only external suppressors of *hisD*3018 that have been characterised so far insert proline[15,16] at a CCCU codon at the frameshift site. We decided to test whether *hisD*3018 can also be suppressed by certain UGA suppressors as has been shown for *trpA*91. Strains were constructed, each containing *hisD*3018, *trpA*91 and one of three different *lac*UGA mutations borne on episomes of *E. coli* origin. Spontaneous revertants selected at 30° C and 37° C in the three strains for (1) Lac$^+$ (lactose utilisation) His$^+$ (histidine independence), (2) Lac$^+$ Trp$^-$ (tryptophan independence), (3) Trp$^+$ His$^+$, and (4) Lac$^+$ alone. At either temperature there were revertants in each category which showed phenotypic suppression of the unselected marker, and without exception all reversion to Trp$^+$ His$^+$ phenotypically cross-suppressed the lactose mutations.

Other combinations of mutations were tested for simultaneous reversion of the frameshift and nonsense phenotypes and did not reveal any such cross suppression, though it may be found in more extensive tests. The combinations tried were: — *hisC*50 (amber)[21] *trpA*91 (FS[frameshift]); *hisC*117 (ochre)[21] *trpA*91 (FS); *hisD*3018 (FS) *trpA*91 (FS)/F′ *lac*×82 (amber); *hisC*3736 (FS)[22] *trpA*91 (FS)/F′*lac* UGA (three different Z^- alleles); *hisF*3704 (FS)[22] *tprA*91 (FS) /F′ *lac UGA* (three UGA alleles); *trpA*872 (FS) *hisD*3018 (FS) /F′ *lac* UGA (three UGA alleles). Mutation *trpA*872 is an internal suppressor of *trpA*91,[7] and is not itself suppressed by *trpA*91 external suppressors. In addition strains were constructed containing *trpA*91/F′ *lac* UGA and either *hisD*3749 or *hisD*2565 (Fig. 1), known +c frameshift mutations[12]. No cross suppressors were recovered when *hisD*2565 was the histidine marker, but we were not able to obtain unambiguous results when *hisD*3749 was the histidine mutation.

a, Amino acid and mRNA sequences of WT surrounding the site of the frameshift mutations.



b, Map position.

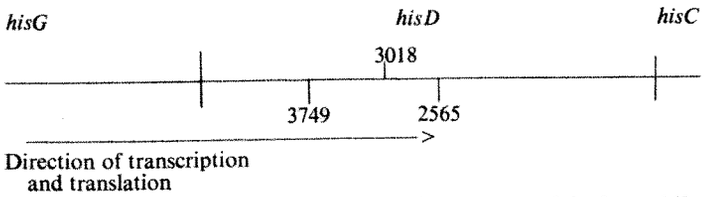


Fig. 1 The nucleotide sequences were deduced from the amino acid sequences of intragenic revertants of the frameshift mutants by Yourno and co-workers[12,14,20]. · Means any base.

Five revertants (nos 610-614) of *hisD*3018*trpA*91/F′*lac*-UGA (Table 1) differing in temperature sensitivity and degree of cross-suppression were selected for further studies. These also had the peculiar property of different abilities to grow on nutrient agar. Since the parent strains grow well at 40° C on nutrient agar, and none of the five revertants do, we selected for back reversions at 40° C on nutrient agar. Revertants with wild type growth rate arose at a frequency of 10^{-8}, and all had simultaneously lost the His$^+$, Trp$^+$ and Lac$^+$ phenotypes as well as their temperature sensitivity. This strongly suggested that all four phenotypic characteristics may be due to a single suppressor-generating mutation. The five revertants (610-614) were then designated *sup*−610 to −614.

Mapping suppressors

Preliminary mapping of *sup*610 to 614 indicated a location in the *argB metK* region. The mutations were further mapped by transduction methods similar to those used previously[7] with P22 or its variants H4 or L4 (ref. 23). The recipients were *hisD*3018 *trpA*91 *pro*−438 *sup*$^+$ (alleles 610–614) /F′ *pro*$^+$ *lac*UGA and the donor phage was grown on *his*C3072 *trpA*91 *leu*-1132 *serA*790 *lys*-554 *sup*$^-$. *sup*$^-$ transductants were selected at 40° C on nutrient agar, approximately 20% of the *sup*$^-$ recombinants were Ser$^-$ Lys$^+$ and 8.5% Ser$^+$ Lys$^-$. Reciprocal transductions with selection for Lys$^+$ and scoring for His$^+$ Trp$^+$ Lac$^+$ and t^s were plated at 30° C and gave consistent results. The suppression and temperature sensitivity characteristics were not separable, thus confirming that in each strain a single mutation was responsible for the pleiotropy. *serA* and *lys* are not cotransducible but the suppressor gene is cotransducible with either marker. Both a dominant frameshift suppressor, *sufD* (ref. 22), and a recessive UGA suppressor, *supK* (ref. 18), have been located in this region between *serA* and *lys*. Derivatives of the *sup*$^+$ (that is, suppressor containing) strains were constructed which were cured of the *F′pro*$^+$*lac*$^-$ episomes, and had the genotypes *hisD*3018 *trpA*91 *serA*790 *sup*$^+$ in their genomes. The strains were infected with the *E. coli* F prime KLF_{16} carrying *serA*$^+$*supK*$^-$*sufD*521*lys*$^+$ with selection for *ser*$^+$. *sufD*52 does not suppress *hisD*3018 or *trpA*91. The F′ ductants were all His$^-$ Trp$^-$ and not t^s. Curing the strains of KLF_{16} restored the His$^+$ Trp$^+$ and t^s characteristic of each strain, thus demonstrating recessivity of the suppressors 610 to 614. This recessivity shows non identity with *sufD*, and is consistent with their being *supK* mutants. Identity with *supK* was confirmed when previously characterised *supK* mutants −584, −1175, and −1176 (ref. 18)

Table 1 Temperature sensitivity and phenotypic suppression characteristics of five His+ Trp+ Lac+ reversions

Phenotypically His+ Trp+ Lac+ Revertants	Phenotype selected originally	Temperature of selection	Growth on nutrient agar at 30° C	at 37° C	at 40° C	Phenotypic suppression of Lac	His	Trp
610	Lac+ Trp+	30° C	Yes	No	No	+++	+	+
611	Lac+ Trp+	30° C	Yes	Yes	No	+++	++	+++
612	His+ Trp+	37° C	Yes	No	No	++	+	+
613	Lac+ His+	37° C	Yes	Yes	No	+++	++	±
614	Lac+	30° C	No	No	No	+	+	+
Parent strains of revertants 610–614			Yes	Yes	Yes	—	—	—

*hisD*3018 *trpA*91 *pro*-438/F′*pro*$^+$*lac*$^-$ UGA (three different UGA alleles)

Strain construction methods were analogous to those described previously[7]. The *pro* mutation was of spontaneous origin and was used to hold in the episome by omitting proline from the medium. The strains were not exposed to any mutagen. All strains were *str*^{s}. +++; single colonies (> 1 mm diameter) in 48 h at the optimum temperature. We estimate +++ as 5–10% efficient based on our own assays of anthranilate synthetase, and comparison with *sup*-584 (see below and ref. 18) for *lac* supression. *F′ pro*+ *lac* UGA were from strains TR289, 294 and 341.

showed similar frameshift and UGA suppression characteristics when cotransduced with *serA* into *his*3018*trpA*91 strains carrying *F'lac*$^-$ *UGA* episome.

Models for *supK* suppression

Cross suppression between UGA and another triplet (UAA) has previously been reported[24,25] but the suppression of UGA and certain frameshift mutations described here is novel. Can these results be explained without invoking non-triplet reading?

One model in which during translation the fidelity of triplet reading may be maintained while at the same time the effect of a frameshift mutation is suppressed is as follows. A frameshift mutation may generate several nonsense codons in the new phase in an mRNA message. Normally the ribosomes do not continue translating the message beyond the first nonsense codon unless either of two conditions are fulfilled: (1) a suitable suppressor for this first nonsense is present in the cell; (2) an internal initiator codon is available in close vicinity. This initiation may be in the neutral, that is, wild-type phase.

Fulfilment of condition (1) at the first nonsense and (2) at the encounter of the second nonsense, if this nonsense is different from the first, would result in the reversal of the effect of a frameshift mutation while maintaining triplet reading. Two polypeptide fragments are then produced.

This model can be applied to suppression of *trpA*91 and *hisD*3018 by the UGA suppressor, *supK* by assuming UGA as the first out of phase nonsense (see Fig. 1) and either UAA or UAG as the second encountered nonsense. Complementation of the two fragments could then give active enzyme. An alternative version of this model, which could lead to the production of overlapping fragments which might be joined into full length polypeptide chains[26], was discussed earlier[7]. Since in both versions of this model the amino acid sequence in the region between the frameshift and the end of the first fragment is unimportant, any amino acid inserted by a UGA suppressor is likely to be acceptable at the UGA site and lead to suppression of the frameshift mutants. We tested three alleles of *supS*, a strong dominant UGA suppressor[27], in partial diploid strains *trpA*91*hisD* 3018*ile*$^-$ *supS*$^-$ /*F* $_{13}$*met*$^+$*ile*$^+$*supS*$^+$ and found no suppression of either frameshift. In addition in an earlier test no internal reinitiation, as detectable by complementation with the available mutants, was found in *hisD* (ref. 28). We therefore favour models in which the suppression of frameshift mutations involves non-triplet reading.

One possibility is that the non-triplet reading occurs not at the frameshift site but at the first nonsense codon encountered thereafter in the out of phase frame, for example UGA· (where · means any base). This could be ruled out if a frameshift which has an amber or ochre as the first downstream out of phase nonsense codon, is suppressed by *supK* suppressors. With the available strains *hisD*3749 and *hisD*2565 we were unable to test this model conclusively (see above).

Alternatively suppression of the frameshift mutants may occur by non-triplet reading close to the frameshift sites. In *supK* mutant strains tRNA is undermethylated and more than one species of tRNA may be affected. The defective modification of one species of tRNA enables it to read the triplet UGA, and of a different species may allow it to read a non-triplet as a codon. It is possible that other components of the translation machinery are undermethylated in *supK* mutants, and may be causing the non-triplet reading. This is, however, unlikely in view of the specificity of the tRNA methylases which have been examined[29]. It is known that *hisD*3018 is a +1 frameshift so presumably its suppression is by quadruplet reading. The sign of *trpA*91 is unknown but the most likely possibilities are that its suppression is by quadruplet or quintuplet reading in *supK* mutant strains (see below).

We find a class of external suppressors for *trpA*91 which are in a gene other than *supK* and which do not also suppress UGA. External suppressors for *trpA*872 have also been isolated. These too are not in the *supK* gene and do not in addition suppress UGA. Since *trpA*91 and *trpA*872 are compensatory frameshift mutants[7], one must be read as a quadruplet and the other may be read as a quintuplet, or possibly as a doublet. A doublet seems unlikely because of the amount of general misreading a doublet suppressor presumably would generate. The reading of four, twice, close to each other to correct the reading is probably only a theoretical possibility in view of the very low efficiency which would be expected. Other types of experiments may have some relevance to how this type of suppression may occur. A ribosome binding experiment gave no evidence for the nature of the adjacent base affecting triplet codon anticodon interaction[30]. But equilibrium dialysis experiments between pentamer oligonucleotides and wild type tRNA show that wild type tRNA does have weak binding to the fifth base (ref. 31 and O. C. Uhelenbeck, personal communication) as well as to the fourth[32]. Presumably a change outside the anticodon loop could make this binding more efficient. This would then be analogous to the situation with the first UGA suppressor tRNA sequenced[33]. But on the basis of the high degree of leakiness of UGA mutants[34,35] compared with that found with the frameshift mutants which have been examined[17], and other considerations it seems more likely that the suppressor activity is due to an insertion in the tRNA anticodon itself.

The study of frameshift mutants in bacteria was initiated when a frameshift specific mutagen, ICR191 an acridine derivative, was found for bacteria[21]. The six frameshift external suppressors which have been characterised in most detail, *sufA–F* suppress frameshift mutations which arose after ICR 191 mutagenesis[22]. *trpA*91 was however isolated after X irradiation and *trpA*872 is of spontaneous origin, and the external suppressors for *trpA*91 and *trpA*872 are in classes which are clearly different (S. R., unpublished) from *sufA–F*. This then raises the possibility that several additional frameshift suppressor classes can be isolated.

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1 Brenner, S., Barnett, L., Crick, F. H. C., and Orgel, A., *J. molec. Biol.*, **3**, 121–124 (1961).
2 Crick, F. H. C., Barnett, L., Brenner, S., and Watts-Tobin, R. J., *Nature*, **192**, 1227–1232 (1961).
3 Terzaghi, E., Okada, Y., Streisinger, G., Emrich, J., Inouye, M., and Tsugita, A., *Proc. natn. Acad. Sci. U.S.A.*, **56**, 500–507 (1966).
4 Barnett, L., Brenner, S., Crick, F. H. C., Shulman, R. G., and Watts-Tobin, R. J., *Phil. Trans. R. Soc. Lond.*, **B252**, 487–560 (1967).
5 Laux, B., Dennis, D., and White, H. B., *Biochem. biophys. Res. Commun.*, **54**, 894–898 (1973).
6 Ames, B. N., Durston, W. E., Yamasaki, E., and Lee, F. D., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2281–2285 (1973).
7 Riyasaty, S., and Atkins, J. F., *J. molec. Biol.*, **34**, 541–557 (1968).
8 Yourno, J., and Heath, S., *J. Bact.*, **100**, 460–468 (1969).
9 Riddle, D. L., and Roth, J. R., *J. molec. Biol.*, **54**, 131–144 (1970).
10 Smith, J. D., *Ann. Rev. Genet.*, **6**, 235–256 (1973).
11 Hartman, P. E., and Roth, J. R., *Adv. Genet.*, **17**, 1–105 (1973).
12 Yourno, J., and Kohno, T., *Science*, **175**, 650–652 (1972).
13 Yourno, J., *Nature new Biol.*, **239**, 219–221 (1972).
14 Yourno, J., and Tanemura, S., *Nature*, **225**, 422–426 (1970).
15 Riddle, D. L., and Roth, J. R., *J. molec. Biol.*, **66**, 495–506 (1972).

[16] Riddle, D. L., and Carbon, J., *Nature new Biol.*, **242**, 230–234 (1973).
[17] Atkins, J. F., Elseviers, D., and Gorini, L., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1192–1195 (1972).
[18] Reeves, R. H., and Roth, J. R., *J. molec. Biol.*, **56**, 523–533 (1971).
[19] Oeschger, N. S., and Hartman, P. E., *J. Bact.*, **101**, 490–504 (1970).
[20] Yourno, J., *J. molec. Biol.*, **48**, 437–442 (1970).
[21] Whitfield, H. J., jun., Martin, R. G., and Ames, B. N., *J. molec. Biol.*, **21**, 335–355 (1966).
[22] Riddle, D. L., and Roth, J. R., *J. molec. Biol.*, **66**, 483–493 (1972).
[23] Smith, H. O., and Levine, M., *Virology*, **31**, 207–216 (1967).
[24] Apirion, D., Phillips, S. L., and Schlessinger, D., *Cold Spring Harbor Symp. quant. Biol.*, **34**, 117–128 (1969).
[25] Ganoza, M. C., Vandermeer, J., Debreceni, N., and Phillips, S. L., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 31–35 (1973).
[26] Apte, B. N. and Zipser, D., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2969–2973 (1973).
[27] Miller, C. G., and Roth, J. R., *J. molec. Biol.*, **59**, 63–75 (1971).
[28] Greeb, J., Atkins, J. F., and Loper, J.C., *J. Bact.*, **106**, 421–431 (1971).
[29] Nishimura, S., *Prog. Nucleic Acid Res. molec.. Biol.*, **12**, 49–85 (1972).
[30] Furuichi, Y., and Ukita, T., *Biochem. biophys. Res. Commun.*, **41**, 797–803 (1970).
[31] Eisinger, J., and Spahr, P.-F., *J. molec. Biol.*, **73**, 131–137 (1973).
[32] Uhlenbeck, O. C., *J. molec. Biol.*, **65**, 25–41 (1972).
[33] Hirsh, D., *J. molec. Biol.*, **58**, 439–458 (1971).
[34] Model, P., Webster, R. E., and Zinder, N. D., *J. molec. Biol.*, **43**, 177–190 (1969).
[35] Roth, J. R., *J. Bact.*, **102**, 467–475 (1970).

LETTERS TO NATURE

PHYSICAL SCIENCES

Narrow-band observations of galactic and extragalactic sources at 1 mm

We report the result of a search for discrete extragalactic sources of continuum radiation near 1.2 mm. The observations were made during a run of one week on the United States National Radio Astronomy Observatory (NRAO) 11-m telescope at Kitt Peak, Arizona.

We attempted observations of a total of 90 known extragalactic objects: three were probably detected (3C84, 3C120, 3C273) and two were possibly detected (3C345, 0727–11). These five objects comprise two quasars, a Seyfert galaxy, an N galaxy and an unidentified radio source. In each case the millimetre emission represents a substantial fraction of the total power of the object, and for 3C120 and 3C345 it may well exceed the power at all other known wavelengths. We also observed a number of well known galactic regions and found millimetre emission in several of them. A detailed description of the observational techniques and discussion of some of the theoretical implications of these observations will be given elsewhere.

Table 1 Probable detections

Source	1.2 mm		Broadband*			
			Present work		Ade, Rather & Clegg	
	Observation time (min)	$\overline{S}\pm\sigma$ (f.u.)	Observation time (min)	$\overline{S}\pm\sigma$	Observation time (min)	$\overline{S}\pm\sigma$
3C84=NGC1275	100	43 ± 11 (3.9σ)	10	28 ± 20	70	25 ± 9.5 (2.7σ)
3C120	70	65 ± 17 (3.8σ)	30	33 ± 9 (3.7σ)		
3C273	140	34 ± 8 (4.2σ)	20	48.5 ± 44	280	22 ± 4 (5.5σ)†
Orion IRB	20	155 ± 23 (6.7σ)‡			130	100 ± 4 (25σ)
Sag B2	10	162 ± 45 (3.6σ)‡				
Sag B2N (position 1′ north of Sag B2)	13	211 ± 35 (6.0σ)‡				

* The flux densities have been calculated assuming a spectrum of the form $P(\nu) \propto \nu^2$ and an effective wavelength of 1.4 mm.
† Earlier detections of 3C273 from broadband observations were reported by Low (ref. 5).
‡ Uncorrected for the effect of an extended nebula.

Table 2 Possible detections

Source	1.2 mm		Broad band			
			Present work		Ade, Rather and Clegg	
	Observation time (min)	$\overline{S}\pm\sigma$	Observation time (min)	$\overline{S}\pm\sigma$	Observation time (min)	$\overline{S}\pm\sigma$
3C345	168	18.5 ± 7.5 (2.5σ)			40	-7 ± 7.5
0727-11	20	201 ± 69 (2.9σ)	7	35 ± 57		
M81=NGC3031			20	40 ± 16 (2.5σ)	40	15.5 ± 8
M82=NGC3034=3C231	60	-12 ± 15	33	17.5 ± 15	250	22 ± 7 (3.1σ)
OR21	40	40.5 ± 14 (2.9σ)			140	32 ± 8 (4.0σ)
OR21N	90	25 ± 10 (2.5σ)				

Table 3 Objects not detected at 1.2 mm. The 2σ upper limits on the flux-densities are ≤ 200 f.u. unless denoted by⁺ (≤ 100 f.u.), * (≤ 50 f.u.), or ** (≤ 30 f.u.)

(*a*) 3C *Quasars:* 3C48*, 138*, 147*, 196*, 220.2*, 232*, 277.1⁺, 279⁺, 286*, 309.1*, 380*
(*b*) 3C *Radiogalaxies:* 3C270, 274**, 348*, 405*
(*c*) *Seyfert galaxies:* NGC1068*, 2782*, 3227*, 3516**, 4051**, 4151*, 5548⁺, Mark 34⁺, 69⁺, 106, 110, 124, 141, 142, 279, 290, 291⁺, 298⁺, 372, 382, 391⁺
(*d*) *N galaxies:* 3C79, 135, 177, 212, 227*, 234*, 318⁺, 371**, 381⁺, 390.3*
(*e*) *QSOs*: BSO2**, B234*, B264, B272*, B340*, B382*
(*f*) *High frequency radio sources:* 4C39.25(Q)*, 0735+17, 1331+170(Q)*, GC1514+19*, OJ287, ON325⁺, OP-192, OQ172(Q)*, NRA0530, OS094(Q)**, BLLAC
(*g,h*) *Galaxies:* NGC253, 676, 2403⁺, 2903, 3368, 3486⁺, 3521, 3623, 4192, 4303, 4472, 4526, 4535⁺, 4654⁺, 4826⁺, 5457⁺, 6946, IC1613⁺
(*i*) *Galactic sources:* Oph A, Crab*

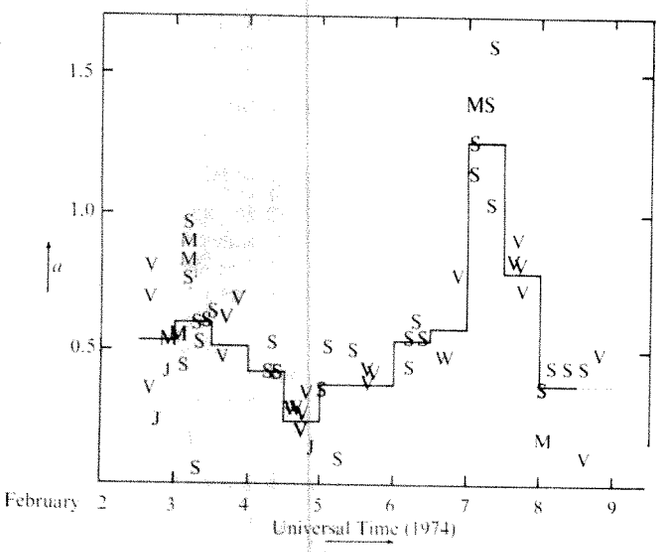


Fig. 1 Atmospheric attenuation coefficient, a, as a function of time during our observing run, from observations of the planets Venus (V), Mars (M), Saturn (S) and Jupiter (J).

In order to study as many types of object as possible, our observing list included: (*a*) all 3C quasars; (*b*) all 3C radio galaxies with $z \leq 0.02$; (*c*) all known Seyfert galaxies with NGC or Markarian numbers and $\delta \geq -2°$; (*d*) all known N galaxies; (*e*) all QSOs with $z \leq 0.2$ in the Braccesi and Formiggini list[1] of optically selected blue stellar objects; (*f*) all known radio sources with $|b| > 10°$ satisfying one or both of the criteria S(1 cm) $\geq$ 4 f.u., S(3 mm) $\geq$ 2 f.u.; (*g*) all galaxies brighter than 11 mag (m_{pg}) with $\delta \geq -2°$; (*h*) all infrared galaxies with S(10 μm) $\geq$ 0.1 f.u., $m_{pg} \leq 12$; (*i*) all galactic radio sources with S(6 cm) $\geq$ 30 f.u. and diameter $\leq 6'$—a total of over 200 objects. Several experimental limitations and problems prevented us from observing more than about half of this list, namely: the location of the Sun; the inability of the telescope to look within 90° of a wind in excess of 20 mile h^{-1} (three nights of our run); intermittent unstable behaviour of the detector/amplifier system; bugs in the computer software. In spite of the resulting selection effects we believe that the sources we observed are a representative sample of the main types of known extragalactic object.

Most of the sources were observed for between 10 and 20 min. A smaller group of 25 sources, comprising the nearest objects in each of the above categories (*a*) – (*h*), together with several radio sources with strong, variable, flat-spectrum emission at high radio frequencies, were observed for between 30 min and 3 h.

We used the NRAO 1-mm continuum receiver (J. D. G. Rather, P.A.R.A., and P. E. C. to be published), beam-switched over 130″. The radiation is detected by a three-part, scandium-doped, germanium bolometer[2]. With its standard low pass filters, this receiver has a response extending from 800 μm to 3 mm. Imperfections in the dish surface act as an additional low pass filter; at 250 GHz, the aperture efficiency is about 12% for an unpolarised feed (the 1-mm receiver is partially polarised). The overall bandpass of the standard filters, atmosphere and telescope will be published elsewhere. Because this bandpass is so large, the mean wavelength depends strongly on the spectral index of the source[3]. For this reason, we used additional filters to restrict the bandwidth. With these filters, the mean wavelength should be close to 1.2 mm for any reasonable spectral index. Thus our observations are the first of extragalactic sources that can genuinely be described as giving narrow band fluxes close to 1 mm (see ref. 4 for line observations of galactic sources at 1.3 mm). For comparison, some sources were observed with only the standard broadband filtering.

We determined the flux scale for the sources, and the attenuation of the atmosphere, by making frequent observations of the planets. Planetary fluxes were computed from the temperatures given by Rather[3], which we believe to be correct to 10%. Venus and Jupiter had to be observed through the wall of the dome, the transmission of which we measured to be 40%. We assumed the attenuation of the atmosphere to be given by $\exp(-a \sec z)$ where z is the zenith distance and a is a function of time. This would be true for monochromatic observations through a plane-stratified atmosphere, and we found this simple model to be a reasonable approximation.

Figure 1 shows how a varied with time during our run, measured from 61 planetary observations taken at different elevations each day. The sudden decrease in transparency on the night of February 7 was due to the arrival of a front, accompanied by light cloud, and shows the importance of frequent calibration at these wavelengths. The solid line shows the mean values of a for 12-h intervals, which we have used in the present preliminary analysis.

The lists of our probable ($\geq 3.5\sigma$) and possible (2.5 to 3.5σ) detections are given in Tables 1 and 2. The few broadband observations we made, and the results of earlier broadband measurements by Ade, Rather and Clegg in February 1973 (previously unpublished) are also shown. The data from each of the several scans on each object were converted to a flux density and corrected by the appropriate attenuation factor, before being combined into an r.m.s. error-weighted mean. ($\overline{S} = (\sum_i S_i/\sigma_i^2)\sigma^2$ where $\sigma^{-2} = \sum_i \sigma_i^{-2}$ and S_i, σ_i are the mean and standard error for an individual scan). A histogram of $\overline{S}/\sigma$ is shown in Fig. 2 for all our observations: apart from the excess of points with $\overline{S}/\sigma \geq 2.5$ the histogram agrees well with the expected Gaussian distribution, indicating that there are no gross systematic errors present. We are confident that the majority of the sources with $\overline{S} \geq 2.5\sigma$ are genuine detections.

Table 3 lists the sources from which no radiation was detected.

Schematic radio, millimetre, infrared and optical spectra have been compiled from the literature for six extragalactic objects (Fig. 3). The Parkes source 0727-11 is at present unidentified: its proximity to the galactic plane means that a galactic origin cannot be ruled out. Although discussion of these spectra must await a more thorough determination of our flux-density scale and effective wavelength, the impression is that we are seeing a similar phenomenon in most of the objects, namely a flux-density rising steeply with frequency in the millimetre region. The fact that 3C273 is the nearest known quasar (this is probably true even if its redshift is not cosmological) and that 3C120 is the nearest N galaxy (it has also been classified as a Seyfert[7] and as an N-type Seyfert[8]) suggests that strong millimetre emission may be a common property among galaxies with active nuclei. (The flux measured from the nearest Seyfert galaxy NGC4051 was 28 ± 12 f.u., on the brink of detectability.) The corresponding millimetre luminosities cannot be determined without more reliable spectral information, but they will be of order

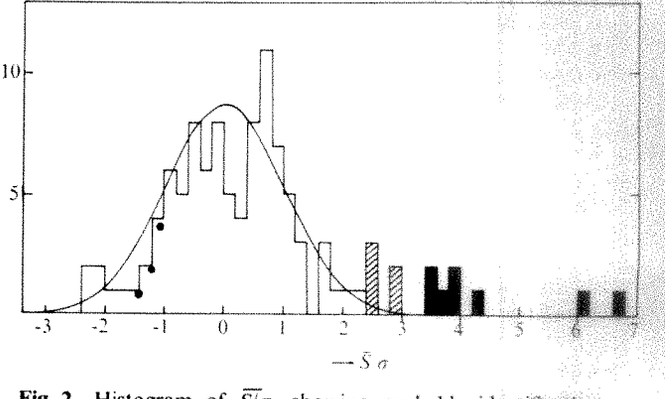


Fig. 2 Histogram of $\overline{S}/\sigma$, showing probable identifications (heavy shading), and possible identifications (light shading).

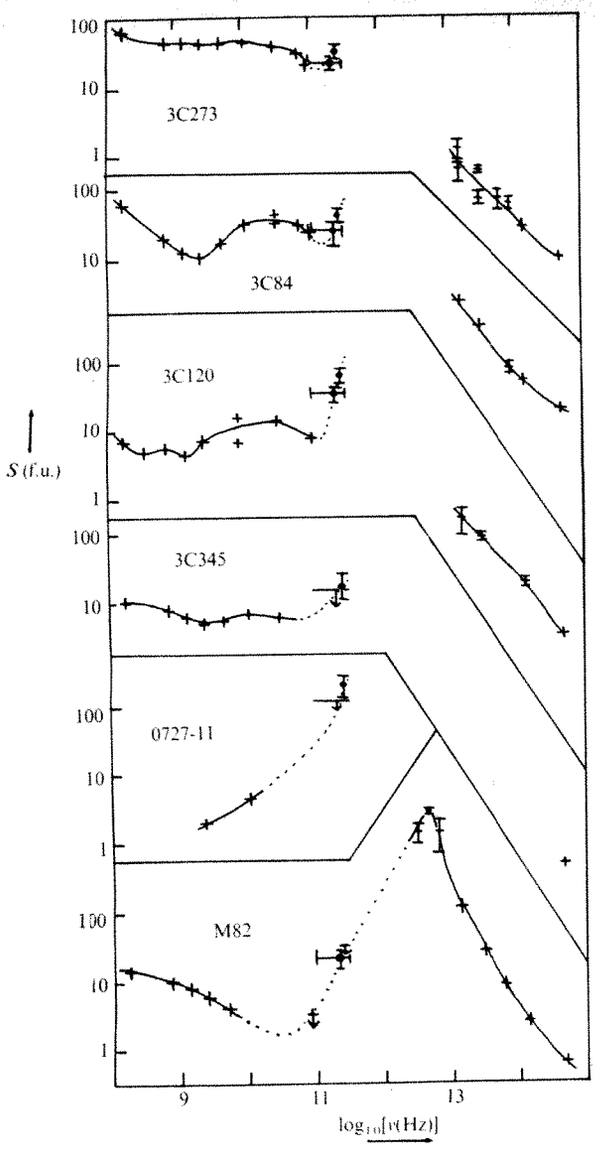


Fig. 3 Schematic radio, millimetre, infrared and optical spectra for detected extragalactic sources. Filled circle with vertical bar, present observations at 1.2 mm; circle with vertical and horizontal bars, broadband.

$P(\nu).\nu$, where $P(\nu)$ is the monochromatic luminosity, and at 1.2 mm, this quantity ranges from $\sim 10^{41}$ erg s^{-1} for M82 to $\sim 10^{47}$ erg s^{-1} for 3C345 if its redshift is cosmological.

The possible detection of the nearby spiral galaxy M81 is extremely surprising, since there is no other optical peculiarity which would explain why it should have a millimetre luminosity more than 10^4 times greater than our own galactic centre. This observation should be treated with caution until a less marginal detection can be achieved.

We stress that the flux densities quoted here are preliminary, although we do not expect the flux scale to be in error by more than 25%. We also emphasise that the search we have made is in no sense complete down to some flux level, owing to the strong dependence of the atmospheric attenuation on zenith distance and time. But among the objects mentioned in this paper, we doubt if we have missed any sources brighter than 200 f.u.

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Note added in proof: Reanalysis of the spectral response of the detector system suggests that the mean effective wavelength for our narrow band observations may be closer to 1.4 mm than 1.2 mm. If this is confirmed, the flux densities in Tables 1–3 need to be reduced by 25%.

[1] Braccesi, A., and Formiggini, L., *Astr. Astrophys.*, **5**, 264 (1971).
[2] Coron, N., Dambier, G., and Leblanc, J., in *Infrared Detection Techniques for Space Research* (edit. by Manno, V., and Ring, J.), (Reidel, 1970).
[3] Ade, P. A. R., Clegg, P. E., and Rather, J. D. G., *Astrophys. J. Lett.* (in the press).
[4] Phillips, T. G., Jefferts, K. B., and Wannier, P. G., *Astrophys. J. Lett.*, **186**, L19 (1973).
[5] Ade, P. A. R., thesis, Univ. London (1973).
[6] Low, F. J., *Astrophys. J.*, **142**, 1287 (1965).
[7] Burbidge, E. M., *Astrophys. J. Lett.*, **149**, L51 (1967).
[8] de Vaucouleurs, G., and de Vaucouleurs, A., *Basic Data on Seyfert galaxies*, Publ. Astr. Dept, Univ. Texas at Austin, Ser. II, Vol. II, No. 7 (1968).
[9] Rather, J. D. G., Ade, P. A. R., and Ulich, B. L., *Icarus* (in the press).

Spontaneous fission on the Ap star HR465

There is an anomalously high abundance of heavy elements on the Cr-Eu subgroup on the Ap star HR465 (refs 1–3). The overall abundance pattern of elements with $Z \gtrsim 40$ indicates that the heavy elements originate from *r*-process nucleosynthesis[3]. In the *r*-process abundance curve there is a broad peak in the rare earth region centred around $Z \simeq 65$ ($A \simeq 165$). The rare earth elements on HR465, however, produce a peak centring near neodymium and samarium at $Z = 60 \sim 62$ (ref. 3); this clearly differs from the trend of the *r*-abundance pattern. Nor does the rare earth peak on HR465 correspond to the *s*-process abundance peak around barium and lanthanum ($A = 135 \sim 140$). Moreover the amount of barium on this star is too small to produce this rare earth peak by the local *r*-process, which begins from the *s*-process peak of barium and lanthanum[4]. Other explanations for the overabundance of rare earth elements on Ap stars do not seem appropriate for HR465 (ref. 1). I examine here an alternative possibility—that the rare earth anomaly on HR465 originates from asymmetric spontaneous fission.

According to the liquid drop model of fission[5], the mass number M_H of the heavy fission fragment produced by the asymmetric spontaneous fission of nuclide (Z, A) is given by

$$M_H = 0.5\,(A - \bar{\nu}) + 0.045A\,(40.2 - (Z^2/A))^{1/2} \qquad (1)$$

where Z^2/A must be less than 40.2, and $\bar{\nu}$ is the average number of prompt neutron per fission event. The $\bar{\nu}$ depends on the excitation energy of fission fragments, but it is approximately given by

$$\bar{\nu} = 0.117A - 25.73 \qquad (2)$$

The numerical coefficients in equation (2) were determined by the least-squares method using the experimental values[6] of $\bar{\nu}$ for spontaneous fission.

The nuclides with $A \gtrsim 180$ can be produced in the Universe only by the *r* process. In the period of *r*-process cooling, delayed spontaneous fission would occur during the successive β decays. Using the value 1.78 for the surface asymmetry constant κ in the expression for the surface energy of a spherical nucleus, implies that all the nuclides in the range $A \gtrsim 265$ ($Z \gtrsim 96$) (ref. 7) would completely disappear in the neutron-rich region far from the β-stability line, because of delayed spontaneous fission with $\lambda_{sf} > > \lambda_\beta$. ($\lambda_x$ is the decay rate of the mode x). As for the nuclides with $250 \lesssim A \lesssim 265$, they become unstable against spontaneous fission faster than they become α-unstable with the approach of the nuclides to the β-stability

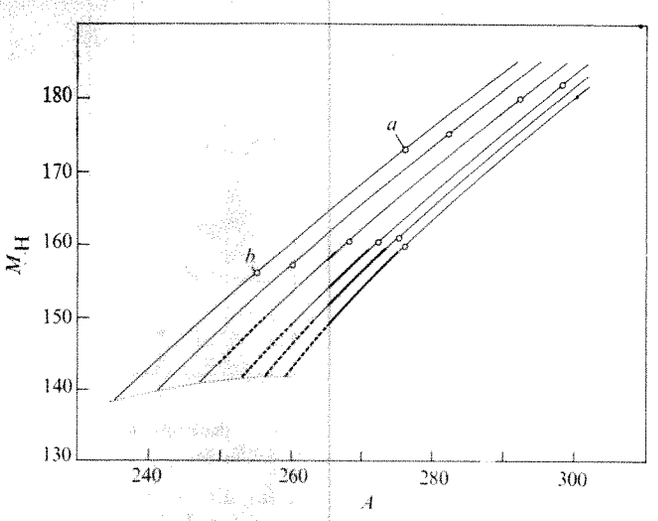


Fig. 1 Mass number M_H of the heavy fragment from the asymmetric spontaneous fission of nuclide (Z, A). Heavy solid lines and heavy dashed lines represent, respectively, the regions for delayed spontaneous fission[7] and regions unstable against spontaneous fission[8]. Small open circles indicate the nearest nearby nuclides to: *a*, Q_n=MeV; *b*, Q_n=4 MeV. . . . . , β-stability line. From left to right, the six curves correspond to: Z=92; Z=94; Z=96; Z=98; Z=99; Z=100.

line ($\lambda_{sf} > > \lambda_\alpha$) (ref. 8). So they would fission completely on the neutron-rich side near the β-stability line. On the other hand, the nuclides with $A \lesssim 250$ can reach the β-stability line safely (see Fig. 1).

The β-stability line was deduced from the relation $Z = (0.3A + 100)A/(A+200)$ (ref. 9) and the nuclides closest to $Q_n = 2$ and 4 MeV are denoted by small open circles. Here Q_n is the binding energy[10] for the last neutron in the nuclide (Z, A), and the *r* process usually proceeds through the neutron-rich nuclides with $Q_n = 2 \sim 4$ MeV (ref. 11). The maximum atomic number attainable on the *r*-process neutron capture path under $Q_n = 2 \sim 4$ MeV is $98 \sim 100$ (refs 7 and 12). The *r*-process cooling takes place from the neutron-rich region of $Q_n = 2 \sim 4$ MeV in the direction of increasing Z, keeping the mass number A constant (vertically downward in Fig. 1). If we adopt $\kappa = 1.78$, the nuclides heavier than 265 completely disintegrate by delayed spontaneous fission in the early period of the cooling to form the abundance peak of heavy fission fragments at around $A = 155 \sim 160$. Almost all the nuclides in the range $250 \lesssim A \lesssim 265$ undergo spontaneous fission in the later period of the cooling and all their fission fragments accumulate around $A = 145 \sim 150$. The Nd-Sm abundance peak on HR465 is situated at $A = 145 \sim 150$; the position of this peak just corresponds to the heavy fragment peak from the spontaneous fission of *r* nuclides with $250 \lesssim A \lesssim 265$. This indicates the possibility of a spontaneous fission origin for the Nd-Sm peak.

In 1960 the measured abundances of these elements on HR465 were Nd≃Sm≃10^6 (ref. 1). I shall express the abundance of elements in a scale of H=10^{12}. To produce the Nd-Sm peak by the spontaneous fission of the nuclides in $250 \lesssim A \lesssim 265$, the average abundance produced, N_1, corresponding to each mass number in the range $250 \lesssim A \lesssim 265$ must be

$$N_1 \simeq 10^6/(Y(265-250)) = 10^6 \quad (3)$$

where Y (≃0.07) represents the fission yield at the heavy fragment peak when the total fission yield is set equal to 2.

The abundance of uranium on HR465 is about 3.5×10^5 (ref. 2). Although we do not have information on the age of this star, the life of main sequence Ap stars is, in general, 10^8 to 10^9 yr. The isotope ^{238}U (4.51×10^9 yr half life) is, therefore, likely to survive almost completely, but the existence of ^{235}U (7.1×10^8 yr half life) is not likely. The numbers of alpha-radioactive precursors for ^{238}U and ^{235}U (including those nuclei) are 3 and 6, respectively[8]. So the range of the average abundance N_2 of the nuclides produced in the range $A \lesssim 250$ is given as follows, assuming two extreme cases of the complete survival and the complete extinction of ^{235}U:

$$3.5 \times 10^5/(3+6) = 4 \times 10^4 < N_2 < 3.5 \times 10^5/3 = 1.2 \times 10^5 \quad (4)$$

From equations (3) and (4), the *r* nuclides with $250 \lesssim A \lesssim 265$ must be produced with abundance higher than that of the nuclides with $A \lesssim 250$ by a factor of 8 to 25.

The abundance peaks of the *r* nuclides at $A \simeq 130$ and 195 correspond to the neutron magic numbers of $N=82$ and 126, respectively. The abundance of the nuclides situated on these peaks is greater than the background abundance by a factor 10 to $10^{3/2}$. Although a precise estimate of the next neutron magic number or submagic number after $N=126$ is difficult at present, $N=164$ (ref. 13) or $N=170$ (ref. 14) have been proposed. If the *r*-process proceeds through the path of $Q_n=3$ MeV, the abundance (sub)peak due to the neutron (sub)magic number would be formed at around $A=250 \sim 252$ when we adopt $N=164$ as a neutron (sub)magic number, and at around $A=258 \sim 260$ when we adopt $N=170$. These peak positions were estimated by using the mass table presented by Seeger and Perisho[10]. In any case the *r*-process make an abundance (sub) peak in the region $250 \lesssim A \lesssim 265$. So the produced abundance of the nuclides in the region $250 \lesssim A \lesssim 265$ would be expected to be higher than that in $A \lesssim 250$ by about a factor 10 to $10^{3/2}$. This explains the high abundance ratio N_1/N_2.

Thus the overabundance in the rare earth region centred near neodymium and samarium can be explained by the asymmetric spontaneous fission of *r* nuclides with $250 \lesssim A \lesssim 265$, which takes place in the later period of the *r*-process cooling. In this case the mass number M_L ($=A-\bar{\nu}-M_H$) of the light fragment from the spontaneous fission is broadly distributed in the range $103 \lesssim M_L \lesssim 112$. Elements such as Rh, Pd, Ag, Cd and In ($Z=45 \sim 49$) are situated in this mass region, and each element is estimated to have the high abundance of at least $\sim 10^6/5 = 2 \times 10^5$. In fact the abundance of palladium on HR465 is extremely high[3]. The abundance determination of Rh, Ag, Cd or In is needed for the more affirmative conclusion for the spontaneous fission origin of the rare-earth peak on HR465. Furthermore we can also expect the high abundance of lead and bismuth on this star since they are the final stable elements of radioactive decay chains in $A \gtrsim 210$.

There are some peculiar A stars which have anomalously high abundances of thorium and uranium[15-18]. An abundance peak centred near neodymium and samarium similar to that on HR465 may be observed also on these Ap stars.

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1 Aller, M. F., *Astr. Astrophys.*, **19**, 248 (1972).
2 Cowley, C. R., and Hartoog, M. R., *Astrophys. J. Lett.*, **178**, L9 (1972).
3 Cowley, C. R., Hartoog, M. R., Aller, M. F., and Cowley, A. P., *Astrophys. J.*, **183**, 127 (1973).
4 Guthrie, B. N. G., *Astrophys. Space Sci.*, **3**, 542 (1969).
5 Swiatecki, W. J., *Phys. Rev.*, **100**, 936 (1955).
6 Gindler, J. E., and Huizenga, J. R., *Nuclear Chemistry, II*, (edit. by Yaffe, L.), 1 (Academic Press, New York, 1968).
7 Boleu, R., *Nucl. Phys.*, **A201**, 401 (1973).
8 Viola, V. E., jun., *Nucl. Phys.*, **A139**, 188 (1969).
9 Myers, W. D., and Swiatecki, W. J., *Nucl. Phys.*, **81**, 1 (1966).
10 Seeger, P. A., and Perisho, R. C., *LA-3751*, (1967).
11 Schramm, D. N., and Fiset, E. O., *Astrophys. J.*, **180**, 551 (1973).
12 Howard, W. M., and Nix, J. R., *Nature*, **247**, 17 (1974).
13 Köhler, M. S., *Nucl. Phys.*, **A162**, 385 (1971); **A170**, 88 (1971).
14 Nilsson, S. G., *et al.*, *Nucl. Phys.*, **A115**, 545 (1968), and **A131**, 1 (1969).
15 Jaschek, M., and Malaroda, S., *Nature*, **225**, 246 (1970).
16 Adelman, S. J., *Astrophys. J. Suppl.*, **26**, 1 (1973).
17 Hartoog, M. R., Cowley, C. R., and Cowley, A. P., *Astrophys. J.*, **182**, 847 (1973).
18 Adelman, S. J., and Shore, S. N., *Astrophys. J.*, **183**, 121 (1973).

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Copernicus spectra of β Lyrae

WE have observed the far ultraviolet spectrum of β Lyrae using the Princeton spectrometer, on the Copernicus satellite[1]. The principal data were scans in the low resolution mode (0.2 Å in the (U2) region 1,000–1,500 Å and 0.4 Å in the (V2) region 2,000–2,900 Å) at phases nearly coincident with the two light minima. Shorter scans (1,100–1,300 Å) were obtained at the two quadrature phases. All observations were made in August and September 1973 but not in the same orbital cycle.

The highest signal-to-noise ratio was obtained in the region 1,000 to 1,300 Å (Fig. 1). Beyond 1,300 Å the instrument response falls rapidly and only the strongest spectral features were detectable. In the longer wavelength region, the signal-to-noise ratio is considerably lower because of high background particle counts; here too, only major spectral features were identifiable.

The spectrum is spectacularly different from B8, the classification given in the visible region. Copernicus scans of normal stars later than about B2 show a rich absorption spectrum, particularly for those of high luminosity. The far ultraviolet spectrum of β Lyrae contains many emission lines, some with P Cygni-type profiles, some of very high excitation, but none with radial velocities associating them with the primary B8 star. It seems that the spectrum in this region is predominantly from a hotter source than the visible primary, supporting several recent suggestions[2–5] that the secondary component in β Lyrae is the hotter object. The recently revived[6] idea of an F-type secondary seems to be eliminated.

A few absorption lines corresponding to the B8 spectrum are observed longwards of 2,400 Å. Multiplet 1 of FeII is present completely in absorption, and estimates of its velocity indicate an amplitude (in phase with the B8 spectrum) of 340 km s^{-1}. The primary star velocity amplitude from the ground based data is 370 km s^{-1} (ref. 7). The strongest transitions from the excited levels of SiIII and SIII are also seen in absorption in this part of the spectrum, with a velocity range of 380 km s^{-1}.

The far ultraviolet spectrum is unique among those so far observed by Copernicus in the number and strength of emission lines. Some 50 are definitely identified; an equal or greater number remains in doubt because of blending effects, or low instrumental response. Figure 1 illustrates a region of the spectrum fairly free of overlap; profiles range from classical P Cygni-type to those which resemble more a broad emission seen on both sides of a shortward-shifted central absorption. (This latter type of profile is also seen at Ha, ref. 8). These profiles are typical of a rotating, outward moving envelope with expansion velocities of ~ 150 km s^{-1} (see refs 9 and 10). A rapidly rotating hot secondary with an outward moving envelope (in its equatorial regions), or an expanding shell around the system, is suggested.

A small velocity variation is found in the absorption and emission turning points of these profiles. In Table 1 we give the mean values derived from some 15 features which are thought to be free of blending effects. These velocity values are similar to those of the shell absorptions and emission peaks in the visible spectrum[7] at, for example, Hγ or HeI 3,889.

These numbers presumably do not represent the motion of the secondary alone, and probably are affected by mass exchange and other motions within the system.

The continuum is generally confused by overlapping emission, but its position may be estimated at 1,220, 1,150, 1,300 and 1,100 Å, in decreasing order of certainty. The flux levels, corresponding to a mean wavelength of 1,200 Å are given in Table 2.

These figures are in good agreement with the turning points of the OAO-2 light curves[2]. They show that the quadratures have unequal light and that the secondary

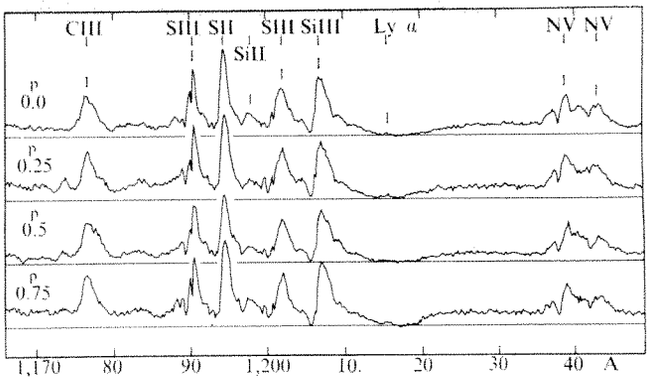


Fig. 1 Portions of Copernicus OAO spectrum scans of β Lyrae at four orbital phases.

minimum is the deeper one in the far ultraviolet. This is in keeping with the evidence that the hot radiation associated with the secondary dominates this part of the spectrum.

The emission lines do not show any marked eclipse effects. But changes are indicated in the absolute peak intensity of some lines and these are generally in the sense of being stronger than normal (by about 30%) at phase 0.0. This is comparable with the continuum changes, and evidence that the emission lines are not associated with the primary.

Table 3 summarises the strong line features indentified. The Lyman lines are very broad (>10 Å), go to zero central intensity and are the only features without emission. They are similar to the Lyman lines seen in other Copernicus data and are presumably interstellar and/or circumstellar.

Helium is not conspicuous; the λ1,085 feature may be ascribed either to HeII or a close resonance multiplet of NII. The feature is strong (peak intensity 8 to 10 times continuum) and complex, so it may be a blend.

The NII and CII lines in the V2 region showing stellar absorption velocities come from excited multiplets. The emission in the U2 region of CII and CIII (and NII if it is present at 1,085 Å) are from zero excitation multiplets.

The most important features are the NV lines, which indicate very high excitation. The presence of these lines was suspected by Houck[11], who found a variable emission redward of Ly α on OAO-A low resolution scans. But he favoured the interpretations at Ly α emission, because of the high excitation of NV. Our data do not display the strong phase variation of these features found by Houck; a nonperiodic change in the system may be indicated. In our data, the profiles and velocities of the NV lines differ somewhat from those of most other lines, so that the features may originate in a different part of the system.

There is no definite evidence for the presence of other lines of high excitation. No lines of NIV, CIV, OIV, or OV lie in the well observed region of the spectrum. There is an OVI line at 1,032 Å, and although a line is seen at this wavelength, there is no sign of the other component of the resonance doublet at 1,037 Å. The 1,032 Å line can be attributed to FeIII.

The Si and S lines identified are all strong features (Fig. 1). Stellar absorption components of SiII and SiIII are suspected in the V2 region. A broad emission at 1,344 Å may be the PIII (1) resonance triplet.

The spectrum of FeIII is strong and all multiplets from 1 to 59 are seen, when their lines are suitably located. The spectrum is unusual in emission in stellar spectra, and it is interesting that P Cygni itself shows FeIII emission in the visual and photographic spectral regions.

Table 1 Mean line radial velocities

Phase	0.0	0.25	0.5	0.75
Absorption (km s^{-1})	−175	−125	−140	−135
Emission (km s^{-1})	105	165	150	160

The spectrum of FeII is very rich in the observed region, but is not definitely present in our data. Lines of FeII (primary absorption) are seen in the V2 region. The presence of other iron peak ions (TiIII, IV, CrIII, MnII, III) is possible, but not certain, primarily because of blending with FeIII.

What do these observations tell us of the nature of the secondary component of β Lyrae? There is little doubt that it is a hotter object than the visible B8 star, from the presence of high temperature lines and continuum, whose velocities and intensities do not follow the behaviour of the visible primary star. The velocity amplitude of the lines suggests that the secondary is the more massive object, by a factor of several times. But, it is evident that the observed line features originate in an extended region around the secondary, which may be highly rotating, and expanding in its outer layers.

Table 2 Continuum light level at 1,200 Å

Phase	0.0	0.25	0.5	0.75
Light	0.81	1.00	0.75	1.10

The spectrum of the massive secondary is invisible in the terrestrial spectrum, either because it is shielded by the extended envelope, or because the B8 star is overluminous. The latter possibility is a strong one if the system is near the end of case B mass exchange. The system would then be rather similar to the Algot-type semidetached eclipsing binaries, and its evolutionary history would be simple. A model of this type was proposed by Kriz[4], using the computations of Kippenhahn and Weigert[12], and more recently by J. Ziolkowski (unpublished). But overall consistency in mass, luminosity, spectral type and rate of period change, has not yet been attained.

Overluminosity of the primary, however, cannot account for all the observations. If the secondary is a BO–B2 main sequence star, as sugggested by Kriz, one must assume rapid and probably differential rotation[5]. R. E. Wilson (unpublished) has interpreted the V light curve of β Lyrae using a model in which the secondary is a highly flattened rotating disk. Most of the radiation generated by the star emerges in the direction of the rotational axis, and the polar regions have the highest effective temperature, of some 11,000 to 15,000 K.

Such a temperature is insufficient to excite NV. A Copernicus survey of early-type spectra, communicated to us by D. C. Morton, shows that the NV lines are seen only in stars of type BO.5 and earlier. A model incorporating a hot spot caused by impact of mass exchange particles on the secondary, or its envelope, seems to be eliminated by the lack of strong phase dependence of the line strengths. In addition, the light curve and geometry of the mass exchange raise further objections to a hot spot hypothesis.

The differential rotation model then would require the NV lines to originate outside the orbital plane, and would also require a higher effective temperature than Wilson's model provides. An adjustment in this sense would result from introduction of a hydrogen deficiency in the primary. A similar star, KS Persei, which has a more marked hydrogen deficiency, has an apparent spectral type A5, at variance with the effective temperature 10,000 K (ref. 13). It is not clear whether such an adjustment would make Wilson's secondary hot enough.

Another serious problem is that of the reversal of eclipse depths at wavelengths below about 1,350 Å (ref. 2 and above). Wilson[3] has pointed out that classical models of thermally radiating stars cannot account for this systematic variation. It is not known whether Wilson's differentially rotating model can achieve this.

An alternative representation of the system is one in which the secondary is a black hole. If matter is transferred

Table 3 Observed features

H	Ly α, β broad interstellar absorption.
HeI	Seen only at 2,829 and 2,945 Å.
HeII	Possibly present at 1,085 Å.
CII, III	Present; also primary absorption in V2 region.
NII	Possibly present at 1,085 Å; primary absorption in V2.
NV	Highest excitation found: 1,238 and 1,242 Å.
MgII	Resonance lines at 2,800 Å; P Cyg profiles.
SiII, III, IV	Well represented.
SII, III, IV	Well represented.
FeIII	Well represented; other iron peak elements?

to the vicinity of a black hole at a rate higher than $10^{-9} M_\odot$ yr^{-1}, an accretion disk will be formed of sufficient density to absorb X radiation from infalling material[14,15]. This radiation then appears as a hot continuum, and radiation pressure is high enough to accelerate the excess of matter transferred over mass accreted, away from the system. They also predict the appearance of strong recombination lines of highly ionised elements, with no apparent ionisation source. The high ultraviolet luminosity also ionises the interstellar hydrogen around it, forming an extended Strömgren region. The observations described, the fact that β Lyrae is a thermal radio source[16], and the mass-exchange rate of 10^{-4} to $10^{-5} M_\odot$ yr^{-1}, fit this picture remarkably well.

A complication of a black-hole model, which β Lyrae shares with other binary candidates such as Cygnus X-1, is its evolutionary history[17]. If, as is often supposed, formation of a collapsed object is associated with a supernova explosion, it is necessary to explain the present small separation and circular orbit of the components. Side stepping this point, we may sketch a possible history of the system as follows. The originally more massive star underwent extensive mass loss to its companion after its main sequence lifetime, leaving a He burning core and producing a He rich envelope on its companion. The He core should finish its evolution (as a black hole) long before the 10^6 to 10^7 yr required for the companion (the present primary) to reach the end of its main-sequence lifetime. We now see the second era of rapid mass exchange, in which the He rich layers of the present primary are transferred to an accretion disk around the collapsed companion.

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1 Rogerson, J. B., Spitzer, L., Drake, J. F., Dressler, K., Jenkins, E. B., Morton, D. C., and York, D. G., *Astrophys. J.*, **181**, L97 (1973).

2 Kondo, Y., McCluskey, G. E., and Houck, T. E., *The Scientific Results from the OAO-2* (edit. by Code, A. D.), 485 (NASA, 1972).

[3] Wilson, R. E., *Nature*, **234**, 406 (1971).
[4] Kríz, S., *Nature*, **245**, 36 (1973).
[5] Stoc ers, R., and Lucy, L. B., *Nature*, **236**, 218 (1972).
[6] Skulsky, M. J., *Soviet Astr.—AJ*, **15**, 606 (1971).
[7] Sahade, J., Huang, S. S., Struve, O., and Zebergs, V., *Trans. Am. Phil. Soc.*, **49**, 1 (1959).
[8] Batten, A. H., and Sahade, J., *Publ. astr. Soc. Pacif.*, **85**, 599 (1973).
[9] Hutchings, J. B., *Mon. Not., R. Astr. Soc.*, **155**, 55 (1970).
[10] Conti, P. S., and Leep, E. M., *Astrophys. J.* (in the press).
[11] Houck, T. E., *The Scientific Results from OAO-2* (edit. by Code, A. D.) 479 (NASA, 1972).
[12] Kippenhahn, R., and Weigert, A. Z., *Astrophys.*, **65**, 251 (1967).
[13] Danziger, I. J., Wallerstein, G., and Böhm-Vitense, E., *Astrophys. J.*, **150**, 239 (1967).
[14] Pringle, J. E., *Nature*, **243**, 90 (1973).
[15] Shakura, N. I., and Sunyaev, R. A., *Astr. Astrophys.*, **24**, 337 (1973).
[16] Wade, C. M., and Hjellming, R. M., *Nature*, **235**, 71 (1972).
[17] McCluskey, G. E., and Kondo, Y., *Astrophys. Space Sci.*, **10**, 464 (1971).

Behaviour of Comet Kohoutek (1973f)

OBSERVATIONS of Comet Kohoutek have revealed the presence of CH_3CN (ref. 1) and HCN (W. Huebner, private communication) in the coma and of H_2O^+ in the tail[2]; although predicted, H_2O was not observed[3].

The spectral identification of CH_3CN and HCN is the first direct support for the hypothesis first proposed by Wurm[4], of chemically stable 'parent molecules' for the less stable radicals and ions seen in the coma and the tail. Either of these molecules could be the parent molecule of CN, which is conspicuous in almost all comets at $\lambda \approx 3{,}883$ Å. Their radiative dissociation schemes are the following[5,6]:

$$CH_3CN + h\nu \rightarrow CH_3 + CN^*(\lambda \lesssim 2{,}160\ \text{Å}) \quad (1)$$

$$CN^* \rightarrow CN + h\nu(0\text{–}0\ \text{band emission near} \approx 3{,}883\ \text{Å})$$

$$HCN + h\nu \rightarrow H + CN\ (\lambda \lesssim 1{,}900\ \text{Å}) \quad (2)$$

These two molecules were among the earliest discovered in dense interstellar clouds[7]. This does not establish an interstellar origin for comets but seems suggestive of the environment in which comets, or more specifically their embryonic grains, condensed out of the gas phase. These two molecules are also among the most abundant products in the gas phase found in sparkings of simulated primitive planetary atmospheres[8].

The H^2O^+ (most probably formed by photoionisation of H_2O at wavelengths shortwards of about 984 Å; ref. 9); strongly suggests the presence of the latter as another parent molecule. Circumstantial evidence for the presence of H_2O as the dominant volatile component in the nucleus has been growing for some time. The strongest line of evidence has come from the recent ultraviolet and near violet observations of Comets Tago–Sato–Kosaka (1969g), Bennett (1969i), P/Encke, and now Kohoutek (1973f), all of which show strong emission from the two species H(Ly α) and OH($\sim$3,040 Å) (refs 10, 11 and unpublished results from J. E. Blamont and M. Festau). Detailed models of the coma computed on the basis that H and OH are the photodissociation fragments of H_2O seem to explain rather well the observed Ly α brightness distribution[12,13] and the distortion of the isophotes[14]. Under typical cometary conditions with a large abundance of H_2O, other parent molecules would be present in the nucleus as clathrate hydrates, since these are thermodynamically more stable than their constituents[15]. Since the potential wells in which the 'guest molecules' are trapped in the H_2O ice lattice in the clathrate are very deep, they can be released only by the destruction of the 'host' lattice and consequently their vaporisation is controlled by the latent heat of vaporisation of H_2O. This explains the almost simultaneous appearance of all the major cometary emission bands (typically around 3 AU for most comets) although the volatilities of the assumed parent molecules differ by more than ten orders of magnitude, provided that the abundance (by number) of the H_2O molecules exceeds all others by about six to one. This is because the clathrate lattice can typically trap only one 'guest' molecule per six 'host' H_2O molecules. The large abundance of OH ($\gtrsim 90\%$ by number of all the radicals) observed in Comets Bester (1949k) (ref. 16) and Tago-Sato-Kosaka (1970g) (ref. 17) supports this view.

The behaviour of Comet Kohoutek cannot be explained by a pure clathrate nucleus with H_2O exceeding the other molecular species by more than 6 to 1. This is because an extensive dust halo was seen at a distance $R \gtrsim 4$ AU where the vapour pressure of H_2O or the clathrate would have been negligible[18]. A much more volatile species is required. Carbon monoxide, which is one of the most abundant molecules discovered in interstellar space and whose ion CO^+ is probably the most abundant species in cometary tails, is a likely candidate. Formaldehyde, another abundant interstellar species which may be a 'parent' for CO according to the scheme[5] $H_2CO + h\nu \rightarrow H_2 + CO(\lambda \lesssim 3{,}000$ Å), is also a possibility. Methane is a not unlikely alternative. Indeed, the behaviour of Comet Kohoutek can be understood in terms of a nuclear model in which the H_2O exceeds the more volatile species by a factor less than 6:1 so that a fraction of these are not trapped in the clathrate lattice. It is also likely that these more volatile species were concentrated in an outer shell because of a slow outward diffusion. As the comet approached the Sun these volatile species would quickly evaporate around $R \approx 5$ AU, throwing out a dust halo which probably consists chiefly of clathrate hydrate grains, possibly also admixed with grains of the volatile species and more refractory, 'earthy' material. The extension (l) of this dust corona is not determined by the lifetime of the grains against evaporation at 5 AU because this would be too large for any reasonably sized grains (being about 5×10^{10} s for 10-μm grains). Rather it would be determined by their velocity. Taking $l \approx 10^4$ km and $v \approx 0.1$ km s^{-1} gives the time duration of mass ejection as $\approx 10^5$ s (≈ 1 d, which seems reasonable). Assuming that the injection rate of dust is β times that of the gas (Q_{gas}) and that the dust coma is optically thin, the 'effective cross section' of the dust coma for scattering of solar radiation is

$$A_c = \beta Q_{gas} \sigma_g \ \Delta t / m_g = \tfrac{3}{4} \beta \frac{(Q_{gas}\, \Delta t)}{(r_g\, \rho_g)} \quad (3)$$

where Δt is the duration of emission of the volatiles and σ_g, r_g, m_g and ρ_g are respectively the average cross section, radius, mass and density of a grain. Since $Q_{gas} = Z_{gas}\, m_{gas}\, A_n$, where A_n is the nuclear cross section, m_{gas} is the molecular mass of the voltaile and Z_{gas} is the vaporisation rate (molecules cm^{-2}s^{-1}), the ratio α of A_c to A_n is

$$\alpha = \tfrac{4}{3} \beta \frac{(Z_{gas}\, m_{gas})}{(r_g\, \rho_g)} \Delta t \quad (4)$$

Assuming that the volatile in question is CH_4 (which is representative of the highly volatile class and for which reliable data is available[19]), and putting $\Delta t \approx 10^5$s, $\beta \approx 0.1$, $r_g \approx 10$ μm, gives $\alpha \approx 40$. In spite of the overall uncertainty in this value arising from our guessed estimates, this shows that the 'effective cross section' at around 5 AU could have been one or two orders of magnitude larger than the nuclear cross section. Consequently, the earlier estimates of the radius could have been too large by a factor of about three to ten, accounting for the later disappointment.

If the highly volatile material involved is small, this activity will quickly cease and a much less volatile cometary nucleus consisting of clathrate hydrates will be exposed, which will then behave in a 'normal' way after the early 'anomalous' brightening

Comet Kohoutek is by no means unique in its behaviour and five out of a set of about 50 long period comets seem to show anomalous brightenings in 4 AU $\leq R \leq$ 5 AU (ref. 20). A much larger sample (about 75% of a total of 79 comets passing perihelion in a period of 40 yr) exhibited flare activity at smaller heliocentric distances, often repeatedly[21,22], and indeed a significant fraction of all long period comets may be first observed during such flares[23]. These flares, as well as the periodic outbursts from P/Schwassman–Wachmann 1, which moves in a nearly circular orbit with perihelion distance $\approx$ 5.5 AU, can be explained in terms of pockets of the highly volatile material contained within the clathrate lattice, being exposed to solar radiation from time to time as the overlying material evaporates. Other explanations of this phenomenon have been suggested[24,25] but they are less direct.

Any attempt such as this to explain the differences of cometary behaviour in terms of differences in composition runs into the difficulty of having to explain why such differences in composition should exist, and this can only lead to further speculation because next to nothing is known about the spatial regions and therefore the physical and chemical environments in which comets condensed[26]. But we require only very small differences in chemical composition to explain very different behaviour. A comet whose volatile component contains 85% H_2O molecules by number will behave in a 'normal' way, whereas one which has only 84% H_2O molecules by number will flare at a large heliocentric distance ($R \geq 5$ AU) and then fizzle out, like Comet Kohoutek. Such small differences in chemical composition could easily be attributed to random statistical fluctuations. This may also be the difference between genuinely 'new' comets coming into the inner Solar System for the first time and those that have come in at least once before.

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[1] Ulich, B. L., and Conklin, E. K., *IAU Circ.* 2607, (1973).
[2] Herzberg, G., and Len, H., *IAU Circ.* 2618, (1974).
[3] Ip, W-H., and Mendis, D. A., *Nature*, **245**, 197 (1973).
[4] Wurm, K., *Mitt. Hamburger Sternw.*, **8**, No. 51 (1943).
[5] Calvert, J. G., and Pitts, J. N., *Photochemistry*, 485 (Wiley, New York, 1966).
[6] Rank, D. M., Townes, C. H., and Welch, W. J., *Science*, **174**, 1083 (1971).
[7] Litvak, M. M., in *Atoms and Molecules in Astrophysics* (edit. by Carson, T. R., and Roberts, M. J.), 2d (Academic Press, London, 1972).
[8] Sagan, C., *Nature*, **238**, 77 (1972).
[9] Hudson, R. D., *Rev. Geophys. Space Phys.*, **9**, 305 (1971).
[10] Code, A. D., and Lillie, C. F., *IAU Circ.* 221, (1970).
[11] Bertaux, J. A., Blamont, J. E., Festau, M., *Astr. Astrophys.*, **25**, 415 (1973).
[12] Mendis, D. A., Holzer, T. E., and Axford, W. I., *Astrophys. Space Sci.*, **15**, 313 (1972).
[13] Ip, W-H., and Mendis, D. A., *Astrophys. Space Sci.*, **26**, 153 (1974).
[14] Keller, H. U., *Mitt. Astr. Gesellschaft*, **30**, 143 (1971).
[15] Delsemme, A. H., and Wagner, A., *Planet. Space Sci.*, **18**, 709 (1970).
[16] Arpigny, C., *Acad. Belgique Cl. Sci.*, **35**, 5 (1965).
[17] Code, A. D., and Savage, B. D., *Science*, **177**, 213 (1972).
[18] Biermann, L., *Nature*, **246**, 400 (1973).
[19] Delsemme, A. H., and Miller, D. C., *Planet. Space Sci.*, **19**, 1229 (1971).
[20] Pittich, E. M., *The Motion, Evolution of Orbits, and Origin of Comets* (edit. by Chebotarev, G. A., and Kazimirchak-Polonskaya, E. I.), 283 (Reidel, Dordrecht, 1972).
[21] Vsekhsvyatskii, S. K., *Nature et Origin des Comets* (edit. by Swings, P.), 53, (Institut Astrophysique, Cointe-Sclessin, Belgium, 1966).
[22] Vsekhsvyatskii, S. K., *Physical Characteristics of Comets* (Israel Program of Scientific Translations, Jerusalem, 1964).
[23] Richter, N. B., *Nature of Comets* (Methuen, London, 1963).
[24] Donn, B., and Urey, H. C., *Astrophys. J.*, **123**, 339 (1956).
[25] Eviatar, A., Joseph, J. H., and Dryer, M., *Cosmic Electrodynamics*, **1**, 239 (1970).
[26] Alfven, H. A., and Mendis, D. A., *Nature*, **246**, 410 (1973).

Monotonic evolution of Boltzmann's H in weakly coupled gravitational systems

GASES with gravitational interactions are expected to evolve away from a state of uniform density and temperature[1,2]. Such behaviour has been strikingly confirmed by numerical experiments on small gravitational systems[3].

We point out here that this evolution does not contradict the criterion:

$$dH/dt < 0 \qquad (1)$$

Indeed this criterion can even be proved. Here,

$$H = N\int d\mathbf{v}\ \psi(\mathbf{v}) \ln \psi(\mathbf{v}) + \text{constant} \qquad (2)$$

is Boltzmann's H, ψ is the velocity distribution of particles with equal masses m. The weak coupling theory of Prigogine and Severne[4,5] leads to a criterion of evolution of the form defined by equation 1, mainly as a byproduct of the simultaneous increase of random kinetic energy and negative correlational potential energy[6]. The criterion is illustrated in Fig. 1, which, following Miller[3], is a schematic plot of the evolution of the random kinetic energy and Boltzmann's H. They do not approach a limiting value. The approach of the velocity distribution towards a Maxwellian one is deflected by an instability of the Maxwellian distributions themselves.

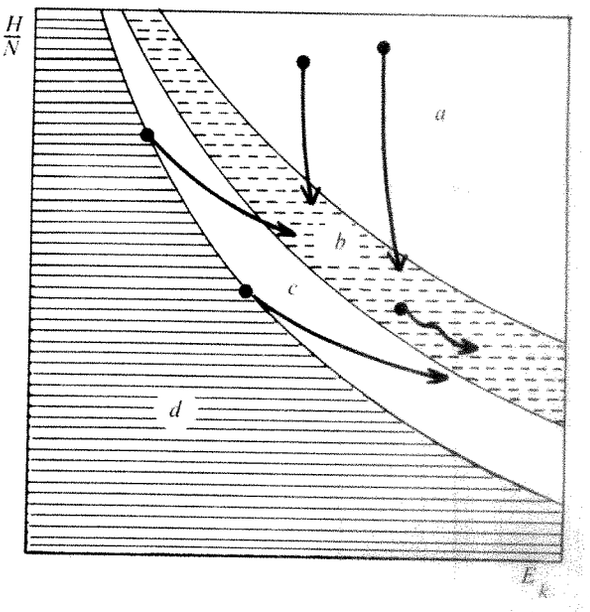


Fig. 1 The monotonic increase in mean kinetic energy per particle, E_k and the monotonic decrease in Boltzmann's H. The size of regions b and c may be much smaller than shown. The line between regions c and d represents Maxwellian velocity distributions ($2E_k dH = -3N dE_k$). Region d contains no allowed distributions. The solid circles represent hypothetical distributions at time t and the arrows show the direction of evolution of H and E_k from these points. Region a is dominated by the usual relaxation of H because of weak binary interactions. Region c is dominated by the kinetic energy production and associated negative potential energy production. The H quantity also decreases there, primarily because of increasing disorder in velocity space as a result of the heating. The evolution in region b is less rigorously established, and the region has not been shown to have boundaries independent of moments of the velocity distribution other than H and E_k. It has, however, been shown that $dE_k/dt > 0$ even in region b, and therefore $dH/dt < 0$ holds everywhere, at least over a period of time.

The Maxwellian is unstable because of distortions associated with the creation of spatial order (ref. 7, and M. J. H., not yet published). This could be crudely described as a dissipative 'flow of entropy' (and of energy) from spatial coordinates to velocity coordinates. Some local order in velocity space develops and is maintained as a byproduct, in spite of the increase in overall dispersion of the velocities.

The spatial order is not reflected in H as it is defined in equation 2, and would have to be taken into account in an appropriate definition of 'total entropy', the evolution of which could be quite different. Work is in progress on the relationship between such a total entropy and the generalised entropy concept being developed by the Brussels school.

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[1] Prigogine, I., *Nature*, **209**, 602 (1966).
[2] Lynden-Bell, D., and Wood, R., *Mon. Not. R. astr. Soc.*, **138**, 495 (1968).
[3] Miller, R. H., *Astrophys. J.*, **180**, 759 (1973).
[4] Prigogine, I., and Severne, G., *Physica*, **32**, 1376 (1966).
[5] Severne, G., *Physica*, **61**, 307 (1972).
[6] Haggerty, M. J., and Severne, G., *Bull. Acad. r. Belg. Cl. Sci.* (in the press).
[7] Haggerty, M. J., *Astrophys. J.*, **166**, 257 (1971).

Sound wave modulation of auroral X rays

A UNIQUELY intense pulsation of auroral-associated X rays, followed a solar cosmic flare in September 1966 and was detected by scintillation counters on a balloon platform at an altitude of about 32 km (ref. 1). The X rays were strongly modulated with a period of 4–6 s. Figures 1 and 2 show a portion of the amplitude-modulated intensity, the ratio of intensities in two neighbouring energy channels, the power spectrum for the X-ray intensity ratio, and the spectrum of intensity variations in the lower energy channel. It can be seen that the X-ray spectrum hardens as the pulsations grow and then softens with the subsequent decay, and that there are two peaks in the X-ray power spectrum.

All these features can be interpreted as an interaction between atmospheric sound waves and a uniform flux of X rays: the intensity modulation results from an atmospheric variable density absorber formed by the sound waves, the energy dependence of the modulation derives from the energy dependence of the X-ray absorption cross section, and the two peaks in the power spectrum are possibly a result of the ducting

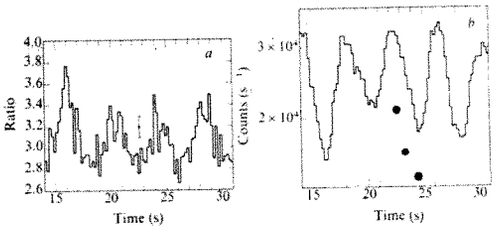


Fig. 1 *a*, Variations in the N(25–50 keV)/N(50–73 keV) ratio and, *b*, N(25–50 keV) intensity for four consecutive peaks in a modulated auroral X ray event, from Brown and Weir[1].

conditions for each of the atmospheric sound channels. First, however, I must digress with a description of the sound ducts.

Infrasonic ducting occurs in the lower and upper atmospheric sound channels, the lower being between altitudes of 5 and 50 km and the upper between 55 and 105 km. Figure 3 shows the lower portion of a sound speed profile based on the COSPAR model atmosphere.

Figure 4 shows the dispersion curves computed by Pfeffer and Zarichney[2] for some of the shorter-period, ducted, acoustic modes. An outstanding feature is the double set of plateaus, a plateau being a region of minimal dispersion—a frequency domain in which wave packets can be formed, propagate and retain identity. At the shorter periods the plateaus alternate between group speeds of 270 and 293 m s^{-1}, the sound speeds in the upper and lower ducts. The mean ratio of periods of neighbouring plateaus, averaged over the third to eighth modes, is about 1.35 (the ratio of the larger central period to the subsequent smaller central period).

The cause of the alternating plateaus, which occur when two partial ducts have a common pressure node at their interface, can be understood from the work of Tolstoy[3]. He shows that approximate eigenvalues for the entire duct can be represented by a lattice of points resulting from the intersections of resonant and antiresonant eigenfunctions for the partial ducts considered separately. The dispersion curve, for the solution of the complete duct, passes through a subset of the lattice points, and as the curve passes from one lattice point to a neighbouring one, most of the energy transfers from one partial duct to the other. If the ducts are excited by an impulsive source, the lattice points acquire the role of coupling frequencies which become the centre frequencies of the principal wave packets.

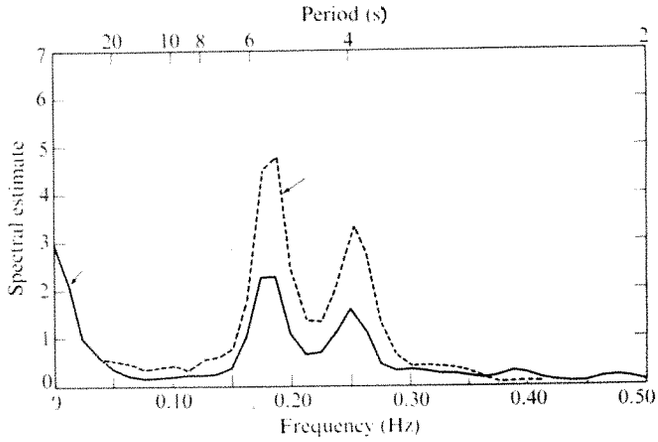


Fig. 2 Power spectra for the X ray intensity ratio N(25–50 keV)/N(50–73 keV). – – –, Intensity variations in the energy range 25–50 keV. (From Brown and Weir[1], 1646.00 to 1649.30 UT.)

In the present case, the lattice points are an ensemble of virtual oscillators with a phase volume so severely restricted that they can be regarded as quantised in period between two levels of group speed (Fig. 4). Such an ensemble, when impulsively excited, will propagate two or more wave packets at group speeds of 270 and 293 m s^{-1}, and the power spectrum of the packets will have two or more peaks. The ratio of the frequencies of sequential peaks, as well as the ratio of the bandwidths of the peaks, is determined by the ratio of the effective widths of the partial ducts which, for the ducts shown in Fig. 3, is 1.35.

Now the ratio of the periods at which maxima occur in the X-ray spectrum is 1.38 and the ratio of the half widths of the two maxima is 22%/16%, or 1.37. These numerical correlations between X ray and ducted sound wave spectra, can be taken as a first suggestion that the X-ray spectrum has a sonic origin. I assume that an upward-looking X-ray telescope, on a balloon platform in the lower duct, will detect modulations

of X rays by sound waves in the lower and upper partial ducts and that the spectra of the modulations will image the sonic spectra.

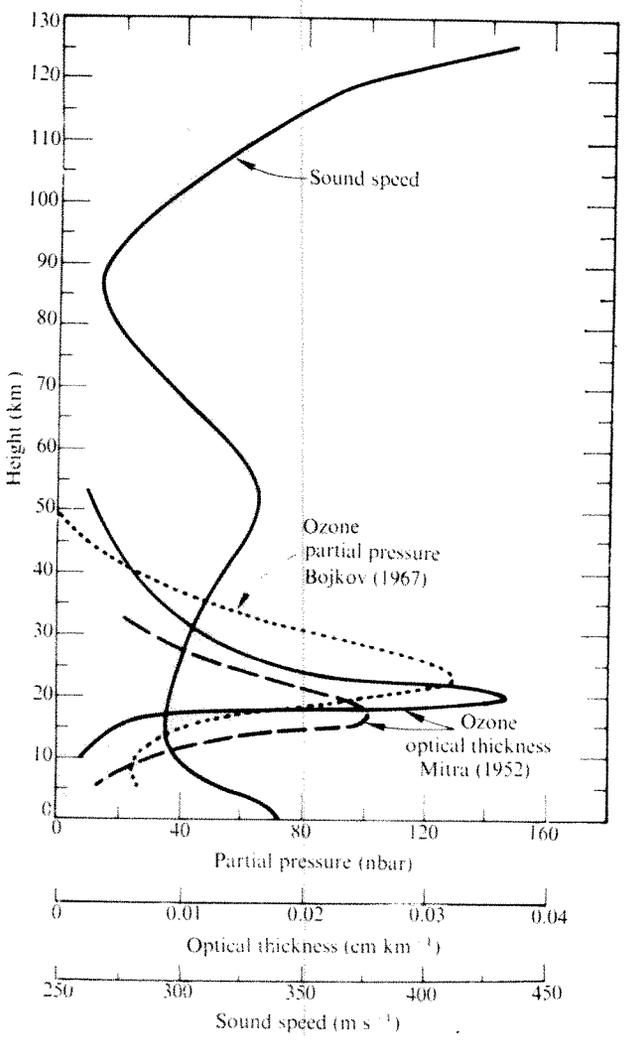


Fig. 3 Sound speed profile based on a COSPAR model atmosphere. Superimposed atmospheric ozone profiles are from Bojkov[10] and Mitra[11].

I shall now consider the magnitude of the sonic condensation required to produce the observed X-ray modulation. Let B_0 be the average X-ray intensity at the height of observation, then the instantaneous value of the intensity, which varies due to change in absorption, will be

$$B = B_0 \exp\left(-\int_{z_0}^{z_1} \xi \mu_1 \rho \, dz\right) \quad (1)$$

where ρ is the ambient atmospheric density, the condensation ξ is $d\rho/\rho$, and μ_1 is the total X-ray absorption cross section in $cm^2\ g^{-1}$. I take $\rho = \rho_0 \exp[-(z-z_0)/H_0]$, assume ξ and μ_1 independent of altitude and integrate equation (1) to obtain

$$\xi = -[\log B/B_0]\,[\mu_1 0_0 H_0)\,(1-\exp-(z_1-z_0)/H_0)]^{-1} \quad (2)$$

where ρ_0 and H_0 are the density and scale height at z_0 the balloon altitude, and z_1 is the height of the top of the lower duct (50 km).

To evaluate equation (2) a weighted mean cross section is the energy interval 25–50 keV must first be computed:

$$\bar{\mu}_1 = \int_{25}^{50} \nu\mu_1 \, dE \Big/ \int_{25}^{50} \nu \, dE \quad (3)$$

where the weighting function, $\nu = \exp[(25-E)/13]$, is based on the e-folding value of 13 keV observed by Brown and Weir. Using the total cross sections in nitrogen given by Siegbahn[4], equation (3) is numerically integrated to obtain $\bar{\mu}_1 = 0.0311$ $cm^2\ g^{-1}$. I determine the ratio $B/B_0 = 1.25$ from the modulations shown in the lower part of Fig. 1, define the rest of the terms in equation (2) for a COSPAR model atmosphere and find that the X ray modulation would be caused by a sound wave condensation $\xi = 0.0835$; however, it is worth correcting this result for the finite angular width of the X-ray telescope, not only to obtain a more accurate condensation but to obtain internal consistency by comparing aperture efficiency with observed percentage modulation.

The aperture width is 26.5° about zenith (R. R. Brown, private communication). I use vertical and horizontal ducted wavelengths of 4.5 and 1.6 km to reconstruct the geometry of the experiment and integrate, graphically, over the solid angle of the aperture to obtain an efficiency of 57%. In making this determination, it is trivial to include a sinusoidal variation in condensation in the integrand in equation (1) because the modulation results principally from oblique alignments of like condensations, much as trees are obliquely aligned in an orchard. I include, however, an obliquity correction for a slight increase in the effective value of scale height. The computed efficiency compares well with the observed modulation, 51%, determined from the observation shown in the lower part of Fig. 1. An efficiency of 57% implies that the condensation required to produce the modulation is 14.6%.

If the ducted disturbance is regarded as the superposition of two obliquely propagating wave trains, then the required condensation in each is a half, or 7.3%. A condensation of this magnitude at 50 km altitude corresponds to 0.21% at ground level, or a pressure disturbance of 27 dyne cm^{-2}. This is surprisingly large but not unusual. Sonic disturbances of comparable magnitude at ground level have been associated with aurora for many years[5].

Can a condensation of 14.6% also produce the energy dependence of the X-ray intensities? The variation of the ratio of the intensities in the intervals 25–50 keV and the 50–73 keV is (d/dp) (N_1/N_2), or

$$d(N_1/N_2) = -(N_1/N_2)\,\xi\rho_0 H_0(\bar{\mu}_1 - \bar{\mu}_2) \quad (4)$$

where $\bar{\mu}_2$ is the weighted mean cross section in the interval 50–73 keV. The negative sign indicates that the fluctuations are out of phase with the density variations, which is confirmed in Fig. 1. Taking the ratio N_1/N_2 to be the average value shown in the top of Fig. 1 and the remaining parameters as used before, I find that a 14.6% condensation will cause a variation in the ratio of X-ray intensities of $d(N_1/N_2) = 0.520$. I have again included a small obliquity correction for effective scale height. This result compares well with the directly observed value of 0.475 (in Fig. 1, half the difference between the maximum and minimum values of N_1/N_2 in the first half cycle).

One additional aspect of the X-ray event can be explained as sound wave modulation. Brown and Weir note that the first part of the event contains the 4-s peak and that the middle of the event the dominant period abruptly changes to 5.5 s. The time difference between the first and second parts of the event is $t = 105$ s. If c_1 and c_2 are the upper and lower duct speeds, then an elementary formula, $r = c_1c_2t/(c_2-c_1)$, that the interval between the two parts of the event can be attributed to the difference in propagation speeds in the two channels from a source at range 362 km.

These sound modulation calculations are also appropriate for that portion of the event which shows modulation characteristic of the upper duct, since modulation that occurs in an elevated layer is invariant to subsequent, lower level absorption. The upper duct, is however, in a more rarified part of the atmosphere and larger condensations would be required to predict the observed modulation. I am inclined to assume that energy in the upper duct extends to or just beyond the interduct boundary, and that greater condensation is not required because modulation occurs at a level near the balloon platform.

When the X-ray observations were made (September 3, 1966, Fairbanks, Alaska, latitude 65° N) the atmosphere was not appreciably different from the reference atmosphere used here. Measurements at 5, 2 and 0.4 mbar, corresponding to heights of about 32, 42 and 55 km, show that differences

between measured and reference temperatures and densities were less than 3 and 1% (ref. 6). Measured wind speeds during this part of the equinoctial period were small, between 10 and 30 knots.

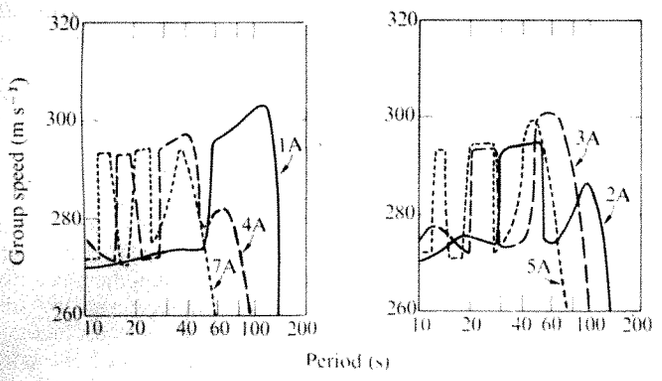


Fig. 4 Machine-computed dispersion curves for ducted sonic modes in a COSPAR model atmosphere, from Pfeffer and Zarichny[2]. 'Plateaus' are the nearly constant speeds at peaks and troughs.

So for a single, especially clear event, it seems that a uniform X-ray flux is density-modulated by ducted atmospheric sound waves. This does not imply, of course, that the X-ray fluxes associated with aurora are always uniform, nor that all X-ray pulsations are the result of modulation by sound waves, ducted or unducted.

There is substantial evidence that ducted sound waves in the upper atmosphere result from volcanic explosions and other natural causes, as well as from nuclear detonations. Baker and Davies[7] report the detection of wave packets generated by nuclear detonations at ranges of about 7,000 km. They conclude that the ionospheric plasma is modulated by ducted sound waves, and the plasma in turn modulates transient radio waves.

If sound waves sometimes modulate X-ray precipitations and ionospheric plasma, other atmospheric phenomena may occasionally be similarly modulated. Little attention has been given to the fluctuations which occur in atmospheric ozone density, possibly because of the use of the Dobson spectrophotometer[8], a device in which short-term variations are averaged. Recently Hering *et al.*[9] studied the fluctuations and concluded that a third to a half of the variance in total ozone content is a result of fluctuations in ozone density at altitudes of 11 to 15 km—the centre of the lower sound duct (Fig. 3).

I recall with pleasure conversations and correspondence with Dr Robert R. Brown.

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1 Brown, R. R., and Weir, R. A. J. *geophys. Res.*, **72**, 5531-5536 (1967); J. *atmos. terr. Phys.*, **29**, 1611–1618 (1967).
2 Pfeffer, R. L. and Zarichny, J. *Geofis. pura appl.*, **55**, 175–199 (1963).
3 Tolstoy, I., J. *acoust. Soc. Am.*, **28**, 1182–1192 (1956).
4 *Beta- and gamma-ray spectroscopy*, (edit. by Siegbahn, K.), (Interscience, New York, 1955).
5 Procunier, R. W., *Geophys. J. R. astr. Soc.*, **26**, 183–189 (1971).
6 *ESSA Technical Report WB* 9, (US Dept Commerce, January 1969).
7 Baker, D. M., and Davies, K., J. *geophys. Res.*, **73**, 448–451 (1968).
8 Dobson, G. M. B., *Proc. phys. Soc. Lon.*, **43**, 324 (1931).
9 Hering, W. S. and Morden, R., Jr., *US Air Force Cambridge Research Papers, No.* 162, (1965).
10 Bojkov, R. D., J. *Geophys.*, **72**, 5585-5593 (1967).
11 Mitra, D. K., *The upper atmosphere*, second ed. (The Asiatic Society, Calcutta, 0000).

Five-year climatic trend in the Northern Hemisphere

Results reported by Starr and Oort[1] reflect changes which have occurred in the instruments, in the methods used at the observing stations to interpret radiosonde measurements, and from the setting up of new stations, rather than from real geophysical changes.

Observations of the upper air are mainly made by measuring signals originating from airborne sensors. The measurements are converted into reports in conventional units by complex processes which incorporate corrections designed to reduce errors inherent in the system. For example, in some systems corrections are applied to the temperature measurements for the effects of lag and solar radiation on the temperature sensor, and to the pressure measurements for the effect of temperature on the pressure sensor. Different instrument systems and different conversion and correction techniques are used at different stations. Moreover, changes of instruments and techniques are introduced piecemeal. The practical effect is that, effectively, different absolute standards are brought into use. Thus, although the units are all called by the same name they have some of the properties of 'floating' international currencies in that both their international exchange rates and their apparent relation to materials vary with time. The changes are partly because of gradual improvements in the quality of techniques for observing the upper air, which are taking place at different rates in different parts of the world.

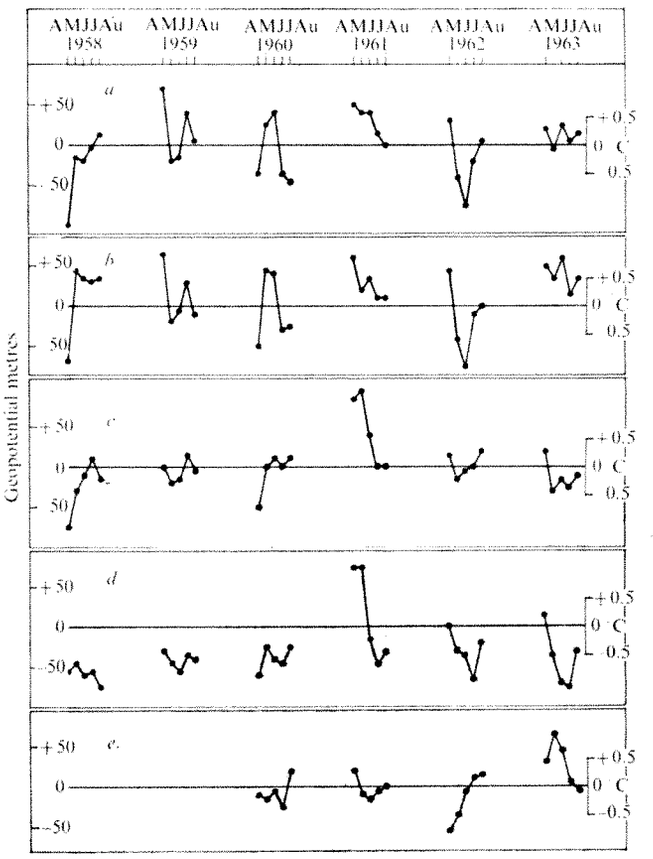


Fig. 1 Deviations of individual values of monthly mean 1,000–100 mbar geopotential thickness from 6-yr averages of the same named month from April to August. *a*, Crawley at 00Z relative to 00Z averages 1958–63; *b*, Crawley at 12Z relative to 00Z averages 1958–63; *c*, Gibraltar at 00Z relative to 00Z averages 1958–63; *d*, Gibraltar at 12Z relative to 00Z averages 1958–63; *e*, Gan at 12Z relative to 12Z averages 1960–65. Equivalent temperature scales for uniform temperature change with height are on the right. A, April; M, May; J J, June July; Au, August.

The most important changes have been aimed at reducing the effects of balloon wake, solar radiation and lag (in a temperature lapse environment), all of which give readings which are high relative to the true air temperature at the level. The net effect of the changes has, therefore, usually been to lower the temperature reported in conventional units relative to that which would have been formerly reported. The introduction of new stations using observing systems different from those of the pre-existing stations can have similar effects on areal analyses. World events such as the IGY 1957 and the IQSY 1964–65 tend to stimulate such changes. Water vapour mixing ratios are calculated from the measurements of pressure, temperature and relative humidity. Consequently, even if relative humidity measurements do not change, apparent changes in air temperature are associated with apparent changes in humidity mixing ratio.

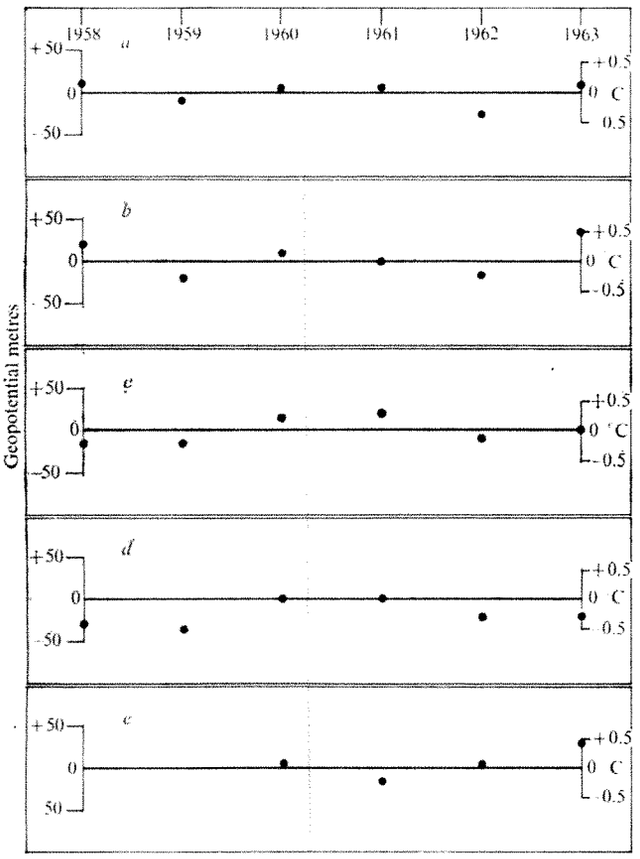


Fig. 2 Twelve-month averages, May to following April, of individual deviations of monthly mean 1,000–100 mbar geopotential thickness from the 6-yr averages of the same named months. Letters as in Fig. 1. Equivalent temperature scales for uniform temperature changes with height are on the right.

Changes such as these invalidate a straightforward geophysical interpretation[1] of the trend in temperature and humidity mixing ratio for the Northern Hemisphere.

Between 1958 and 1963 changes introduced into British radiosonde practice are likely to have had little effect upon the systematic errors of measurements from British controlled stations up to 75 mbar. Examination of quantities such as the monthly mean 1,000–100 mbar geopotential thickness for named months (preferably in summer) at such stations situated where Starr and Oort[1] (Fig. 3 of ref. 1) indicate a material trend in the vertical mean mass-weighted temperature, should serve as a direct check of the validity of a geophysical interpretation of their result. The 1,000–100 mbar geopotential thickness is a sensitive measure of vertical mean height-weighted temperature and a decrease of 1° C in temperature, uniformly distributed in the vertical, is associated with a decrease of 67 geopotential metres (g.p.m.) in the 1,000–100 mbar geopotential thickness. The relationship between vertical mass-weighted mean temperature and 1,000–100 mbar thickness depends upon the distribution of temperature change with altitude. If, however, negative temperature changes tend to increase with altitude (as for the most part indicated by Starr and Oort, Fig. 2) a change of −1° C in the mass-weighted mean temperature would correspond to more than the −67 g.p.m. in 1,000–100 mbar thickness associated with −1° C for a uniform change of temperature with altitude.

A check has been carried out using data for individual months for Crawley (51°N 00°W), Gibraltar (36°N 5°E), and Gan (01°S 73°E). Starr and Oort[1] indicate trends close to −1° C in the vertical mean mass-weighted temperature between 1958 and 1963 at each of these stations. Each of the series showed only minor variations and no trend (Figs 1 and 2). Whether or not the last points in Fig. 2 are considered, the evidence indicates that no persistent trend in the average atmospheric temperature occurred. In both figures the mean values of 1,000–100 mbar thickness at 12Z in Gibraltar are systematically lower than associated values at 00Z. This contrasts with the result from Crawley. (If it is a real result it implies that the atmosphere over Gibraltar is a little colder near noon than near midnight.) It arises from differences on the 12Z soundings at the two stations in the mean albedo underlying the sondes. (At the time this albedo was assumed to be 0.4 for solar radiation correction techniques. When, as often happens at Gibraltar in summer, the albedo actually experienced by the sonde in 12Z soundings was less than 0.4 there was an over-correction for solar radiation effects.) It illustrates one of the ways quantities measured in the upper air and reported in the same nominal units can apparently vary in an unreal manner.

Using Gan as a new station with soundings which yield temperatures systematically lower than those of Indian radiosondes, will probably have the effect of reducing analysed temperatures within its area of influence relative to those indicated before Gan was used.

Similar apparent changes (mainly downwards) in the mean temperature of the upper air and associated changes in mean humidity-mixing ratio can be expected in some regions long after 1963.

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[1] Starr, V. P., and Oort, A. H., *Nature*, **242**, 310–313 (1973).

Drs Starr and Oort Reply: Hawson's contention that local changes in instruments and in the method of reducing the data would explain the downward trend in hemispheric mean temperature seems highly improbable. These factors may be important in the case of a few radiosonde stations used in the analyses. In our sample of more than 600 upper air stations, however, the instruments and procedures between different countries vary so widely that the quite systematic changes are more compatible with the interpretation of a real 5-yr trend.

Furthermore, the computed values at our lowest level (1,000 mbar) agree well with those computed from surface records[1]. Neither set of data shows any appreciable 5-yr temperature trend close to the surface. As the independent observations[1] were made with the aid of surface instruments which, presumably, are not affected by the radiation corrections mentioned by Hawson, the agreement suggests

Table 1 Comparison of the yearly-mean temperature deviations computed by Dronia[4] and by ourselves

	Temperature deviation from 6-yr normal for lower half of atmosphere (Dronia)						Percentage of latitude circle included	Temperature deviation from 5-yr normal for mass between surface and about 18 km (present authors). Entire latitude circle is included in computation			
	1958	1959	1960	1961	1962	1963		1958–59*	1959–60*	1960–62*	1961–62*	1962–63*
80° N	−0.14	0.76	0.36	0.26	−0.29	−0.94	100	−0.06	0.54	0.24	−0.26	−0.46
70° N	−0.22	0.38	0.38	−0.11	−0.12	−0.32	100	−0.10	0.30	0.30	−0.10	−0.40
60° N	0.01	0.11	−0.09	0.01	−0.04	0.01	100	0.06	0.06	0.16	−0.04	−0.24
50° N	0.25	−0.05	−0.35	0.25	−0.05	−0.05	89	0.14	−0.06	−0.06	0.14	−0.16
40° N	0.17	0.08	0.08	−0.02	−0.12	−0.22	56	0.22	0.02	−0.08	0.02	−0.18
30° N	0.18	0.08	0.28	−0.28	−0.12	−0.12	47	0.28	0.08	−0.02	−0.02	−0.22
20° N	0.39	0.14	0.14	−0.31	−0.21	−0.16	28	0.40	0.20	0.10	−0.20	−0.50

*May, 1958–April 1959, May, 1959–April, 1960 and so on.

that these factors do not play an important role in the upper air data which we used.

Hawson's data points, together with our values for the nearest grid point, are reproduced in Fig. 1. (For practical reasons it was not possible to extract the data at exactly the same location.) The two sets of data are in reasonably good agreement in spite of the smoothing introduced by our objective scheme of data interpolation. The trend at each grid point is evidently small in comparison with the background 'noise' from natural variability. This supports the view that significant results cannot be expected from the study of only a few months of data for a few selected points. Large areas must be considered before possible climatic trends can be delineated.

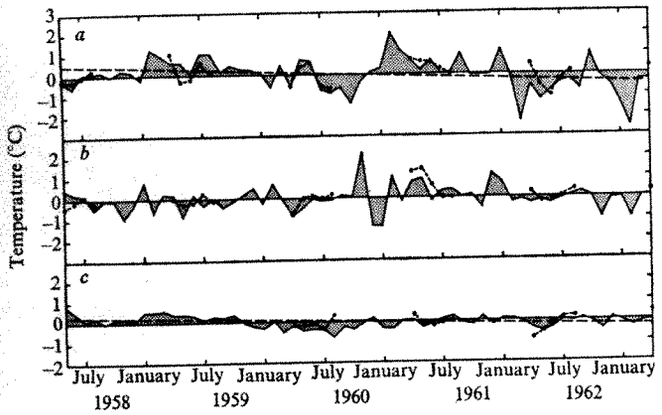


Fig. 1 Temperature deviations (00Z) from the normal annual cycle during the period May 1958–April 1963 for: *a*, Crawley (51.0°N, 0.1°W) trend = 1.0° C every five years; *b*, Gibraltar (36.1°N, 5.2°W) trend = 0.0° C; *c*, Gan (0.4°S, 73.1°E) trend = 0.5° C; short-dashed line-segments, according to Hawson; full line deviations at the nearest grid points in the present analysis (52.7°N, 0.0°W; 33.9°N, 5.2°W and 0.4°S, 73.3°E, respectively); linear trend lines computed from the present data. (Our earlier map showed a trend of about −1° C every 5-yr in Gibraltar. This was because of a minor inaccuracy in the drawing of the isolines.)

Dronia[2] has studied a large set of mean thickness maps of the 1,000 to 500 mbar layer of about 50% of the geographical area in the Northern Hemisphere. This thickness is a measure of the vertical mean temperature in the lower half of the atmosphere. Our results are in excellent agreement with Dronia's results (Table 1) in spite of differences in data reduction and analysis procedures.

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[1] Reitan, C. H., thesis, Univ. Wisconsin (1971).
[2] Dronia, H., *Beil. Berliner Wett. Karte* (1973).

Frictional constraints on thrust, wrench and normal faults

IT is generally true that in ancient fault zones, quartzo-feldspathic mylonites which indicate partial plastic yield throughout a volume of rock[1], are most extensively developed in association with thrust faults. They are less widespread along wrench faults (unless the finite displacement is very large) and are comparatively rare along normal faults[2]. The release of seismic energy at shallow depths follows the same general pattern. More than 90% is released in areas of compressive plate interaction, mostly where underthrusting is the dominant mechanism, whereas only 6% is derived from the midocean ridges, where tensional rifting is operative[3].

The analysis presented here, which accounts for these observations at least in part, quantifies a suggestion put forward by Anderson[2]. It is applicable to any existing fault the behaviour of which is approximately governed by the linear frictional failure criterion

$$\tau > \tau_f = \mu\sigma_n$$

where τ is the applied shear stress, τ_f is the frictional shear resistance, μ is the coefficient of static friction, and σ_n is the normal stress across the fault. This will include faults made up of discrete planes and also low cohesion crush zones which can

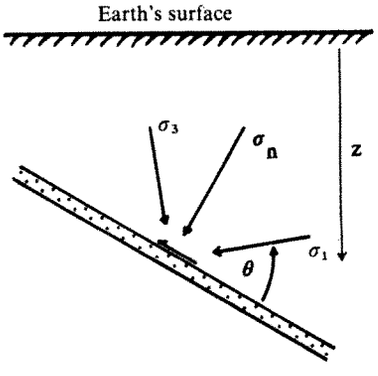


Fig. 1 Stresses around a fault zone at a depth z in the crust.

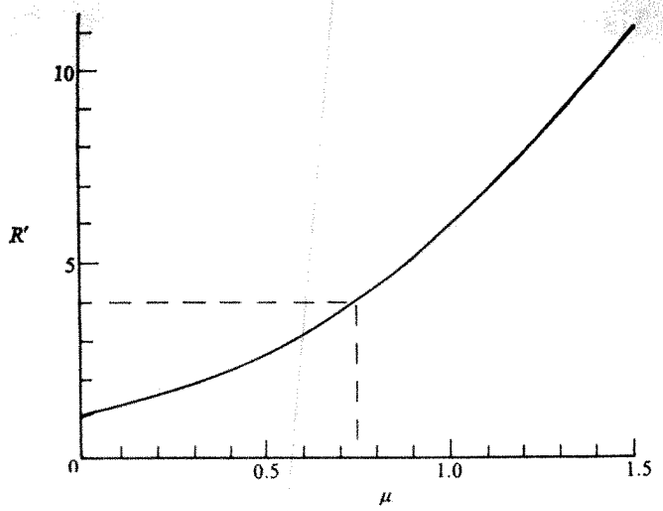


Fig. 2 Minimum ratio of principal stresses (R') required to initiate sliding on a plane with coefficient of static friction, μ.

be treated as granular aggregates of quasi-elastic rock particles[4]. (In fact, the addition of a low-value term for intrinsic cohesion across the fault will not greatly affect the outcome of this argument.) Figure 1 illustrates the stresses acting on a planar fault zone at a depth z in the crust. Throughout this discussion, σ_1 and σ_3, the greatest and least principal compressive stresses respectively, are considered as acting in a plane normal to the

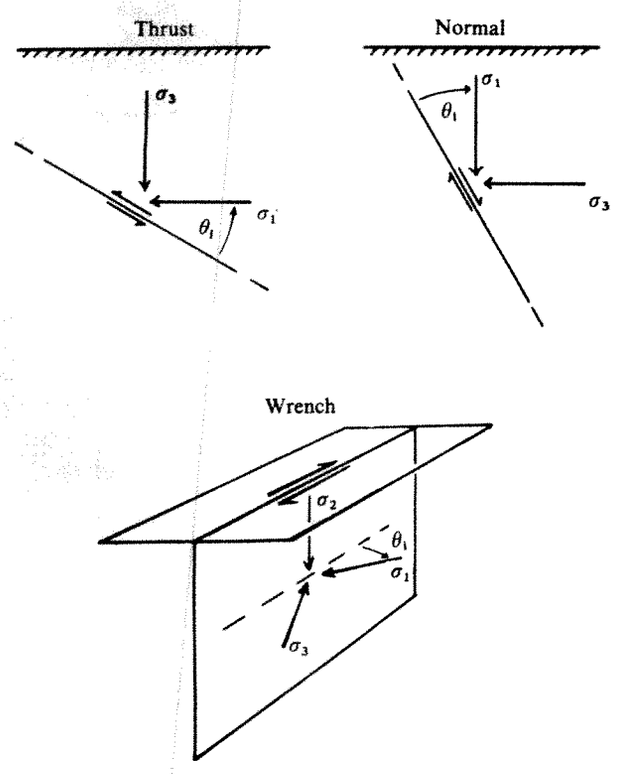


Fig. 3 Anderson's dynamic classification of faults.

fault. The condition for sliding may be rewritten in terms of the the principal stresses as

$$(_1\sigma-\sigma_3)\sin 2\theta = \mu[(\sigma_1+\sigma_3)-(\sigma_1-\sigma_3)\cos 2\theta]$$

and putting $R = \sigma_1/\sigma_3$,

$$R = [\sin 2\theta+\mu(\cos 2\theta+1)]/[\sin 2\theta+\mu(\cos 2\theta-1)]$$

By differentiating this expression and putting $dR/d\theta = 0$, it is found that the minimum value of R required to initiate sliding is given by

$$R' = [-(1+\mu^2)-\mu)]^{-2}$$

(see Fig. 2) and occurs only when σ_1 is at an angle

$$\theta_f = \tfrac{1}{2}\tan^{-1}(1/\mu)$$

to the fault. At all other orientations, the ratio of principal stresses needed to initiate sliding will be greater.

Thus, in general, the limiting condition for frictional sliding is

$$\sigma_1 \geqslant R'\sigma_3$$

or

$$(\sigma_1-\sigma_3) \geqslant (R'-1)\sigma_3$$

Typical crustal rocks will have a static frictional coefficient, μ, of about 0.75 (refs. 4 and 5) and therefore $R' = 4$ and the corresponding value of θ_f is about 27°.

It has been shown[2] that many geological faults fall into three classes termed thrust, wrench and normal faults the orientation of which can be explained dynamically in terms of the Navier–Coulomb failure criterion ($\tau = \tau_0+\mu_i\sigma_n$; where τ_0 is the cohesion and μ_i is the 'internal friction' of the rock mass), assuming that one of the principal stresses is vertical and the other two lie in a horizontal plane (Fig. 3). When the fault first forms, it does so at an angle θ_i to σ_1, where $\theta_i = \frac{1}{2}\tan^{-1}(1/\mu_i)$. In most cases θ_i will not differ greatly from θ_f, the optimum angle for frictional sliding.

The effective overburden pressure at a depth, z, in the crust is given by

$$\sigma_v = \rho g z(1-\lambda)$$

where ρ is the crustal density, g is the acceleration due to gravity, and the pore-fluid factor $\lambda = P/\rho g z$, P being the pore-fluid pressure.

Thus, in a thrust fault regime, where $\sigma_v = \sigma_3$, the limiting inequality for frictional sliding is

$$(\sigma_1-\sigma_3) \geqslant (R'-1)\rho g z(1-\lambda)$$

In a wrench fault regime, $\sigma_v = \sigma_2$, and σ_2 may be written in the form

$$\sigma_2 = \sigma_3+k(\sigma_1-\sigma_3)$$

where $0 < k < 1$. In this case, the limiting inequality for frictional sliding is

$$(\sigma_1-\sigma_3) \geqslant \{(R'-1)/[k(R'-1)+1]\}\,\rho g z(1-\lambda)$$

and if $\sigma_2 = (\sigma_1+\sigma_3)/2$, (that is, $k = \frac{1}{2}$), the inequality becomes

$$(\sigma_1-\sigma_3) \geqslant [2(R'-1)/(R'+1)]\,\rho g z(1-\lambda)$$

In a normal fault regime, where $\sigma_v = \sigma_1$, the limiting inequality for frictional sliding is

$$(\sigma_1-\sigma_3) \geqslant [(R'-1)/R']\,\rho g z(1-\lambda)$$

Thus at a given depth, z, and pore-fluid factor, λ, the ratio of differential stresses required to initiate sliding on thrust, wrench ($k = \frac{1}{2}$) and normal faults is, respectively

$$(R'-1):2(R'-1)/(R'+1):(R'-1)/R';$$

and if $R' = 4$ ($\mu = 0.75$), the ratio is 4:1.6:1 (Fig. 4). (Note that as k varies from 0 to 1, the minimum differential stress required for wrench faulting increases from that required for normal faulting to that needed for thrust faulting.)

The expression for the distortional elastic strain energy per unit volume can be written in the form

$$E_d = [(\sigma_1-\sigma_3)^2/6G]\,(1-k+k^2)$$

where k is as already defined and G is the rigidity modulus. Clearly, if R' is again 4, and for identical λ values, the volume density of distortional strain energy stored at the same depth around thrust, wrench and normal faults respectively (all with $k = \frac{1}{2}$) just before slip, is in the ratio 16: 2.56:1. Stress regimes associated with thrust, wrench and normal faults respectively, are broadly equivalent to those inferred at crustal depths for the different types of plate boundary, arcs, transforms and ridges. Thus, the observed pattern of seismic energy release at shallow depths, and also the fact that the largest magnitude shocks occur in arc regions where underthrusting is operative, are not solely caused by varying areas of contact between lithospheric plates as has been suggested[3], but are to some extent a consequence of the different stress regimes associated with the three types of plate boundary. This conclusion is of course dependent on the assumption that the shallow earthquake mechanism is indeed a process, such as stick-slip, which is controlled by friction.

Assuming that there is some upper limit to differential stress, above which it is easier for rocks to deform plastically by distributed shear throughout a volume, than by frictional sliding on a plane[5], then the critical depth for this transition

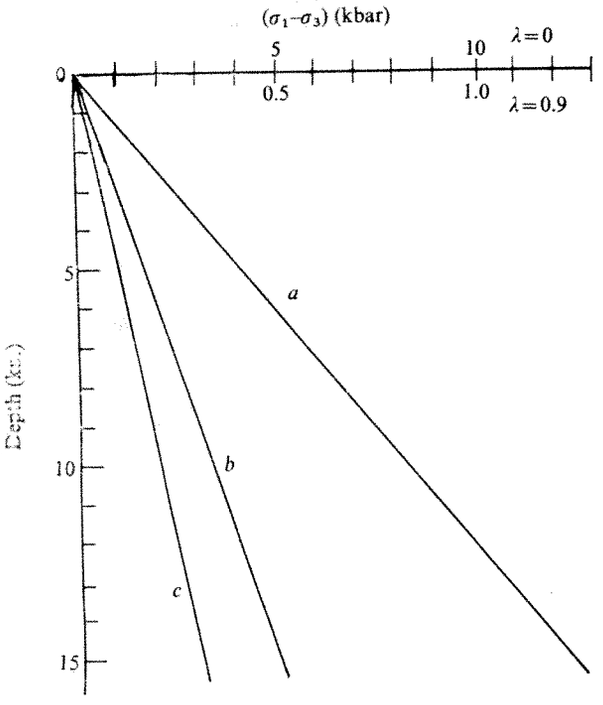


Fig. 4 Minimum differential stress required to start sliding on ideally oriented thrust (*a*), wrench ($k = 0.5$) (*b*), and normal faults (*c*), against depth in the crust. ($\rho = 2.8 \times 10^3$ kg m^{-3}; $g = 9.8$ m s^{-2}; $\mu = 0.75$; $\theta_f = 27°$.)

to plastic yielding will be in the ratio 1 : 2.5 : 4 around thrust, wrench ($k = \frac{1}{2}$) and normal faults respectively, if temperature effects are neglected, $R' = 4$, and λ remains the same throughout. This explains rather well the observed distribution of mylonites in fault zones.

Absolute values for the shear stress within crustal fault zones are thought not to exceed a few hundred bars[6,7]. It is therefore unlikely that the associated differential stress will exceed 1 kbar at the very most. If this is so, it is apparent from Fig. 4 that high pore-fluid pressures are an absolute necessity if faulting (especially thrust faulting) is to take place by frictional stick-slip to a depth of 20 km or more[8]. This condition is most likely to be maintained in the forefront of island arcs where 'wet' sediments are thought to be continually dragged down by the underthrusting process. With large wrench fault zones, it is hard to escape the conclusion that water must be continually injected into the zones from the deep crust or upper mantle, at pressures considerably greater than the hydrostatic head.

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1 White, S. H., *Nature*, **244**, 276–278 (1973).
2 Anderson, E. M., *The Dynamics of Faulting*, second ed. (Oliver and Boyd, Edinburgh, 1951).
3 Isacks, B., Oliver, J., and Sykes, L. R., *J. geophys. Res.*, **73**, 5855–5899 (1968).
4 Jaeger, J. C., and Cook, N. G. W., *Fundamentals of Rock Mechanics* (Methuen, London, 1969).
5 Orowan, E., *Mem. geol. Soc. Am.*, **79**, 323–345 (1960).
6 Chinnery, M. A., *J. geophys. Res.*, **69**, 2085–2089 (1964).
7 Wyss, M., *J. geophys. Res.*, **75**, 1529–1543 (1970).
8 Evison, F. F., *Tectonophysics*, **9**, 113–128 (1970).

Marscoite and the origin of andesites

THE present communication points out certain discrepancies between the arguments of those who dispute a contamination origin for andesitic lavas and the evidence provided by one specific and well documented example of contamination.

Green and Ringwood[1] have suggested a model for the primary derivation of andesite from mantle rocks, which relies heavily on the fact that andesites have low 'mantle-type' $^{87}Sr/^{86}Sr$ ratios, which cannot be explained by "theories involving melting of sialic material in orogenic zones, or the contamination of mantle-derived basalt magmas with sialic material". Mainly on this basis, they propose a model of mantle derivation.

In a study of Quaternary lavas from the Cascade range, Peterman *et al.*[2] come to a similar conclusion using two main arguments. First, that the low ratios of around 0.7032 for $^{87}Sr/^{86}Sr$ are very similar to those of the accompanying basaltic rocks and "suggest a mantle origin" (see Table 2); second, that "the extremely high Sr contents of the andesites . . . indicate that these rocks could not have been formed by . . . mixing of basaltic magma and greywacke or partial fusion of greywacke." This is seen from Table 2, and in Peterman's Fig. 1, which show that mixing of reasonable ratios of basalt and greywacke to give the major element composition of andesite would result in a rather lower Sr content for the hybrid than is actually seen in andesites.

I believe that both arguments can be answered by evidence provided by Moorbath and Bell[3] in their study of strontium ratios in igneous rocks from Skye. At the classic locality of Marsco, Wager *et al.*[4] have shown that marscoite results from the mixing at depth of a ferrodiorite magma, which they believe to be a differentiate of the Cuillin intrusion, and a porphyritic felsite magma.

Marscoite shows several analogies with the presumed manner of andesite generation by contamination theories. Both chemistry and mineralogy (Table 3) and the K-feldspar, sodic andesine, quartz and hornblende assemblage, indicate that it is an andesitic rock.

Table 1 Geochemistry of Marsco rocks

Rock type	Range of $^{87}Sr/^{86}Sr$	Average $^{87}Sr/^{86}Sr$	Average Rb (p.p.m.)	Average Sr (p.p.m.)
Ferrodiorite	0.7112–0.7133	0.7123	47	256
Marscoite	0.9124–0.7139	0.7129	78	238
Felsite	0.7123 ± 0.0020	0.7213	113	15

The most noticeable difference is that the MgO/(FeO + MgO) ratio is 0.173 in the marscoite and 0.443 in the hornblende andesite, this may be partly because whereas andesite, on the contamination model, is assumed to have a basaltic parent, marscoite has a more differentiated tholeiitic parent, which would be expected to impart a lower Mg/Fe ratio to a hybrid derived from it.

The acid parent of marscoite, porphyritic felsite, has a very high $^{87}Sr/^{86}Sr$ (Table 1) ratio which is typical of rocks derived by partial melting of crustal material (probably Lewisian in this case). The mixing of ferrodiorite and porphyritic felsite magmas is therefore analogous to the presumed contamination model of andesite in which basaltic magma mixes either with crustal material or with liquids produced by partial melting of such material, and as the analyses show, the Marsco case at least produces an andesitic rock. In addition, Carmichael[6] has cited evidence of magmatic mixing in dacites from the Cascade Range, and considers that it is equivalent to contamination.

With reference to Peterman's two main arguments against the contamination origin, two statements from Moorbath and Bell are pertinent (see Table 1).

First, "a mixture of 60 parts ferrodiorite and 40 parts porphyritic felsite" (which is the ratio suggested by Wager *et al.*[4] as necessary to give the observed major element composition of marscoite) "would have an initial $^{87}Sr/^{86}Sr$ ratio of 0.7127, which is still within the limit of error of both the ferrodiorite and marscoite. In spite of the fact that the initial $^{87}Sr/^{86}Sr$ ratio of the porphyritic felsite is considerably higher than that of the ferrodiorite, the ratio of common strontium (porphyritic felsite/ferrodiorite) was presumably too low to affect the initial ratio of the ferrodiorite significantly when the rocks were mixed in the above ratio". Second, "a mixture of 60 parts ferrodiorite and 40 parts felsite would have a Sr content of 60 parts per million (p.p.m.). This is considerably lower than the value observed for marscoite and suggests that marscoite contains an excess of common strontium from some extraneous source."

The implications of these statements can be summarised thus: Marscoite shows clear evidence of being a hybrid of a basic and an acid magma, and has the same isotopic composition, within experimental error, as its basic parent; andesites, petrologically similar to marscoite, have the same isotopic composition as basalt. The hybrid origin of andesite is, therefore, not precluded by this strontium ratio data. The data on Sr concentration for marscoite and its presumed parents are inconsistent, and so these data cannot preclude a hybrid origin for andesite, particularly as the inconsistency is in the same direction for both cases.

Table 2 Geochemistry of Cascade rocks

Rock type	Range of $^{87}Sr/^{86}Sr$	Range Rb (p.p.m.)	Range Sr (p.p.m.)
Olivine basalt	0.7032–0.7039	6.6–22	200–501
Pyroxene andesite	0.7029–0.7030	31–35	1,390–1,490
Hornblende andesite	0.7032	17	1,300
Greywacke	0.705–0.708	Not given	About 200

The present analogy has its weaknesses, such as the great difference in tectonic setting between marscoite and circum-Pacific andesites, and there is not the same paucity of Sr in Peterman's greywackes relative to olivine basalt as there is in the porphyritic felsite relative to the ferrodiorite (Table 2). This is, however, somewhat countered by the simultaneously smaller difference in the $^{87}Sr/^{86}Sr$ ratios of the Cascade basalt and greywacke, so that the increase in Sr ratio produced by contamination is again limited, but from another quarter. A similar 60/40 mixing ratio of basalt and greywacke, each with 200 p.p.m. Sr, would produce a hybrid with a ratio around 0.705, that is, only 0.001–0.002 higher than the basalt.

In order to test this argument further, rather more precise data on the Marsco occurrence are necessary, and it may be that the accurate methods now available will allow the detection of the small (0.01 %) contribution of the porphyritic felsite to the strontium ratio of marscoite.

The 'extraneous source' of strontium would seem to be the main obstacle in the path of further application of this argument. Moorbath and Bell[3] state that the extraneous strontium could not have had a crustal origin, and this reflects favourably on the argument applied to the Cascade results, because it would not then be expected to alter the andesite ratio significantly, assuming that this is the reason for their high strontium contents.

Table 3 Chemical analysis of marscoite and a typical hornblende andesite

	Marscoite[3]	Hornblende andesite[5]
SiO_2	60.07	61.5
Al_2O_3	14.22	14.98
Fe_2O_3	3.71	1.51
FeO	6.65	4.05
MgO	1.39	3.22
CaO	4.35	4.56
Na_2O	4.02	3.59
K_2O	2 75	2.80

Further data on other cocurrences of known hybridisation would add greatly to the understanding of this problem. In the meantime I suggest that the arguments against a contamination origin for andesite through strontium isotopic work are by no means as watertight as many papers seem to imply.

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1 Green T. H., and Ringwood, A. E., *Earth planet. Sci. Lett.*, **1**, 307 (1966).
2 Peterman, Z. E., Carmichael, I. S. E., and Smith A. L., *Bull. geol. Soc. Am.*, **81**, 311 (1960).
3 Moorbath, S., and Bell, J. D., *J. Petrology*, **6**, 37, (1965).
4 Wager, L. R., Vincent, E. A., Brown, G. M., and Bell, J. D., *Phil. Trans. R. Soc.*, **A257**, 273 (1965).
5 *Mem. geol. Surv. UK*, Glencoe and Ben Nevis (Institute of Geological Sciences, London, 1960).
6 Smith, A. L., and Carmichael, I. S. E., *Contr. Miner. Petrol.*, **19**, 212 (1968).

Possible thermal explanation of contrasting Archean and Proterozoic geological regimes

THE Earth underwent a remarkable change of behaviour at the Archean–Proterozoic boundary. Greenstone belts intruded by granites dominate the geological record before about 2.5×10^9 yr ago. After that time, essentially no such greenstone belts were formed, and long linear orogens characterised by high-grade metamorphic rocks made their first appearance.

A typical Archean greenstone belt has the following characteristics. Early volcanic activity commonly ranges from ultramafic to mafic, implying high and variable degrees of partial melting of the underlying mantle. Series of volcanic rocks becoming progressively more silicic along calc-alkaline differentiation trends overlie the ultramafic flows. Clastic sedimentary rocks are sporadically interbedded with the volcanic rocks, but predominate towards the top of the pile which can reach a thickness of about 100,000 feet (ref. 1). Diapiric granite plutons cut the greenstone belts which are often preserved only as deep keels between the plutons. Structures within the greenstone belts and granitic rocks indicate that they were deformed by vertically acting forces. The rocks show only low grade metamorphism typically in the greenschist facies, although locally amphibolite and granulite grades are reached. Large areas of platformal sediments are lacking in Archean terrains.

In contrast, the Proterozoic terrains show a clear division into mobile belts and platforms. The mobile belts are long linear features characterised by high grade reworking of pre-existing rocks as well as of newly deposited rocks. Deformation is intense and complex with flow-folding predominating. Transcurrent dislocations characterise many Proterozoic mobile belts. In short, the Proterozoic mobile belts are more similar to Phanerozoic orogens than are the Archean greenstone belts.

The contrasts between the Archean and Proterozoic regimes are commonly attributed to a thin Archean sialic crust[1,2]. But there is evidence to show that the Archean crust may have been as thick as that of the present day[3]. Large areas of continental crust are underlain by rocks 2.5 $\times 10^9$ yr old, or older, yet today many of these areas have sialic crust of normal thickness. Either this was the Archean

thickness or sial has been added to the base of the crust since Archean times. Sial added to the base of the crust would cause isostatic rise of the continent and subsequent erosion, so that the gain in thickness of the crust would be substantially less then the amount of underplated sial. Furthermore, post-Archean isostatic uplift and subsequent erosion would expose rocks with lower K/Ar mica ages, so that Archean ages should not be preserved. We consider it much more likely that the continents have grown by lateral accretion since the Archean, possibly with their thickness being controlled essentially by sea level.

Nevertheless, the differences between Archean and Proterozoic geologic regimes outlined above require an explanation, one which is consistent with the relative sharpness and worldwide nature of the change between the two. We suggest that an explanation lies in the thermal behaviour of the Earth.

There is ample evidence to show that the Earth is cooling[1], presumably because of a decrease in heat production by radioactive decay, and also degassing to form the oceans and atmosphere. The supposedly continuous nature of this cooling has perhaps obscured its potential in causing discontinuous geologic behaviour such as the Archean–Proterozoic transition. But a simple possible explanation lies in the intersection of phase boundaries and the geotherm.

Figure 1 shows schematically the relations between geotherms which, as a result of cooling, would migrate downwards with time, and a hypothetical peridotite solidus which would be progressively higher with lower water pressure, that is, would migrate upwards because of mantle degassing. Partial melting occurs only when the geotherm and solidus intersect, and the degree of partial melting will be proportional chiefly to the excess heat involved. Thus during the Archean, with a high geothermal gradient and high P_{H_2O}, there would be melting over a wide P_{H_2O} interval, with adiabatic rise of numerous mantle diapirs probably in a random geological pattern though perhaps locally controlled by crustal fracture systems. This, along with lower pressure differentiation under high P_{H_2O}, would account for the wide range of both extrusive and intrusive magma compositions, as well as the geological patterns of the Archean greenstone belts.

As the extent of intersection of the geotherm and the solidus is decreased as a result of cooling and mantle degassing, the degree of partial melting should decrease. One might thus expect to see a gradual change in magma compositions (a more restricted range and less hydrous nature) with time in the Archean. But as this process continues there would be a rapid change of behaviour at the point where the geotherm and solidus no longer intersect, such as at point A in Fig. 1. Magma at this point would have a very restricted composition and with further cooling melting would cease. This situation is suitable to explain the nature of the Archean–Proterozoic transition. After this condition obtained there would be no further worldwide magmatism, since it would be restricted to local areas of perturbation of the geotherm or to pockets of high water content. Geothermal perturbations could result from adjustments of the lithosphere (possibly an early form of plate tectonics), thus accounting for the linear patterns of Proterozoic magmatism and tectonics, or possibly in zones of concentrated heat flux (deep mantle plumes) for which there seems to be little Proterozoic evidence. We suggest the former process and further suggest this as an explanation for the relatively constant tholeiitic composition of Proterozoic magmas.

This paper arose from discussion with J. G. Thurlow, S. Swinden, and P. L. Dean.

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[1] Anhaeusser, C. R. R., Mason, M. J., and Viljoen, R. P., *Bull. geol. Soc. Am.*, **80**, 2175–2200 (1969).
[2] Cloud, P., *Am. J. Sci.*, **272**, 537–548 (1972).
[3] Condie, K. C., *Bull. Geol. Soc. Am.*, **84**, 2981–2992 (1973).
[4] Ernst, W. G., *Am. J. Sci.*, **272**, 657–668 (1972).
[5] Naldrett, A. J., *Bull. Can. Inst. Min. Metall.*, **66**, 45–63 (1973).

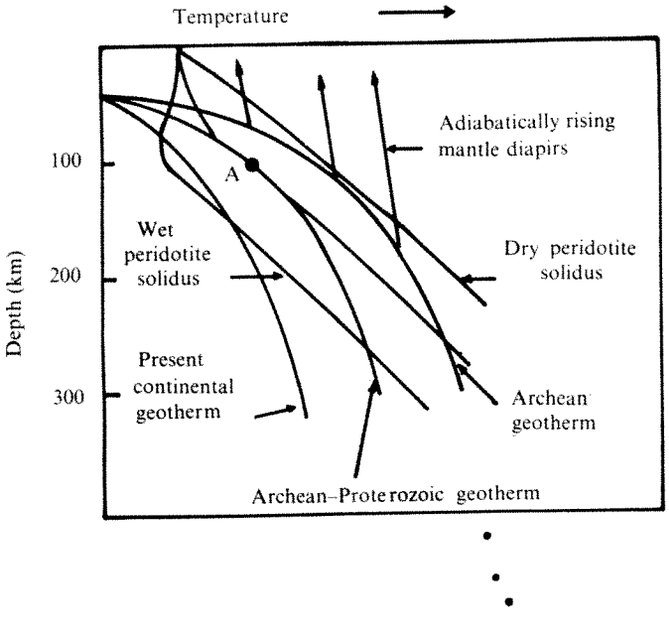


Fig. 1 Schematic relations between peridotite solidus at different water pressures, and geotherms at different times in a cooling Earth. Designed to show the nature of these effects but not precise temperatures or water pressures. See text for further explanation. (Modelled after Fig. 4 of ref. 5).

Pseudo-holography using scattered X rays

A major obstacle to the formation of images with scattered X rays is the lack of a suitable X-ray lens. Radiation at such short wavelengths is not appreciably refracted by matter, which means that a lens, in the optical sense of the word, cannot exist. To overcome this difficulty, a concept can be applied which has previously been used to form an image of sources which emit their own radiation rather than scattering radiation from another source. Basically, the concept consists of a two-step process in which the radiation is used to cast shadows of a coded aperture which are recorded on film. The encoded image is then decoded by one of several possible methods, depending on the type of aperture used. This technique was first proposed for use in X-ray astronomy[1] and has been applied to imaging distribution of γ emitters (refs 2–5, and H. H. Barrett, and F. A. Horrigan, not yet published). We have used a Fresnel zone plate as a coded aperture. This is by no means the only possible aperture, but it does offer some advantages over other types. The advantages involve primarily the ease with which decoding can be accomplished.

The Fresnel zone aperture consists of concentric annular zones of equal area which are alternately transparent and opaque to X rays. Figure 1 illustrates its operation. A

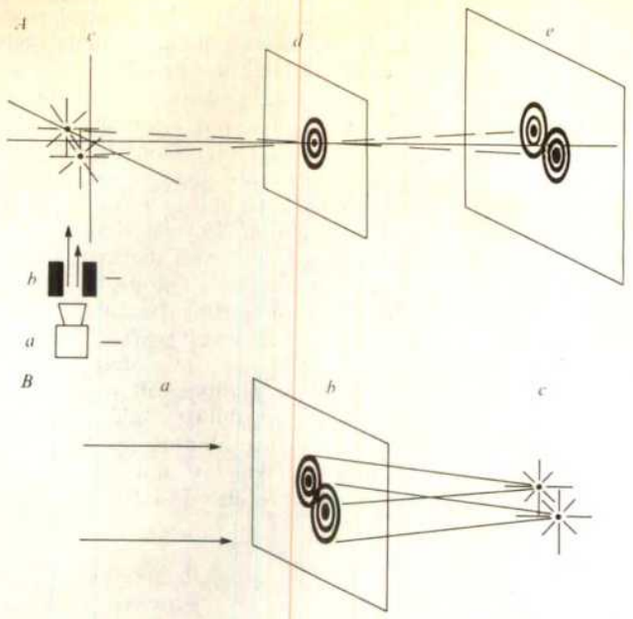


Fig. 1 *A*, Apparatus used to form a shadow of the scatterers: *a*, X-ray generator; *b*, collimator; *c*, scatterers; *d*, Fresnel zone plate; *e*, recording medium. *B*, Formation of an image of the original scatterer: *a*, beam of coherent light; *b*, reduced hologram; *c*, image of point scatterers.

point scatterer is placed at some position on one side of the zone plate. The scattered radiation field casts a shadow of the zone plate on a recording medium such as a sheet of X-ray film. The location of the shadow depends linearly on the position of the scattering point and on the distances between zone plate and film. The shadow, copied at a suitable reduction in size, will be a small Fresnel lens capable of focusing (by diffraction) a beam of coherent light to a point image of the original scatterer (Fig. 1*B*). The focal point of the Fresnel lens is dependent on the size of the shadow and thus on the distance of the scatterer from the aperture. For an actual extended object each point in the object will act as a point scatterer, producing its own Fresnel lens shadow and each lens will reproduce its

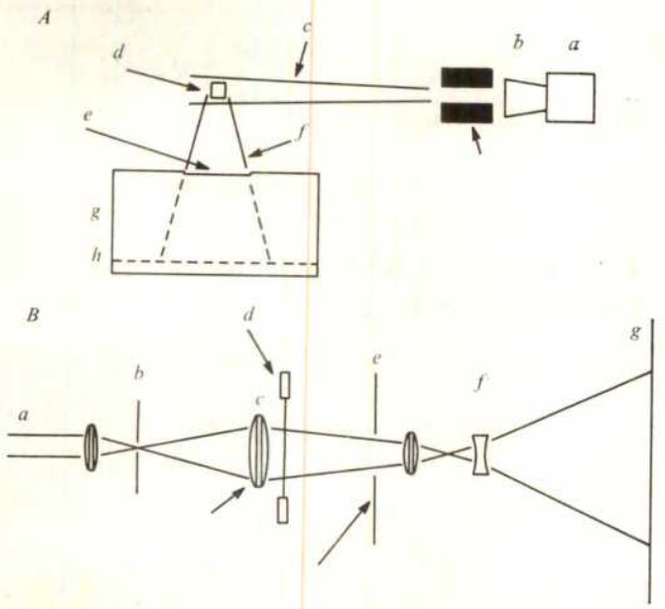


Fig. 2 *A*, Imaging system: *a*, X-ray generator; *b*, collimator; *c*, primary field; *d*, scattering object; *e*, zone plate; *f*, scattered field; *g*, shielding; *h*, X-ray cassette. *B*, Optical reconstruction system: *a*, laser beam; *b*, spatial filter; *c*, transform lens; *d*, hologram; *e*, iris and shutter; *f*, telescope; *g*, image plane.

associated point scatterer independently of all of the others. The superposition of the point images will yield an image of the extended scattering object, and because each plane of the object is at a different distance from the zone plate, it will focus at a different distance from the shadow transparency. As a result, the image will have three-dimensional information which can be viewed tomographically (plane by plane).

The Fresnel zone pattern is familiar as the hologram of a point, and the optical hologram of an extended object can be thought of as the summation of many point holograms (Fresnel zones) formed by each point of the object. This analogy with optical holography implies that the complex shadows of the Fresnel zone aperture can be thought of as a pseudo-hologram of the scattering object, formed by shadowing rather than interference. Decoding can be thought of as the reconstruction of the actual scattered wavefronts.

The imaging system is illustrated in Fig. 2*A*. The primary source was a standard industrial X-ray generator with the beam collimated to a 3-cm circular cross-section and located about 30 cm from the scatterer. For most of the exposures the generator was run at a potential of 90 k*V* and a beam current of 5 mA for an average exposure time of 45 s. The zone plate had 30 zones with a maximum diameter of 10 cm and a minimum width of 0.86 mm. It was used in the on-axis mode and positioned about 8 cm from both the centre of the scatterer and the film in such a way as to view the 90° scattered component. This configuration



Fig. 3 The scattering object.

of the imaging system has theoretical resolution of approximately 2.0 mm as determined by the relative position of the zone plate and the width of the outermost transparent ring.

The opaque rings were composed of a lead powder and glue matrix with an aluminium backing plate comprising the transparent rings. The recording system consisted of an X-ray film cassette with high speed intensifying screens and fast film. The resulting transparencies were copied at a reduction of approximately 64: 1 and placed in the optical reconstruction system (Fig. 2*B*). Light from the helium-neon laser was passed through a spatial filter to clean up the beam and then reconverged through the pseudo-hologram. The real image was initially very small and was therefore magnified and focused on a high speed photographic film packet on which it was recorded.

The scattering object is shown in Fig. 3. The letters were made of aluminium, each approximately 25 mm high, 18 mm wide and 6 mm thick. The aluminium support rod was 2.5 mm in diameter and the letters were positioned with lateral displacement 6 mm and depth separation 12 mm. The purpose of the depth separation was to demonstrate the

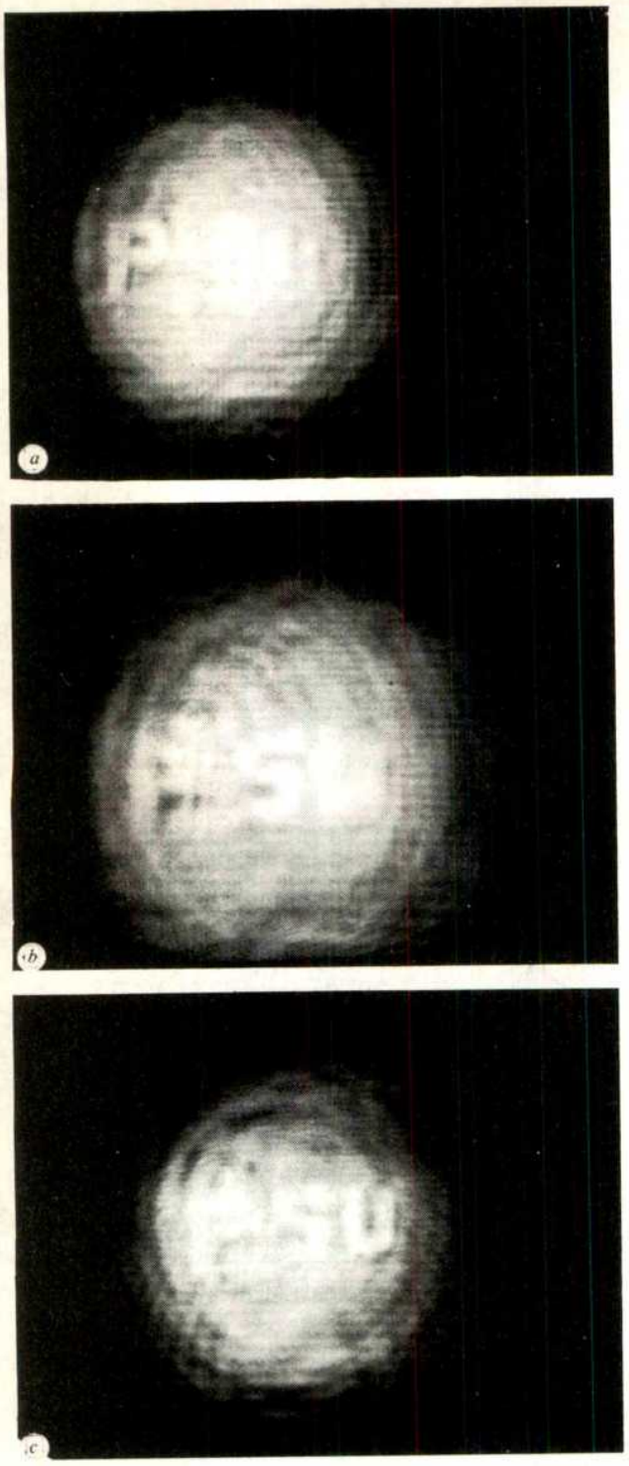


Fig. 4 Reconstructed images of the scattering object (Fig. 3) showing the three-dimensional capabilities of this type of imaging: *a*, focused in the 'P' plane; *b*, focused in the 'S' plane; *c*, focused in the 'U' plane.

three-dimensional capabilities of this method of imaging and its effects can clearly be seen in the reconstruction.

Figures 4*a*, *b*, and *c* show the reconstructed images. The tomographic effect is clearly demonstrated, with each reconstruction corresponding to a different image plane. The theoretical resolution of the system was calculated to be approximately 2.0 mm. This is clearly achieved as the dimensions of the centre of the 'P' are 4 mm by 6 mm. The background appearing in the reconstruction is caused by the undiffracted light passing through the pseudo-holograms. Removal of the background will result in higher quality images and can best be accomplished through the use of the zone plate in the off-axis rather than the on-axis mode. In the off-axis mode the undiffracted component and the image are laterally separated.

The results of this work clearly demonstrate that the use of coded apertures permits imaging with scattered radiation of a very different character from visible light. Although this particular work was done using scattered X rays, it is important that the technique is by no means restricted to that type of radiation. Indeed, similar results have been achieved in our laboratory with thermal neutrons scattering from hydrogeneous material. The use of penetrating radiation is particularly interesting because most of the photons which carry information scatter from the interior of the object. This allows those details of the object which are hidden from photons of visible wavelengths to be viewed. Such a capability could prove very useful in both medical and industrial radiography.

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1 Mertz, L., and Young, N. O., *Proc. Int. Conf. on Optical Instrum., London*, 305 (1961).
2 Barrett, H. H., *J. nucl. Med.*, **13**, 382 (1972).
3 Barrett, H. H., Wilson, P. T., and DeMeester, G. D., *Opt. Commun.*, **5**, 398 (1972).
4 Barrett, H. H., *Proc. IEEE.*, **60**, 723 (1972).
5 Rogers, W. L., Han, K. S., Jones, L. W., and Beierwaltes, W. H., *J. nucl. Med.*, **13**, 612 (1972).
6 Dicke, R. H., *Astrophys. J.*, **153**, L101 (1968).

BIOLOGICAL SCIENCES

Tight binding of RNA polymerase to rDNA genes in *E. coli*

THE synthesis of ribosomal RNA (rRNA) in exponentially growing bacteria accounts for approximately half of the total RNA synthesis, although the ribosomal DNA (rDNA) genes make up only 0.3–0.4% of the genome[1]. Electron-microscopic observations[2] and biochemical data[3,4] suggest that the frequency of transcription initiation on these genes is much higher than on any other part of the bacterial chromosome. If the same enzyme is responsible for the transcription of all kinds of bacterial RNA, this difference in the initiation frequency might be due either to a specific regulatory factor or to the structure of the relevant interacting macromolecules themselves. If the latter were true, the DNA promoter sequence responsible for the initiation of transcription on rRNA genes should be more active than other promoters. This hypothesis is supported by the

discovery of preferential *in vitro* rRNA synthesis with purified RNA polymerase in the absence of any external factor[5–7].

Several attempts have been made to characterise the interaction of RNA polymerase with the DNA promoters. Conditions could be found under which RNA polymerase was bound only on distinct sites of the DNA of several simple DNA phages[8,9] and these sites seemed to be the promoters[10]. Fragmentation of DNA did not disturb the specificity of binding[11]. Kinetic analysis of dissociation observed at various ionic strengths revealed that the stabilities of these specific enzyme–DNA complexes were different[8].

The filter binding technique of Jones and Berg[12] as modified by Hinkle and Chamberlin[8] offered the possibility for us to study the interaction of RNA polymerase with fragments of the much more complex bacterial DNA. The easy and extremely sensitive technique of RNA–DNA hybridisation allowed us to differentiate between rDNA and non-rDNA fragments. We reasoned that if the rDNA promoters are indeed more active, then, under certain conditions which favour the dissociation of less stable complexes, that is, at raised ionic strengths, the proportion of rDNA should increase in the bound fraction, provided the fragments are of appropriate size.

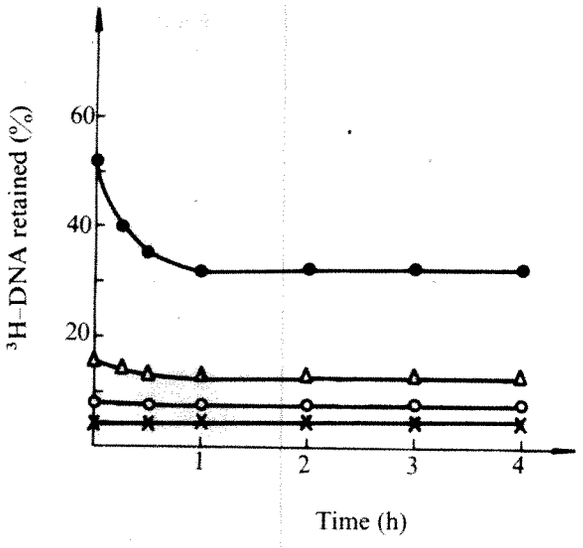


Fig. 1 Highly stable complex of RNA polymerase holoenzyme and bacterial DNA. ^{3}H-labelled DNA from *E. coli* MRE 600 (specific activity 14,200 c.p.m. μg^{-1}) was sheared by passing it several times through an injection needle (No. 16) with syringe to get DNA fragments of average double stranded molecular weight 12×10^6 as determined by gradient centrifugation in neutral sucrose density gradient[17]. ^{32}P-labelled λ phage DNA was applied as molecular weight standard. In order to remove single-stranded ends from the DNA fragments 50 μg sheared DNA was incubated for 5 min at 37° C in 200 μl of reaction mixture containing 0.015 M sodium acetate *p*H 4.6, 0.05 M NaCl, 1 mM $ZnSO_4$, 5% glycerol and 5 μg nuclease S_1 prepared from *Aspergillus oryzae* crude alpha-amylase[18]. The reaction was stopped by the addition of 10 μl M Tris HCl *p*H 8.4, to bring the *p*H to 7.0. RNA polymerase holoenzyme was prepared from *E. coli* MRE 600 according to Burgess[19] as modified by Nüsslein *et al.*[20]. The enzyme synthesised 8–10% rRNA on total *E. coli* DNA template. 4.5 μg nuclease S_1-treated ^{3}H-DNA was pre-incubated for 15 min at 37° C in 0.8 ml binding buffer[8]. Binding reaction was started by the addition of RNA polymerase. After an additional 20 min incubation, 45 μg unlabelled T_7 phage DNA was added to dilute out the dissociated enzyme molecules. During subsequent incubation 100 μl aliquots were removed at the times indicated, diluted with binding buffer to 1 ml, filtered immediately through Millipore HAMP 02500 (0.45 μm) filters under 2 cm of mercury vacuum, washed with 3 ml of binding buffer, dried and counted in a toluene base scintillation fluid. A blank (0.2–0.3%) representing the amount of ^{3}H-labelled DNA retained by the filter in the absence of RNA polymerase were subtracted from all points. The amounts of RNA polymerase holoenzyme added were 1 μg ●; 0.35 △; 0.15 μg ○; 0.1 μg ×.

When ^{3}H-labelled *Escherichia coli* DNA was mixed with highly purified RNA polymerase holoenzyme in a proportion of 4.5:1 by weight, approximately 50% of the DNA was retained on the nitrocellulose filters as DNA-protein complex. On dilution with unlabelled homologous or heterologous T_7 phage DNA the filter-bound radioactivity rapidly dropped to 30% but the dissociation did not go further (Fig. 1). The non-dissociated complex corresponds to the highly stable complex defined by Hinkle and Chamberlin[8]. At a lower polymerase: DNA ratio practically all the enzyme molecules could be found in such a highly stable complex. The proportion of rDNA in this highly stable complex was approximately the same as in the total DNA. If, however, the ionic strength of the medium was increased, the complex progressively dissociated (Fig. 2) and the amount of DNA retained on the filters decreased. The rDNA content of the filter-bound DNA was inversely proportional to the total amount of DNA bound (Table 1). In order to prove that in DNA–RNA polymerase complexes observed at different ionic strengths the enzyme was bound to specific regions of DNA (that is, to promoters) dissociation experiments were repeated in the presence of heparin which inactivates free and non promoter-bound RNA polymerase[13,14]. Heparin destroyed a fraction of enzyme–DNA complexes and the rDNA enrichment in the heparin and salt-resistant complexes was more pronounced (Table 1). Our results do not prove that the observed stability is an exclusive property of rDNA promoters. But in view of the magnitude of rDNA enrichment and the average size of fragments it is obvious that these promoters belong

Table 1 Tight binding of RNA polymerase to rDNA

Salt-resistant fraction M $(NH_4)_2SO_4$	Without heparin		With heparin	
	^{3}H-DNA retained (c.p.m.)	rDNA content (%)	^{3}H-DNA retained (c.p.m.)	rDNA content (%)
0.0 (control)	7,030	0.33	4,940	0.41
0.125	3,630	0.79	2,630	1.20
0.250	1,350	1.87	1,030	2.41
0.375	590	4.26	490	4.95
0.500	370	8.45	310	10.60

14 μg ^{3}H-DNA (average double-stranded molecular weight 12×10^6) treated with nuclease S_1 as described in Fig. 1 was preincubated in 0.8 ml binding buffer for 15 min at 37° C. Binding reaction was started with the addition of 0.6 μg RNA polymerase holoenzyme. After 20 min binding buffer or heparin (1 mg ml^{-1}) was added and 10 min later dissociation was initiated with ammonium sulphate. To measure the amount of salt-resistant enzyme-DNA complex 100 μl aliquots were diluted after 60 min incubation with binding buffer to 1 ml and filtered. For the estimation of rDNA content the rest of the reaction mixture was diluted with 3 ml of binding buffer and filtered. Approximately 95–97% of this filter-bound DNA can be recovered by incubating the filters for 3 h at 37° C in 2 ml of 0.15 M NaCl, 0.015 M Na citrate, *p*H 7.0 containing 200 μg ml^{-1} self-digested pronase. The rest of the bound DNA was liberated by soaking the filters in 1 ml of 0.3% sodium dodecylsulphate at 100° C for 20 min. The pronase and SDS fractions were pooled, exhaustively dialysed against 0.01 M NaCl, 0.01 M Tris HCl, *p*H 7.0 DNA was denatured by alkali and hybridised with ^{32}P-labelled rRNA, specific activity $1.25.10^6$ c.p.m. μg^{-1} on Millipore filters[21]. ^{32}P-labelled rRNA was prepared from *E. coli* MRE 600 growing on 121 pepton medium[22] containing 12.5 mCi KH_2 $^{32}PO_4$ per 100 ml of culture. Before collecting the cells a 200-fold excess of potassium phosphate was added for a generation to chase radioactivity from the messenger RNA. Ribosomes were prepared and purified[23]. rRNA was prepared by phenol extraction and ethanol precipitation and further purified on methylated albumin-Kieselguhr column with a linear NaCl gradient. The hybridisation blank of rRNA prepared in this way was less than 0.02% of input radioactivity.

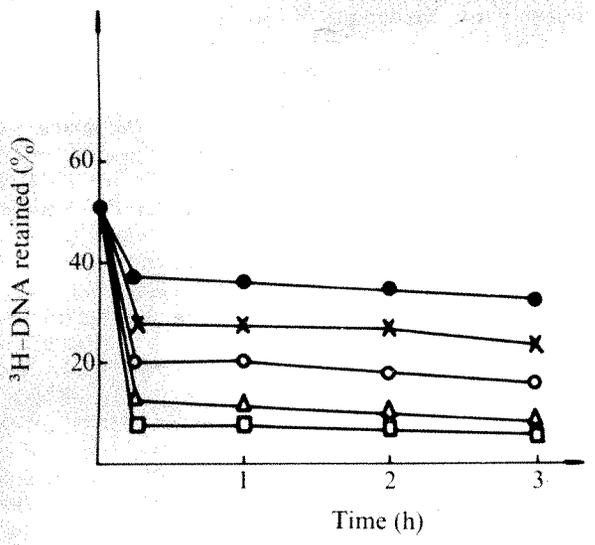


Fig. 2 Effect of ionic strength on dissociation of the highly stable complex. 1 μg RNA polymerase and 4.5 μg ^{3}H-DNA treated with nuclease S_1 was mixed to prepare the enzyme-DNA complex as described in Fig. 1. The complex was dissociated by adding increasing concentrations of ammonium sulphate in the presence of 45 μg unlabelled T_7 phage DNA. At the times indicated 100 μl aliquots were removed, diluted with binding buffer to 1 ml and filtered. The final concentrations of ammonium sulphate were 0.05 M ●; 0.10 M ×; 0.15 M ○; 0.20 M △; 0.25 M □.

to a small group of promoters, representing only a fraction of the total, which might be characterised by extremely tight binding.

As expected, the rDNA enrichment in the salt-resistant complex was smaller if the DNA fragments were larger (Table 2). Under a molecular weight of about 10^6 however, the enrichment effect could not be enhanced, small fragments bound very poorly and the effect was not reproducible.

Elution of the tightly bound complex from the filters was very difficult. One hundred per cent recovery could be achieved only after prolonged pronase digestion plus hot SDS treatment. If the pronase treatment was shorter, only 95% of the bound DNA could be eluted and this fraction was not enriched with rDNA; the bulk of the rDNA was found in the partly pronase-resistant 5% (Table 3). It is known that RNA polymerase becomes more resistant to proteolytic enzymes after binding to DNA, and this is thought to be due to a conformational change[15,16]. Our result suggests that the strong binding of the polymerase at the rDNA promoters might effect this conformational change, rendering the complex even less susceptible to pronase attack.

The demonstration of an extremely strong binding of RNA polymerase to DNA fragments carrying the rDNA genes strongly supports the notion that DNA structure itself might be responsible for the high frequency of transcription on these genes observed both *in vivo* and *in vitro*, although it does not exclude additional regulatory factors.

Table 2 Effect of template molecular weight on rDNA enrichment

Salt-resistant fractions M $(NH_4)_2SO_4$	rDNA content (%) Molecular weight of DNA 34×10^6	20×10^6	12×10^6
0.0 (control)	0.27	0.34	0.32
0.125	0.39	0.50	0.68
0.250	0.61	0.93	1.40
0.375	0.87	1.86	3.43
0.500	1.05	2.15	8.37

Salt-resistant fractions of the RNA polymerase holoenzyme–DNA complex were prepared as described in Fig. 1 applying DNA of different molecular weights. The rDNA content of these fractions was determined by hybridisation with ^{32}P-labelled rRNA.

Table 3 Distribution of rDNA sequences between pronase sensitive and pronase resistant fractions of enzyme-DNA complex

	rDNA content (%)
Pronase-sensitive fraction	0.19
Pronase-resistant fraction	3.1
Total DNA (control)	0.3

A highly stable complex of RNA polymerase holoenzyme and DNA was prepared as described in Fig. 1. The whole reaction mixture without addition of ammonium sulphate was diluted with 3 ml of binding buffer and filtered. Ninety-two per cent of the filter-bound DNA was recovered after incubation for 60 min at 37° C in 0.15 M NaCl, 0.015 M Na citrate, *p*H 7.0, containing 200 μg ml^{-1} self-digested pronase. The rest of the filter-bound DNA, the pronase-resistant fraction, was liberated by an additional pronase digestion for 2 h and hot SDS treatment. Both pronase-resistant and pronase-sensitive fractions were hybridised with ^{32}P-labelled rRNA.

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1 Lazzarini, R. A., and Winslow, R. M., *Cold Spring Harbor Symp. quant. Biol.*, **35**, 383 (1970).
2 Hamkalo, B. A., and Miller, Q. L., jun., *A. Rev. Biochem.*, **42**, 379 (1973).
3 Pato, M. L., and von Meyerburg, K., *Cold Spring Harbor Symp. quant. Biol.*, **35**, 497 (1970).
4 Doolittle, W. F., and Pace, N. R., *Proc. natn. Acad. Sci., U.S.A.*, **68**, 1786 (1971).
5 Haseltine, W. A., *Nature*, **235**, 329 (1972).
6 Pettijohn, D. E., *Nature new Biol.*, **235**, 204 (1972).
7 Hussay, C., Pero, J., Shorenstein, R. G., and Losick, R., *Proc. natn. Acad. Sci., U.S.A.*, **69**, 406 (1972).
8 Hinkle, D. C., and Chamberlin, M. J., *J. molec. Biol.*, **70**, 157 (1972).
9 Nakano, E., and Sakaguchi, K., *FEBS Lett.*, **14**, 139 (1971).
10 Chamberlin, M. J., and Ring, J., *J. molec. Biol.*, **70**, 221 (1972).
11 Nakano, E., *FEBS Lett.*, **22**, 139 (1972).
12 Jones, O. W., and Berg, P., *J. molec. Biol.*, **22**, 199 (1966).
13 Willmund, R., Kneser, H., *Molec. gen. Genet.*, **126**, 165 (1973).
14 Schäfer, R., Krämer, R., Zillig, W., and Cudny, H., *Eur. J. Biochem.*, **40**, 367 (1973).
15 Kolesin, R. B., Astaurova, O. B., Shemyakin, M. F., Komzolova, S. G., and Manyakov, S. G., *Molec. Biol.*, **1**, 617 (1967).
16 Novak, R. L., and Doty, P., *J. biol. Chem.*, **243**, 6068 (1968).
17 Burgi, E., and Hershey, A. D., *Biophys. J.*, **3**, 309 (1963).
18 Vogt, V. M., *Eur. J. Biochem.*, **33**, 192 (1973).
19 Burgess, R. R., *J. biol. Chem.*, **244**, 6160 (1969).
20 Nüsslein, C., and Heyden, B., *Biochem. biophys. Res. Commun.*, **47**, 282 (1972).
21 Gillespie, D., and Spiegelman, S., *J. molec. Biol.*, **12**, 829 (1965).
22 Torriani, A., in *Procedures in Nucleic Acid Research* (edit. by Cantoni, G. L., and Davies, D. R.), 224 (Harper and Row, New York, 1966).
23 Stanley, W. M., jun., and Wahba, A. J., in *Methods in Enzymology* (edit. by Colowick, S. P., and Kaplan, N. O.), 524 (Academic Press, New York and London, 1967).

Probable linkage of LCAT locus in man to the α haptoglobin locus on chromosome 16

LECITHIN: cholesterol acyltransferase (LCAT) catalyses the esterification of cholesterol in plasma. Familial LCAT deficiency was first described in 1967 (ref. 1); in 1970 a second[2] family and in 1973 a third, with the same disease, were found in Norway. Seven patients with ages of 26–60 yr

are now known in that country[3].

Clinically the disease is characterised by proteinuria, corneal opacities and normochromic anaemia with decreased erythrocyte life span. The plasma cholesteryl ester and lysolecithin concentrations are decreased, and free cholesterol and lecithin usually increased. Studies of the LCAT-deficient patients have increased our knowledge of normal lipoprotein metabolism in man[4–7]. The fact that the patients develop atherosclerotic lesions when young, even though they have extremely low levels of plasma cholesteryl esters, may help our understanding of the development of atherosclerotic lesions.

The first three families with this disorder have been traced back five to eight generations and most members have come from the same area[8,9]. No inbreeding occurred between the maternal and paternal branches of the families. Moreover, no direct connections have been found between the families. The familial occurrence, the horizontal distribution within families and the fact that the disease manifests itself in both sexes make an autosomal, recessive inheritance pattern likely. The presumed heterozygous carriers (parents and children of the patients) are clinically and biochemically normal. One possibility is that the genetic basis of this disease is the presence of an LCAT-deficiency gene in double dose. This defective gene, operating in all three Norwegian families, is probably the result of one mutational event which occurred at least 250–300 yr ago.

In an attempt to characterise further the genetic defect operating in LCAT deficiency we carried out linkage studies to genetic marker systems currently in common use. The following markers were examined in the three families: A_1A_2BO, Rh (CDEce) MNS, Kell, Fy(b), haptoglobin (Hp), group specific component (Gc), transferrin (Tf) third component of complement (C3), phosphoglucomutase (PGM_1), erythrocyte acid phosphatase (EAP), alanine aminotransferase (GPT) and HL-A.

The studies excluded close linkage between the LCAT gene and the genes determining the marker systems Rh, Duffy, Gc, C3, PGM_1, EAP and HL-A. With respect to the other markers, these families were not sufficiently informative with one exception: nonrandom assortment was observed between LCAT deficiency and Hp types (Fig. 1). The appearance and inheritance pattern of the Hp phenotypes were quite regular in all family members studied, including 15 more distant relatives. When tests for linkage were carried out using a computer program (MOSM) described by Gedde-Dahl *et al.*[10], a combined lod score of 2.81 at a recombination fraction of 0.00 was found. At a recombination fraction of 0.05 the lod score is 2.48. This is very close to a lod score of 3.00 which is usually regarded as good evidence for chromosomal linkage. Given close linkage no recombinants can be counted between the α haptoglobin locus and the LCAT locus.

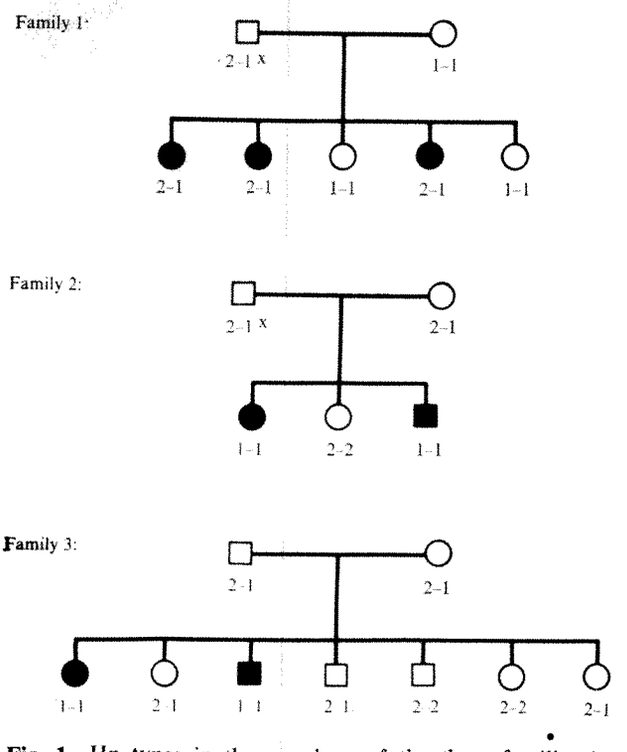


Fig. 1 Hp types in the members of the three families in which LCAT deficiency has been found. ×, Hp types deduced from the types of the other family members; ● ○, females; ■ □, males. ■, ●, Patients with LCAT deficiency; □, ○, family members with no signs of disease.

The total posterior probability of linkage calculated according to Smith[11] was 0.81. A linkage disequilibrium exists, with five out of the six parents handing over the LCAT deficiency gene to their children together with the Hp 1 type. The probability of such a constellation appearing by chance is less than 0.04. Through the period of at least six to eight generations that this gene has existed, only one proven crossover event has taken place. This speaks in favour of close linkage, with a probable recombination fraction of less than 0.05 between the two loci.

Robson *et al.*[12] have shown that the α haptoglobin locus, which determines the genetic variability of haptoglobin, is situated between the middle and terminal point of the long arm of chromosome 16. No case of linkage to this locus has been reported so far.

Our work indicates rather strongly that the LCAT locus is situated on chromosome 16, close to the α haptoglobin locus. This is of interest to geneticists as a new linkage group is added to the human chromosome map. Prenatal diagnostic are not possible at present because haptoglobin is not formed in significant quantities in the foetus.

Our results suggest that the basis of this disease, which reveals an inborn error of lipid metabolism, is a single genetic defect causing a lack of LCAT activity in plasma.

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1 Gjone, E., and Norum, K. R., *Fedn. Eur. biochem. Soc. 9th Meet July 3–7, 1967, Oslo*, Abstracts of Communications, 141 (Universitetsforlaget, Oslo 1967).
2 Norum, K. R., Børsting, S., and Grundt, I., *Acta med. scand.*, **188**, 323 (1970).
3 Gjone, E., *Acta med. scand.*, **194**, 353 (1973).
4 Forte, T., Norum, K. R., Glomset, J. A., and Nichols, A. V., *J. clin. Invest.*, **50**, 1141 (1971).
5 Glomset, J. A., *Blood lipids and lipoproteins*, 745–787 (Wiley, New York, 1972).
6 Glomset, J. A., Norum, K. R., and King, W., *J. clin. Invest.*, **49**, 1827 (1970).
7 Norum, K. R., Glomset, J. A., Nichols, A. V., and Forte, T., *J. clin. Invest.*, **50**, 1131 (1971).
8 Torsvik, H., thesis Universitetsforlaget, Oslo, 22–23 (1972).
9 Gjone, E., Skarbøvik, A. J., Blomhoff, J. P., and Teisberg, P., *Scand. J. clin. Invest.* (in the press).
10 Gedde-Dahl, T., jun., Fagerhol, M. K., Cook, P. J. L., and Noades, J., *Ann. hum. Genet.*, **35**, 393 (1972)
11 Smith, C. A. B., *Am. J. hum. Genet.*, **41**, 289 (1959).
12 Robson, E. B., Polani, P. E., Dart, S. J., Jacobs, P. A., and Renwick, J. H., *Nature*, **223**, 1163 (1969).

Photoreactivation of sister chromatid exchanges induced by ultraviolet irradiation

In contrast to the inefficiency of ionising radiations in the induction of sister chromatid exchanges (SCEs)[1,2], ultraviolet light can cause a striking increase in the SCE frequency in mammalian chromosomes[3,4]. It has been shown in Chinese hamster cells that the SCE formation requires postirradiation DNA synthesis, and that a single ultraviolet irradiation can induce SCE repeatedly for several cell cycles[4]. As pyrimidine dimers are the major DNA lesions caused by ultraviolet light and as Chinese hamster cells have a poor ability to excise them[5], the delayed formation of SCE seems to be due to unexcised pyrimidine dimers. This possibility was tested here with the use of a rat kangaroo cell line possessing a photoreactivating enzyme[6-8] to see if the frequency of ultraviolet-induced SCE was actually reduced when cells were post-treated with visible light.

The female rat kangaroo cell line, Pt-kl, had a doubling time of about 38 h in our culture conditions[4]. In the first experiment, exponentially growing cells were labelled with ^{3}H-thymidine (0.2 μCi ml^{-1}, specific activity 12.1 Ci mmol^{-1}) for 8 h, washed twice with fresh medium containing 10 μM cold thymidine, drained, and then exposed to ultraviolet light at doses ranging from 20–80 erg mm^{-2} at an incident dose rate of 5 erg mm^{-2} s^{-1}. The cultures were then incubated in normal medium containing cold thymidine. One set of duplicate cultures was kept in the dark, while the other was illuminated for 12 h with visible light from a 20-W fluorescent 'blue light' bulb (National) located 10 cm above the culture dishes. After exposure to visible light, the cultures were returned to the dark. The cells were collected 68 h after ultraviolet irradiation. Colcemid (0.05 μg ml^{-1}) was added to culture medium 8 h before collection, chromosome preparations being made according to the conventional air-drying method. At cultivation, most labelled cells were in the second postlabelling mitosis. Slides were coated with emulsion (Sakura, NR-M2) and, after appropriate exposure times, were developed and stained with Giemsa. The frequency of SCE in the largest (No. 1) chromosomes is plotted against ultraviolet dose in Fig. 1. Ultraviolet irradiation at 80 erg mm^{-2} caused about a four-fold increase in the SCE frequency above a control level, whereas illumination of ultraviolet irradiated cells with visible light lowered the SCE frequency markedly. Thus, at 80 erg mm^{-2} of ultraviolet light, it was only twice the control level. The only ultraviolet-induced DNA lesion that is known to be reversible by photoreactivation is pyrimidine dimer[9]. Now that most SCEs induced by ultraviolet light are photoreactivable, it may be safely concluded that the lesions leading to the formation of SCEs are pyrimidine dimers.

This conclusion was confirmed by experiments in which ultraviolet irradiated cells were kept in the dark for various times before being submitted to photoreactivation treatment and ^{3}H-labelling. The results are shown in Fig. 2. When cultures were kept in the dark, the SCE frequency decreased gradually with time, but it was significantly higher than the control level even 164 h after irradiation. By that time the cells might have undergone division at least three or four times, as mitotic delay caused by the ultraviolet dose used was found to be less than 24 h during this culture period.

These results indicate that a substantial number of pyrimidine dimers remains unexcised, even though the rat kangaroo cells are reported to carry out repair replication after ultraviolet exposure[7], and that the cells might have a repair mechanism that renders them tolerant to the presence of unexcised pyrimidine dimers. These dimers, if not repaired by photoreactivation, would repeatedly provoke SCE before they are either removed by the excision mechanism and/or diluted through consecutive cell divisions. Previous work has shown that induction of SCE by ultraviolet light requires a postirradiation replication and is inhibited by caffeine post-treatment[4]. The involvement of pyrimidine dimers in this phenomenon was confirmed in our study. These features seem to indicate that SCE is closely related to postreplication repair of ultraviolet-induced DNA damage[10-13].

The exact mechanism underlying the induction of SCEs is still unknown. At least, it is probable that the formation of SCEs involves double-strand exchange: assuming that each chromatid contains a single DNA duplex[14,15], only a parental strand in one of the sister chromatids is assumed to contain ^{3}H. A switch of silver grains on an autoradiogram might therefore be an outcome of exchange between the labelled parental strand in one chromatid and an unlabelled daughter strand in another. To achieve this step while avoiding an unwinding difficulty, simultaneous exchange between the remaining two strands of the same polarity in the two sister chromatids would be required. Considering this aspect, Whitehouse's model[16], which was originally proposed to explain genetic crossing over, also seems to be a feasible explanation for formation of SCEs. According to his model, the initial step of crossing-over is the formation of staggered breaks of two nascent, complementary strands from two DNA molecules, which is then followed by a limited replication of the broken ends along the parental template strands. In the induction of SCEs by ultraviolet

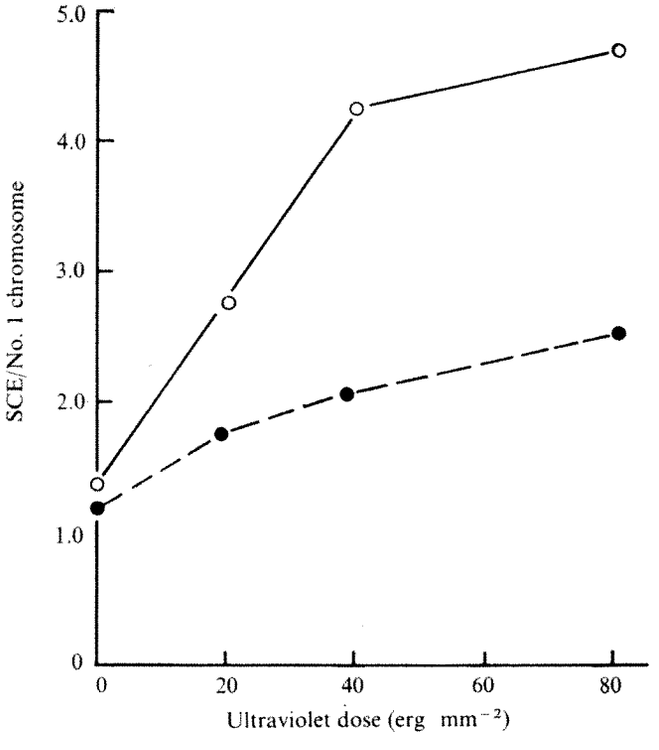


Fig. 1 Dose response curves of (SCEs) with or without photoreactivation treatment. SCE was scored only in No. 1 chromosomes in cells at a diploid level which constituted about 60% of the cell population. Any unlabelled region following a labelled segment of a chromatid and matched by the opposite pattern in its sister chromatid was scored as one exchange. The presence of an unlabelled region within a labelled segment together with the presence of at least three grains on the sister chromatid opposite the region was regarded as evidence that two exchanges had occurred. In ultraviolet irradiated cultures, a considerable number of cells had aberrant chromosomes, so that only intact chromosomes were selected for scoring. No account was taken of whether other chromosomes within the cell were aberrant or not. Isolabellings were omitted from the score. O, Ultraviolet only; ●, ultraviolet and visible light.

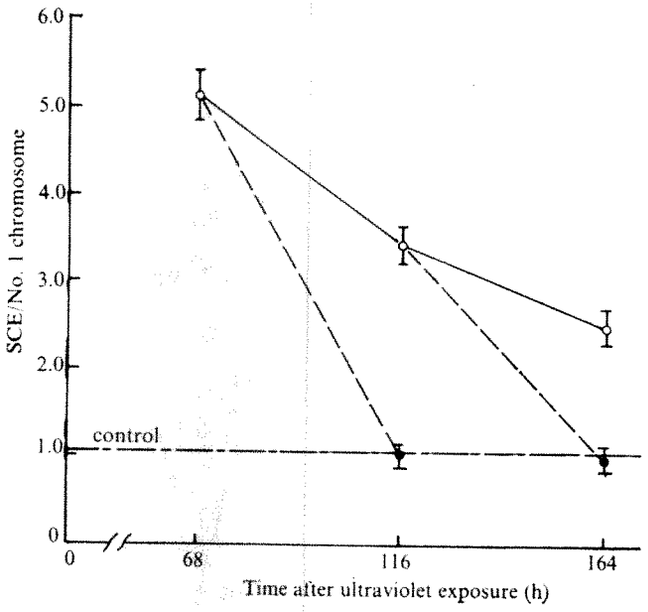


Fig. 2 Delayed formation of SCEs after a single ultraviolet exposure at a dose of 60 erg mm^{-2}. The exchange frequency was determined for only No. 1 chromosomes in the second postlabelling metaphase cells. One set of cultures was labelled with ^{3}H-thymidine for 8 h and exposed to ultraviolet light at 60 erg mm^{-2}. It was incubated in the dark and fixed for chromosome preparation at 68 h after irradiation. Other sets of cultures were first exposed to ultraviolet light at 60 erg mm^{-2} and incubated in the dark for times before being submitted to photoreactivation treatment and ^{3}H labelling: one of duplicate cultures was for 12 h illuminated with visible light from 28 h to 40 h after ultraviolet irradiation, while the other was kept in the dark. Both were then labelled in the dark for 8 h, incubated further and fixed 68 h later, that is, 116 h after ultraviolet irradiation. A similar procedure was applied to the remaining set of cultures: one of them was illuminated with visible light from 76 h to 88 h after ultraviolet irradiation, while the other was kept in the dark before ^{3}H labelling. — — —, Visible light; ———, ultraviolet light.

light these steps may correspond to the formation of gaps in the two newly synthesised strands at adjacent positions and to the *de novo* DNA synthesis detected previously[10,11], although it may still be that the staggered breaks are actually simple nicks made enzymatically, provided that, as claimed by Chiu and Rauth[17], most dimer sites do not involve gap formation after exposure to small amounts of ultraviolet light. The most important part of his model is his proposal that cross-over molecules form by pairing of the two overlapping sectors of the daughter strands of the broken ends. This process effects a crosswise reunion of daughter DNA strands of the two future sister chromatids. In the light of recent findings about the middle repetitive nucleotide sequence in eukaryote DNA[18–20], it is tempting to speculate that such a pairing process may be facilitated by short repetitive nucleotide sequences interspersed between unique sequences.

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1 Marin, G., and Prescott, D. M., *J. Cell Biol.*, **21**, 159 (1964).
2 Gibson, D. A., and Prescott, D. M., *Expl Cell Res.*, **74**, 397 (1972).
3 Rommelaere, J., Suskind, M., and Errera, M., *Chromosoma*, **41**, 243 (1973).
4 Kato, H., *Expl Cell Res.*, **82**, 383 (1973).
5 Trosko, J. E., Chu, E. H. Y., and Carrier, W. L., *Radiat. Res.*, **24**, 667 (1965).
6 Cook, J. S., and Regan, J. D., *Nature*, **223**, 1066 (1969).
7 Krishnan, D., and Painter, R. B., *Int. J. radiat. Biol.*, **21**, 97 (1972).
8 Krishnan, D., and Painter, R. B., *Mutation Res.*, **17**, 213 (1973).
9 Rupert, C. S., and Harm, W., in *Advances in Radiation Biology* (edit. by Augenstein, L. G., Mason, R., and Zelle, M.), **2**, 1 (Academic Press, New York, 1966).
10 Lehman, A. R., *J. molec. Biol.*, **66**, 319 (1972).
11 Buhl, S. N., Setlow, R. B., and Regan, J. D., *Int. J. radiat. Res.*, **22**, 417 (1972).
12 Cleaver, J. E., and Thomas, G. H., *Biochem. biophys. Res. Commun.*, **36**, 202 (1969).
13 Trosko, J. E., and Chu, E. H. Y., *Chem.-biol. Interactions*, **6**, 317 (1973).
14 Laird, C. D., *Chromosoma*, **32**, 378 (1971).
15 Kavenoff, R., and Zimm, B. H., *Chromosoma*, **41**, 1 (1973).
16 Whitehouse, H. L. K., *Nature*, **199**, 1034 (1963).
17 Chiu, S. F. H., and Rauth, A. M., *Biochim. biophys. Acta*, **259**, 164 (1972).
18 Bonner, J., and Wu, J-R., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 535 (1973).
19 Davidson, E. H., Hough, B. R., Amenson, C. S., and Britten, R. J., *J. molec. Biol.*, **77**, 1 (1973).
20 Smyth, D. R., and Stern, H., *Nature new Biol.*, **245**, 94 (1973).

Molecular basis of control of mitotic cell division in eukaryotes

MITOTIC division in *Physarum polycephalum* is preceded by a marked increase in the phosphate content of very lysine-rich histone (Pp. F1) (ref. 1) due to phosphorylation of serine residues probably at specific points in the amino acid sequence[2,3]. The phosphate content of F1 histone has been measured by a number of groups under a variety of conditions in an attempt to understand the role of F1 phosphorylation and three facts have been established.

First, F1 phosphate content is positively correlated with cell replication rate in calf[4], cultured hepatoma cells[5,6], normal and regenerating rat liver[7] and a variety of rat and mouse tumours[8], giving an important indication that the correlation is universal.

Second, some F1 phosphorylation takes place during S phase. In regenerating rat liver F1 phosphorylation occurs in the few hours before mitosis[7–9] but it is not possible to distinguish, in this system, between S phase and G2 phase and this high phosphate content could not be correlated either with DNA synthesis or with initiation of mitosis. Histone phosphorylation has been studied in cultured mammalian cells synchronised at mitosis using a blocking agent and mitotic selection[10] which gives synchrony through G1 phase and into S phase but not in G2 phase[11]. Pulse labelling experiments have shown increased phosphorylation of F1 in S phase[10]. Different experiments using long term double labelling and the precise natural synchrony in *P. polycephalum* have shown that there is no change in the overall phosphate content per molecule of Pp. F1 during S phase[1].

It is important to determine whether these data are consistent with the mammalian pulse labelling experiments and so the *P. polycephalum* data have been transformed to predict the results of a pulse label experiment such as those carried out in mammalian systems. The transformation (Fig. 1) shows that a peak of phosphorylation, as measured by pulse labelling, would appear in S phase in *P. polycephalum*, in agreement with the mammalian observations. The peak is due to histone synthesis, which requires some phosphorylation to occur in order to maintain the constant overall phosphate content per molecule. These results throw considerable doubt on the significance of the S phase phosphorylation although the possibility that different sites of

phosphorylation are involved cannot yet be ruled out. The doubt has been strengthened recently by studies of the coupling of S phase phosphorylation and DNA synthesis[12], and by the work of Marks *et al.*[13] with synchronous HeLa S-3 cells.

Third, most Pp. F1 phosphorylation occurs in late G2 phase (Fig. 1) and at this time there is also a large increase in overall phosphate content per molecule[1]. Increased F1 phosphorylation has also been observed in mammalian cells in late G2 phase[11], mostly in the 3 h before metaphase [14], exactly in agreement with the *P. polycephalum* results. Further, an increased F1 phosphate content occurs in mammalian metaphase cells[15] and dephosphorylation occurs when these cells move into G1 (ref. 16). These data are consistent with the very precise timing in *P. polycephalum* which located the peak of maximum Pp. F1 phosphate content at the time of chromosome condensation[1] and this is proposed as the initiation step for mitosis[17]. The nuclear F1 histone phosphorylating enzyme activity has been found to peak dramatically at the time during the mitotic cycle when the net rate of phosphorylation of Pp. F1 is a maximum implying that the change in enzyme activity 'triggers' the phosphorylation[17]. Further we have shown that there is a remarkable correlation between the observed changes in enzyme activity and the data in the literature concerning the expected behaviour of the mitotic trigger in *P. polycephalum* and it has been proposed that these events are linked[17]. As a direct test of the proposal, the effect of the addition heterologous F1 histone phosphokinase on the cycle time of the naturally

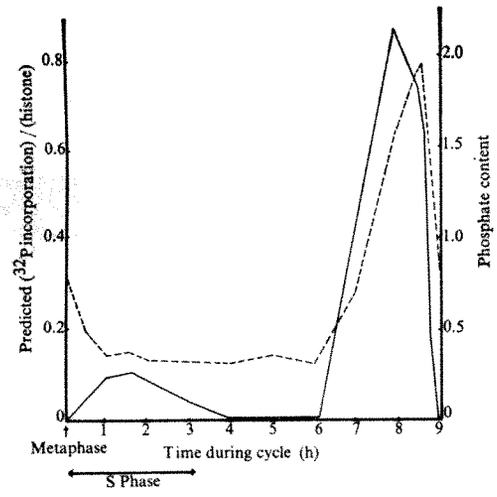


Fig. 1 The pulse length was 1/8 of a generation time in the mammalian experiments[10] which is equivalent to 1 h in the *P. polycephalum* cycle. If the net rate of phosphorylation of F1 is dP/dt then a 1 h pulse would show an incorporation

$\int_{t-1}^{t} (dP/dt)\,dt = P_t - P_{t-1}$ where P_t is the total phosphate content of F1 at time t. The *P. polycephalum* experiments measured $P_t/N_t = \chi_t$, say, where N_t = total number of F1 molecules at time t[1]. The incorporation expected during a 1 h pulse ending at time t is

$$P_t - P_{t-1} = N_t\chi_t - N_{t-1}\chi_{t-1} = N_t\partial\chi + \chi_t\partial N - \partial\chi\partial N$$

where $N_{t-1} = N_t - \partial N$ and $\chi_{t-1} = \chi_t - \partial\chi$. Therefore incorporation per F1 molecule,

$$(P_t - P_{t-1})/N_t = (N_t\partial\chi + \chi_t\partial N - \partial\chi\partial N)/N_t = \partial\chi + \chi_t\partial N/N_t, \text{ neglecting } \partial\chi\partial N$$

The first term, $\partial\chi$, represents incorporation due to a change in the overall phosphate content of the F1 and the second term represents net incorporation due to maintaining the overall phosphate content per molecule constant in the face of synthesis of new F1. Any turnover of F1 phosphate would raise the level of the curve but this has been ignored. N_t and ∂N were estimated for *P. polycephalum* by assuming a constant ratio of Pp. F1 histone synthesis to main band nuclear DNA synthesis[25] and using published data on DNA synthesis[16]. χ_t and $\partial\chi$ were obtained from our previous measurements of Pp F1 phosphate content[1]. ———, Calculated phosphorylation (simulated label experiment); — — — measured phosphate content[1].

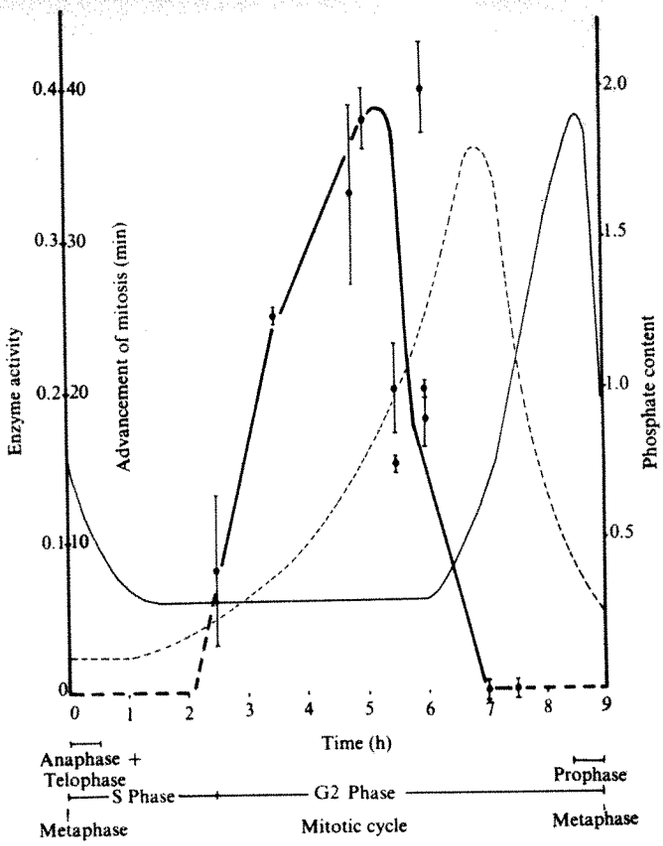


Fig. 2 Synchronous plasmodia of *P. polycephalum* were prepared by fusion of microplasmodia on filter paper and grown at 26° C in the dark[27]. At a known time between mitosis 2 (the second mitosis after fusion) and mitosis 3, the filter paper was cut into quarters and transferred to Petri dishes. Each culture constituted a complete experiment with its own control, conducted in duplicate. Duplicates of the same plasmodium divided within 5 min of each other. Plasmodia were treated by carefully pipetting on their surface 0.1 ml of solution. Enzyme solutions, containing approximately 1.5 mg ml^{-1} protein had been dialysed overnight against 0.02 M Tris HCl buffer *p*H 7.2 (ref. 19). The treated plasmodia were starved for 1.5 h to facilitate absorption[19]. Fresh medium was then added and the time of the third mitosis determined by microscopic examination of glycerol/ethanol fixed smears under phase contrast[28]. Advancement of mitosis by F1 phosphokinase is shown as a function of the time in the cycle when the enzyme was added, (●—●) with the errors calculated from the differences between duplicates as in Table 1. For comparison, the level of endogenous enzyme activity[17] (— — — —) and Pp F1 phosphate content (————) are also shown together with the timing of the main events in the *P. polycephalum* mitotic cycle[26].

synchronous cultures of *P. polycephalum* has been investigated.

F1 histone phosphokinase was prepared by extraction of Ehrlich ascites cell chromatin with 0.4 M NaCl, followed by ammonium sulphate fractionation of the extract in the presence of 10^{-5} M cyclic AMP. The protein precipitating between 17.5 and 35% saturation was collected and dissolved in 0.4 M NaCl, 0.01 M Tris *p*H 7.5. The enzyme activity is similar to that described in metaphase arrested cells by Lake[18]. It phosphorylates F1 *in vitro* mainly in the N and C terminal regions at sites whose phosphorylation is associated with growth. It does not require cyclic AMP. The The preparation contains only traces of F1 histone kinases, HK_1 (cyclic AMP dependent) and HK_2 (cyclic AMP independent)[2].

Heterologous enzyme was used because of its availability and because the general features of the process are almost certainly universal. Work is in progress to obtain and use pure homologous and heterologous enzyme, but this is a

Table 1 Control experiments

Treatment begun, (h before expected MIII)	Treatment Segments A	Segments B	Advancement of A before B (min)	Error due to differences* between duplicates (min ±)
3.5	F1 phosphokinase	Buffer	15	0
3.0	F1 phosphokinase	0.1 x phosphokinase	40	3
5.5	F1 phosphokinase	F1 phosphokinase inactivated by freezing and thawing	25	0
4.2	F1 phosphokinase	F1 phosphokinase inactivated by dialysis and storage	33	6
3.0	F1 phosphokinase	F1 phosphokinase inactivated by dialysis and storage	20	0
3.0	F1 phosphokinase	creatine phosphokinase	18	2
4.0	F1 phosphokinase	albumin	38	2

* This error is obtained as $[(\text{difference between mitosis times of A segments})^2 + (\text{difference between mitosis times of B segments})^2]/2$

long term project.

For each *in vivo* experiment at least two plasmodia of *P. polycephalum* were used. One was not treated in any way and was used as a control for measurements of the normal cycle time. The second was cut into four approximately equal 90° segments at a known time after its second mitosis. Two segments were treated with a blank solution and two with the enzyme solution. The four segments were starved for 1.5 h and then transferred to fresh growth medium (for experimental details see Fig. 2 and ref. 19). Provided the enzyme was added at the correct time in the cycle the enzyme treated segments entered mitosis precisely together up to 40 min before the blank treated duplicate segments which themselves entered mitosis precisly together. The rate of progression through mitosis itself was not affected. The effect of adding the enzyme at different times during the cycle is shown in Fig. 2. This curve correlates with the time course of F1 phosphorylating activity in *P. polycephalum* nuclei, since at about 2 h before mitosis this activity falls off sharply and adding enzyme is found to have no effect, either because the added enzyme is also inactivated or because the enzyme activity is no longer needed by the nucleus. Moreover, added enzyme is most effective when the endogenous enzyme is at one-third to two-thirds of its maximum level. The ineffectiveness of added enzyme early in the cycle is probably due to its instability or to some other preparation for mitosis, such as DNA synthesis, not being complete. The correlation of time of enzyme addition for maximum effect with the previous measurements of endogenous enzyme activity provides strong support for the contention that the observed effect is due to a specific action of F1 phosphokinase and is not a general growth promotion.

The *in vitro* activity of the actual enzyme preparations used for the experiments of Fig. 2 was assayed directly using calf thymus F1 histone as substrate. The specificity of the enzyme for F1 histone in chromatin was demonstrated by incubating calf thymus chromatin in 5 mM $MgCl_2$+50 mM tris-HCl *p*H 7.5 with γ^{-32}P-ATP. In the absence of added enzyme, extensive phosphorylation of nonhistone proteins occurred but no histone phosphorylation was observed. In the presence of added enzyme reduced phosphorylation of nonhistone proteins occurred and a large incorporation of ^{32}P into F1 histone, but not into other histones, was observed[20]. The significance of the reduced incorporation into nonhistone proteins is unknown but the enzyme clearly phosphorylates specifically F1 histone, in chromatin. It is just conceivable that the unpurified F1 phosphokinase preparation contained some other substance or combination of substances which produced the effects reported (Fig. 2). This possibility can be ruled out completely only by the use of pure homologous F1 phosphokinase. However, some control experiments have been caried out to check if such a coincidence is likely. The enzyme after dialysis against 0.02 M Tris *p*H 7.2, can be specifically inactivated by storage for 2 weeks at 4° C or by freezing and thawing four times. Such inactivated enzyme preparations have no effect on the timing of mitosis. Albumin and creatine phosphokinase are also without effect (Table 1). Positive results have only (and always) been obtained with active F1 phosphokinase preparations. Further progress on these lines must await the availability of pure enzyme.

Taken together with the previously reported data on Pp. F1 phosphate content[1], chromatin condensation (for example ref. 21), F1 phosphorylating activity in *P. polycephalum* nuclei[17] and the mitotic trigger[22–24], the results reported here provide direct evidence for our previous proposal that F1 phosphokinase triggers an increase in F1 phosphate content which is the initiation step in mitosis. Acceleration of initiation of mitosis has been shown before, by plasmodial fusion[22,23], ultraviolet irradiation[23] and plasmodial extracts[19] but this is the first successful attempt to accelerate the initiation of mitosis by a specific substance, F1 histone phosphokinase. The data already accumulated on the mechanism of this control process mediated by F1 phosphokinase fit into a consistent hypothesis (unpublished), and experiments are in progress to test it further and clarify the details. It is interesting that this process probably applies to cancer as well as normal cells.

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1 Bradbury, E. M., Inglis, R. J., Matthews, H. R., and Sarner, N., *Eur. J. Biochem.*, **33**, 131 (1973).
2 Langan, T. A., *Ann. N.Y. Acad. Sci.*, **185**, 166 (1971).
3 Dixon, G. H., Louie, A. J., McLeod, A. R., and Sing, M. T., *Trans. Biochem. Soc.* (in the press).

4 Panyim, S., and Chalkley, R., *Biochemistry*, **8**, 3972 (1969).
5 Oliver, D., Balhorn, R., Granner, D., and Chalkley, R., *Biochemistry*, **11**, 3921 (1972).
6 Balhorn, R., Chalkley, R., and Granner, D., *Biochemistry*, **11**, 1094 (1972).
7 Balhorn, R., Riecke, O., and Chalkley, R., *Biochemistry*, **10**, 3952 (1971).
8 Balhorn, R., Balhorn, M., Morris, H. P., and Chalkley, R., *Cancer Res.*, **32**, 1775 (1972).
9 Stevely, W. S., and Stocken, L. A., *Biochem. J.*, **110**, 187 (1968).
10 Balhorn, R., Bordwell, J., Sellers, L., Granner, D., and Chalkley, R., *Biochem. biophys. Res. Commun.*, **46** 1326 (1972).
11 Gurley, L. R., Walters, R. A., and Tobey, R. A., *Biochem. biophys. Res. Commun.*, **50**, 744 (1973).
12 Balhorn, R., Tanphaichitr, N., Chalkley, R., and Granner, D. K., *Biochemistry*, **12**, 5146 (1973).
13 Marks, D. B., Paik, W. K., and Borun, T. W., *J. biol. Chem.*, **248**, 5600 (1973).
14 Gurley, L. R., Walters, R. A., and Tobey, R. A., *J. Cell. Biol.* (in the press).
15 Lake, R. S., and Salzman, N. P., *Biochemistry*, **11**, 4817 (1972).
16 Lake, R. S., *Nature new Biol.*, **242**, 145 (1973).
17 Bradbury, E. M., Inglis, R. J., and Matthews, H. R., *Nature*, **247**, 257 (1974).
18 Lake, R. S., *J. Cell Biol.*, **58**, 317 (1973).
19 Oppenheim, A., and Katzir, N., *Expl Cell Res.*, **68**, 224 (1971).
20 Inglis, R. J., thesis, Univ. London (1974).
21 Bradbury, E. M., Carpenter, B. G., and Rattle, H. W. E., *Nature*, **241**, 123 (1973).
22 Chin, B., Friedrich, P. D., and Bernstein, I. A., *J. gen. Microbiol.*, **71**, 93 (1972).
23 Sachsenmaier, W., Remy, U., and Plattner-Schoebel, R., *Expl Cell Res.*, **73**, 41 (1972).
24 Brewer, E. N., and Rusch, H. P., *Expl Cell Res.*, **49**, 79 (1968).
25 Mohberg, J., and Rusch, H. P., *Archs Biochem. Biophys.*, **138**, 418 (1970).
26 Rusch, H. P., *Adv. Cell Biol.*, **1**, 297 (1971).
27 Guttes, E., and Guttes, S., in *Methods in Cell Physiology* (edit. by Prescott, D. M.) 43 (Academic Press, New York, 1964).
28 Daniel, J. W., and Baldwin, H. H., in *Methods in Cell Physiology* (edit. by Prescott, D. M.) 9 (Academic Press, New York, 1964).

Inhibitor of protein synthesis isolated from ribosomes of unfertilised eggs and embryos of sea urchins

IT is not yet clear what mechanisms turn on protein synthesis in the first stages of embryonic development. In the sea urchin two mechanisms are debated: activation of ribosomes[1,2] and activation of mRNA[3,4], possibly through adenylation[5,6]. An inhibitor of protein synthesis has been isolated from ribosomes of unfertilised eggs of *Paracentrotus lividus*[7,8] by extraction of ribosomes with high concentrations of salt. Metafora *et al.*[7] found that this inhibitor is a protein, that it is not a ribonuclease and that it decreases ribosomal binding of a synthetic messenger (poly(U)) and of phenylalanine tRNA. They found that this inhibitor was absent from extracts of ribosomes of *P. lividus* blastulae, suggesting that it is inactivated or lost when the eggs are fertilised.

In view of the importance of such a finding for understanding the restriction of protein synthesis in unfertilised eggs, I have investigated similar inhibitors from eggs and embryos of *Strongylocentrotus purpuratus*, and determined the distribution of inhibitor between monoribosomes and polyribosomes of echinoid embryos. While confirming the occurrence of inhibitor in unfertilised eggs, I have found it in embryos as well, particularly in monoribosome fractions.

Ribosomal extracts were tested for inhibitory potential in protein synthesis in a cell-free system using both endogenous mRNA and poly(U). Extracts were prepared from ribosomes of unfertilised eggs and blastulae by the method of Metafora *et al.*[7] using 1 M NH_4Cl. Protein synthesis was measured as incorporation of ^{14}C-phenylalanine in an *in vitro* system containing ribosomes from either unfertilised eggs or blastulae. Only blastula ribosomes had enough natural mRNA attached to give detectable endogenous synthesis (Table 1, experiment 2). Endogenous incorporation with these ribosomes was inhibited strongly by either of the ribosomal extracts (Table 1, experiment 1). Incorporation with ribosomes from both unfertilised eggs and blastulae was stimulated strongly by addition of poly(U). This stimulation was inhibited by ribosomal extracts from both unfertilised eggs and blastulae.

Table 1 Effect of ribosomal extracts on cell-free protein synthesis directed by endogenous mRNA and poly(U)

Type of mRNA	Source of extract	*in vitro* ^{14}C-phenylalanine incorporation (c.p.m. per assay)	
		Using egg ribosomes	Using blastulae ribosomes
Experiment 1			
Endogenous mRNA	none	—	6,520
,,	UF egg ribosomes*	—	2,516
,,	blastula ribosomes†	—	2,620
Experiment 2			
Endogenous mRNA	none	0	3,740
,, + poly(U)	none	7,430	21,500
,, + ,,	UF egg ribosomes‡	48	4,940
,, + ,,	blastula ribosomes§	200	5,860

Eggs and sperm of *Strongylocentrotus purpuratus* were collected and cultivated[19]. Ribosomes and their 1 M NH_4 Cl extracts were prepared as described by Metafora *et al.*[7] except that triethanolamine-HCl (K and K Laboratory, Inc.) was substituted for Tris HCl. The ribosome and extract solutions were stored at −80° C. A *p*H 5 fraction prepared from rat liver supernatants[3] contained aminoacyl activating enzymes, tRNA, amino acids, and soluble enzymes. The *in vitro* assay mixture contained in 250 μl: 0.1 M KCl, 5mM $MgCl_2$, 0.05 M triethanolamine-HCl (*p*H 7.4), 6mM mercaptoethanol, 2.5 mM ATP, 0.2 mM GTP, 4 mM 2-phosphoenol pyruvate, 0.35 μg pyruvate kinase, 0.31 μg ^{14}C-L-3-phenylalanine (5.3 mCi mmol^{-1}), *p*H 5 fraction from rate liver at optimal concentrations (about 30 μl containing about 0.8 mg protein), 2.6 A_{260} units (defined in Fig. 1) of ribosomes, ribosomal extract, 46 μg *, 42 μg †, 58 μg ‡, and 23 μg §, or a compensating buffer and sometimes 50 μg (poly(U)). The reaction mixture was incubated with vigorous shaking for 60 min at 27° C, stopped by the addition of 3 ml of ice-cold 6% trichloroacetic acid (TCA) containing L-phenylalaline, incubated for 10 min at 90° C, chilled in ice, filtered onto membrane disks, then washed with 6% TCA, dried, and counted in a toluene based scintillant. The assay was linear for 50 to 60 min with poly(U) as the messenger and for 20 min with endogenous blastula messenger. Protein content was estimated by the method of Lowry *et al.*[20].

As the inhibitor could have a regulatory function in development, I have measured its concentration in sea urchin eggs and embryos at various stages. High salt extracts from ribosomal pellets were prepared simultaneously from unfertilised eggs, 32-cell morulae, 500-cell blastulae (B1-500) and early mesenchyme blastulae (B1My-1), by the method of Metafora *et al.*[7] (Fig. 1). Extracts from ribosomes of all early stages were inhibitory. Extracts from equal quantities of ribosomes were progressively less inhibitory when taken from embryos of increasing age. I found that extracts of blastula ribosomes contained about half as much protein as those from unfertilised egg ribosomes. When relative incorporation was plotted against the amount of protein in each extract, the inhibition by extracts from the various stages was similar (Fig. 2). Since there is little change in the total number of ribosomes from fertilisation until mesenchyme blastula stage (about 25 h at 14° C), I conclude that there is a continuous decrease in the amount of extractable inhibitor per embryo during this time.

The ribosomal extracts from these early stages were resolved into between 9 and 11 protein bands on 6 M urea electrophoretic gels[10]. Seven of these bands were common to

extracts of unfertilised eggs, morulae and blastulae, and I do not know which might have inhibitory activity.

Previous studies have shown that the polyribosome: monoribosome ratio increases after fertilisation of sea urchin eggs and increases throughout the blastula stage[11,12]. To see if there was any correlation between the observed amounts of extractable inhibitor and the polyribosome: monoribosome ratio of these embryos, I prepared monoribosomes and polyribosomes of blastulae by sucrose gradient centrifugation. Embryos were homogenised in 0.2 M salt[13] instead of the 0.4 M salt of Fig. 1. With the lower concentration of salt fewer nonribosomal ribonucleoprotein particles (RNP particles) cosedimented with the monoribosomes and small polyribosomes. The high speed pellets of these fractions were used to prepare the extracts whose inhibitory capacity is shown in Fig. 3. The points for the unfertilised egg extract from Fig. 1 are included for reference although those ribosomes were isolated using different salt concentrations. To obtain an equivalent amount of inhibition by monoribosome and polyribosome extracts, almost three times as many polyribosomes as monoribosomes were needed. For example, 2.4 A_{260} units (defined in Fig. 1) of monoribosomes or 6.7 A_{260} units of polyribosomes gave 40% inhibition.

To determine whether this inhibitor is absent from non-embryonic tissues, extracts were prepared from the ribosomes of adult rat liver, which are known to contain about 90% polyribosomes[14]. The effect of these extracts on *in vitro* synthesis of proteins is given in Fig. 3. I found that ribosomes from adult rat liver contained little extractable inhibitor, about 0.1 that of blastulae ribosomes. In summary, Fig. 3 shows that high salt extracts of blastula monoribosomes contain substantially more extractable inhibitor than those of polyribosomes, implying that *in situ* the monoribosomes are inhibited, and that the inhibitor is more abundant in eggs and embryos than in adult tissue.

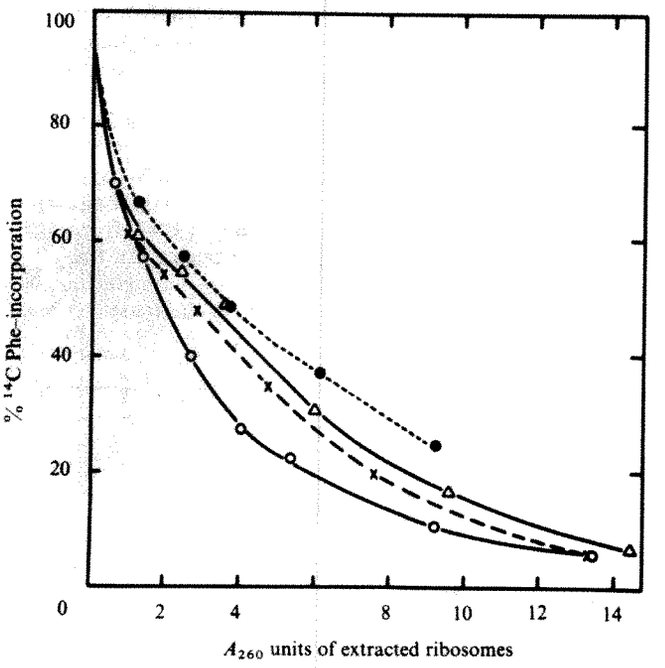


Fig. 1 Quantitative determination of the inhibitory capacity of ribosomal extracts from unfertilised eggs and embryos. The percentage of ^{14}C-phenylalanine incorporation stimulated by poly(U) plus endogenous mRNA is plotted against amounts of ribosomal extracts taken from known quantities of ribosomes. Methods were as described in Table 1 except that for the assays 1.2 A_{260} units of morula ribosomes were added and the incubation time was 45 min. The amount of ribosomal extract varied as noted in the abscissa. The quantity of extract added is measured by the 260 nm absorbance of the ribosomes from which they were taken. This absorbance was measured after the ribosomal pellet had been stirred overnight at 4° C in 1 M NH_4Cl, and after the unsuspended material had been removed by low speed centrifugation. According to convention, 1 A_{260} unit of ribosomes equals the absorption of ribosomes if they were contained in 1 ml and read in a 1 cm cuvette at 260 nm. Ribosomal extracts from unfertilised eggs (0); from 32-cell morulae (×); from 500-cell blastulae (△); and from hatched blastulae at stage B1My-1[21] (●).

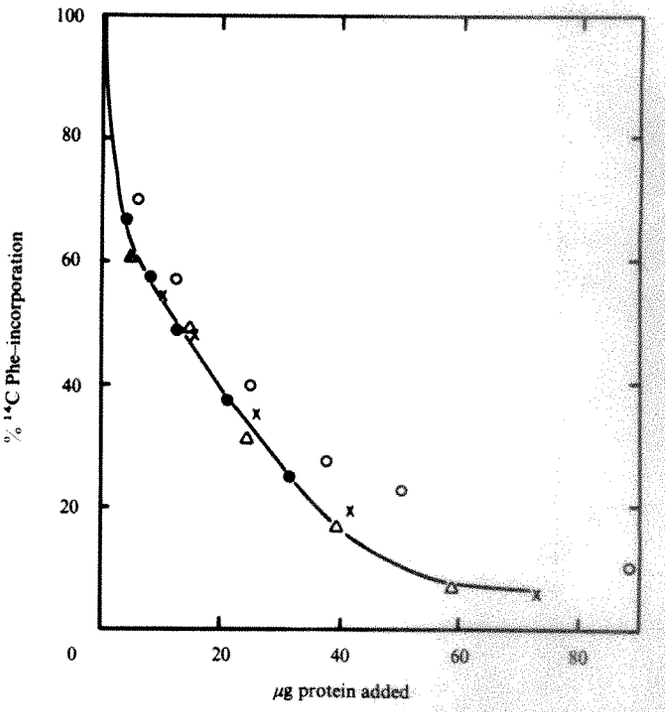


Fig. 2 Inhibition of ^{14}C-phenylalanine incorporation per μg of protein added from ribosomal extracts. Observations and notations are as in Fig. 1. The protein concentration was estimated by a micro Biuret method[25].

Preliminary results with RNP particle-enriched fractions do not rule out the possibility that RNP particles which cosediment with monoribosomes also contain inhibitory proteins. The presence of an inhibitory protein on preformed messages in such particles could prevent their attachment to ribosomes or their translation once attached.

Ribosomal extracts were tested for possible RNase activity by Ball's method[15]. Extracts from unfertilised eggs and blastulae containing 20–40 μg of protein, sufficient to inhibit amino acid incorporation by 60–85%, had less than 1×10^{-10} g RNase. Extracts of rat liver ribosomes sufficient to inhibit amino acid incorporation by 15% also contain negligible amounts of RNase. The rat liver supernatant fraction used in the assays contributed about 2.5×10^{-10} g of RNase. It is unlikely that any observed inhibition is due to RNase activity, for the amount of RNase activity did not correlate with the percentage of inhibition.

The inhibitor is not a GTPase since increasing the amounts of GTP two- and six-fold did not alter the amount of inhibition.

What is the normal function of this inhibitor? While these results and those Metafora *et al.*[7] and Gambino *et al.*[8] agree in demonstrating the existence of the inhibitor, these authors indicate that the inhibitor disappears after fertilisation, thereby triggering activation of protein synthesis. In contrast, I have found a gradual diminution in the amount of inhibitor. Therefore, I suggest that this slow loss of inhibitor is responsible for the continuous increase of rate of incorporation of labelled amino acids seen until mesenchyme blastula or early gastrula[16–18]. The inhibitor seems to be acting at the monoribosome level, so that a

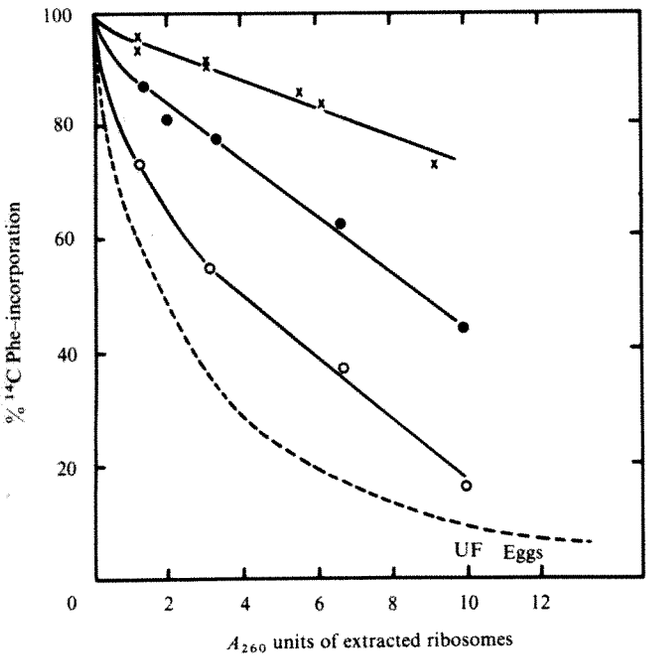


Fig. 3 Inhibitory potency of extracts from different ribosomal fractions, and from adult rat liver. Embryos were slightly older than any described in Fig. 1, being stage B1My-2/Ga-1[21]. Homogenisation and sucrose gradient preparation of monoribosomes and polyribosomes were described by Hogan and Gross[13]. Rat liver ribosomal pellets were prepared as described by Stavy and Gross[3]. Extracts were prepared as described in Table 1. Assay conditions and quantification were as described in Fig. 1. Ribosomal extracts from monoribosomes (O); from polyribosomes containing from 4 to 25 ribosomes per polysome (●); and from rat liver ribosomes (×); unfertilised egg extract of Fig. 1 (- - - -).

gradual loss of inhibitor with time would also account for the continuous increase in the polysome:monoribosome ratio until the mid-blastula stage[11,12].

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1 Monroy, A., Maggio, R., and Rinaldi, A. M., *Proc. natn. Acad. Sci. U.S.A.*, **54**, 107-111 (1965).
2 Maggio, R., Vittorelli, M. L., Caffarelli-Mormino, I., and Monroy, A., *J. molec. Biol.*, **31**, 621-626 (1968).
3 Stavy, L., and Gross, P. R., *Proc. natn. Acad. Sci. U.S.A.*, **57**, 735-742 (1967).
4 Humphreys, T., *Devl Biol.*, **26**, 201-208 (1971).
5 Slater, I., Gillespie, D., and Slater, D. W., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 406-411 (1973).
6 Wilt, F. H., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2345-2349 (1973).
7 Metafora, S., Felicetti, L., and Gambino, R., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 600-604 (1971).
8 Gambino, R., Metafora, S., Felicetti, L., and Raisman, J., *Biochem. biophys. Acta*, **312**, 377-391 (1973).
9 Giudici, G., *Developmental biology of the sea urchin embryo*, (Academic Press, New York, 1973).
10 Reisfeld, R. A., Lewis, U. J., and Williams, D. E., *Nature*, **195**, 281-283 (1962).
11 Monroy, A., and Tyler, A., *Archs Biochem. Biophys.*, **103**, 431-435 (1963).
12 Infante, A. A., and Nemer, M., *Proc. natn. Acad. Sci. U.S.A.*, **58**, 681-688 (1967).
13 Hogan, B., and Gross, P. R., *J. Cell Biol.*, **49**, 692-701 (1971).
14 Falvey, A. K., and Staehelin, T., *J. molec. Biol.*, **53**, 1-19 (1970).
15 Ball, W. D., *Biochem. biophys. Acta.*, **341**, 305-317 (1974).
16 Markman, B., *Expl Cell Res.*, **23**, 197-200 (1961).
17 Berg, W. E., *Expl Cell Res.*, **49**, 379-395 (1968).
18 Gross, P. R., Malken, L. I., and Moyer, W. A., *Proc. natn. Acad. Sci. U.S.A.*, **51**, 407-414 (1964).
19 Whiteley, A. H., McCarthy, B. J., and Whiteley, H. R., *Proc. natn. Acad. Sci. U.S.A.*, **55**, 519-525 (1966).
20 Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.* **193**, 265-275 (1951).
21 Whiteley, A. H., and Baltzer, F., *Publ. Staz. zool Napoli*, **30**, 402-457 (1958).
22 Zamenhoff, S., *Methods in Enzymology*, **3**, 696-704 (1957).

Stability of the nucleoside composition of tRNA during biological ageing of mice and mosquitoes

IN several organisms, alterations in tRNA have been reported to occur during physiological changes. Both qualitative and quantitative changes have been demonstrated in isoaccepting species of various tRNAs[1-4]. Chromatographic changes have often been attributed tc changes in nucleoside composition. In *Drosophila*, changes in the chromatographic patterns of certain tRNA species at different developmental stages have been correlated with an enzymatic alteration producing a minor nucleoside, Q[4]. Such differences could be involved in control of protein synthesis at the translational level directly by tRNA species, and (or) in regulation of protein activity by interaction of tRNA with a previously formed protein[5,6].

During an investigation of the role of translational macromolecules in biological ageing, column chromatography of aminoacyl-tRNAs from 'mature' and 'old' adults of *Aedes aegypti* revealed variations in patterns of certain isoaccepting tRNAs[7]. A similar study on aminoacyl-tRNA from ageing mice revealed no chromatographic changes[8]. In the liver of ageing rodents, including mice, however, glycine N-methyltransferase activity was reported to increase[9]. It was

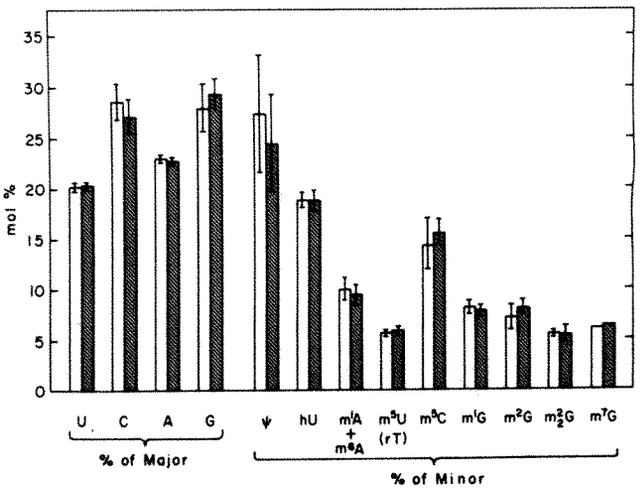


Fig. 1 Nucleoside composition of tRNA from mature and old mosquitoes. Nucleoside compositions were determined by the chemical labelling technique as described in the text. Values plotted are mean ± s.d. for four determinations on each age with the exception of m⁷G, for which only two analyses were done on mosquito tRNA. Minor/major ratios: mature, 0.109±0.005; old, 0.106±0.004. Open columns, mature (10-d-old); hatched columns, old (30-d-old) adult female *Aedes aegypti*.

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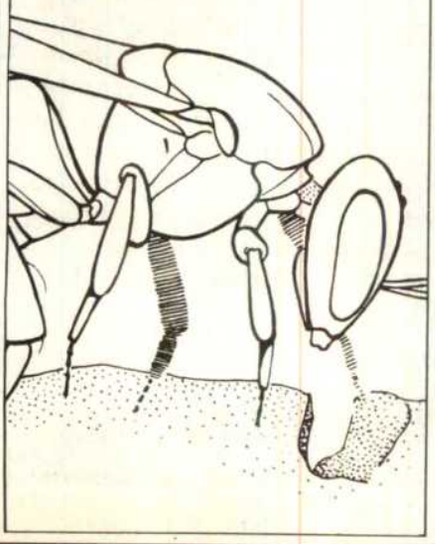
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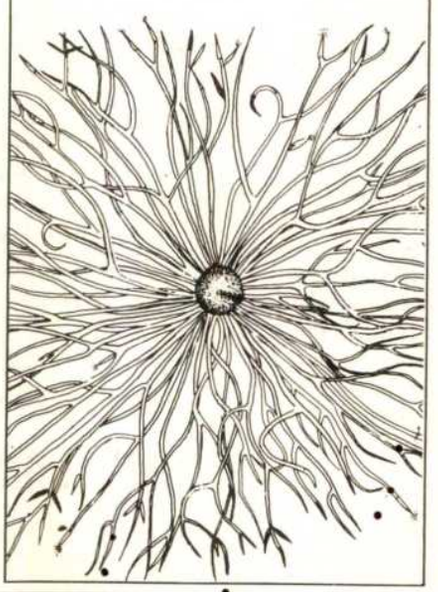
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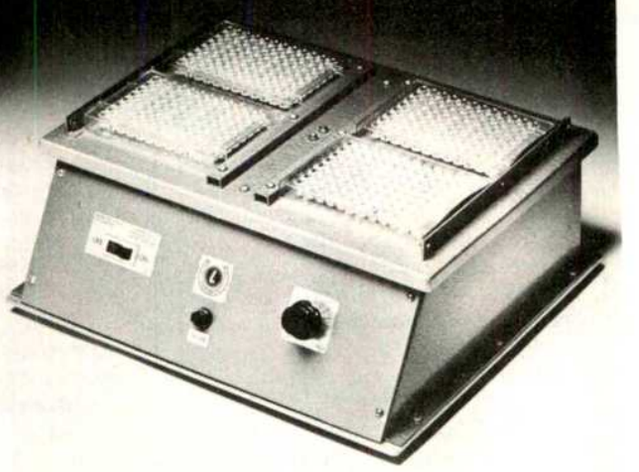
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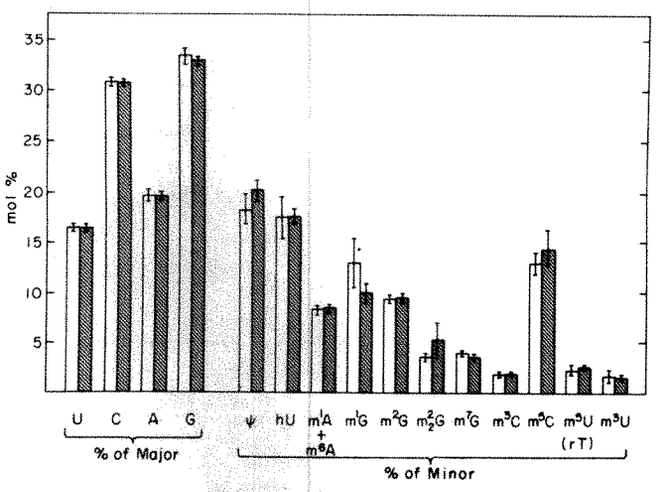


Fig. 2 Nucleoside composition of tRNA from mature and old mice. Determined as in Fig. 1. Minor/major ratios: mature, 0.190±0.013; old, 0.193±0.010. Open columns, mature (12 months old); hatched columns, old (30 months old) female C57BL/6J mice.

suggested that this could lead to undermethylation of tRNA in ageing animals by depletion of S-adenosylmethionine, and by production of S-adenosylhomocysteine which inhibits tRNA methyltransferases[10].

With these observations as background, we have investigated the nucleoside composition of tRNA from mature and old C57BL/6J mice and *Aedes aegypti* to determine if there is any change in this parameter as mature animals age. On the basis of survivorship curves, we have defined mature mosquitoes as 10-d-old adults, and mature mice as 12 months old. Old animals are from the 50% survivorship level, being 30 d for adult mosquitoes and 30 months for mice.

Samples of tRNA were prepared from whole mosquitoes and from mouse liver by phenol extraction, DEAE-cellulose chromatography, and Sephadex G-100 gel filtration. Purity of the preparations was assessed by electrophoresis on polyacrylamide gels and subsequent ultraviolet scanning. All preparations were at least 90% tRNA, the principal contaminant being 5S rRNA.

Nucleoside composition analyses were carried out by chemical tritium labelling exactly as described by the Randeraths (ref. 11 and references therein). We were able to quantitate 13 components in mosquito tRNA and 15 in mouse liver tRNA. Inosine seemed to be present in both organisms, but on our chromatograms it was overlapped by the guanosine trialcohol spot and is included with guanosine. A portion of 1-methyladenosine is converted to N^6-methyladenosine during the analysis, and so these two components were combined in the calculation of composition.

Inspection of fluorograms showed no discernible qualitative differences in nucleoside composition during ageing, that is the same nucleosides were present in tRNA from mature and old animals (data not shown). Figures 1 and 2 show that there are also no significant age-related quantitative changes in tRNA nucleoside composition nor in degree of tRNA modification in the two animal models studied.

It seems remarkable that, at a time when the population has declined physiologically to the point of highest mortality rate, the complex enzyme systems necessary for tRNA maturation are still producing a fully modified product. This indicates that the nucleoside composition of tRNA is an important and tightly regulated parameter for a given organism, or organ therein.

Our results argue against tRNA modifying enzymes having a role in biological ageing. They also point up the danger of interpreting chromatographic changes in tRNA or changes in activity of tRNA methylases in terms of the final degree of modification without actually analysing the tRNA.

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1 Nishimura, S., in *Prog. nucleic acid Research molec. Biol.*, **12** (edit. by Davidson, J. N., and Cohn, W. E.), 49 (Academic Press, New York, 1972).
2 Smith, R. L., and Forrest, H. S., *Devl Biol.*, **33**, 123 (1973).
3 White, B. N., Tener, G. M., Holden, J., and Suzuki, D. T., *J. molec. Biol.*, **74**, 635 (1973).
4 White, B. N., Tener, G. M., Holden, J., and Suzuki, D. T., *Devl Biol.*, **33**, 185 (1973).
5 Jacobson, K. B., *Nature new Biol.*, **231**, 17 (1971).
6 Twardzic, D. R., Grell, E. H., and Jacobson, K. B., *J. molec. Biol.*, **57**, 231 (1971).
7 Hoffman, J. L., *Fedn Proc.*, **31**, Abstr. 866 (1972).
8 Frazier, J. M., and Yang, W.-K., *Archs Biochem. Biophys.*, **153**, 610 (1972).
9 Mays, L. L., Finch, C., and Borek, E., *Nature*, **243**, 410 (1973).
10 Kerr, S. J., *J. biol. Chem.*, **247**, 4248 (1972).
11 Randerath, K., and Randerath, E., *J. Chromatog.*, **82**, 59 (1973).

Relationship between tritiated poly-L-lysine binding and template activity of human chromosomes

IT has been reported that cytological preparations of chromosomes can serve as templates in RNA polymerase reactions[1,2]. The newly synthesised RNA on chromosomes was readily digested with RNase in contrast to RNA which was synthesised in a similar manner but using as a template denatured DNA immobilised on nitrocellulose filters[1]. When cytological preparations of chromosomes were pretreated with poly-L-lysine (PL), no synthesis of RNA could be demonstrated[2]. The implication was that bound PL blocked those sites on DNA template which were responsible for transcription.

We used human metaphase spreads prepared by a standard technique[3] as templates for RNA synthesis and for binding tritiated poly-L-lysine (^{3}H-PL). We wished to establish whether both of these processes occur on the same or on different segments of chromosomes. Typical karyotypes of metaphase chromosomes with bound ^{3}H-PL or bound ^{3}H-RNA are shown in Fig. 1. The results of the labelling are summarised in Table 1. In both sets of experiments over 98% of total grains was located over the chromosomes and the remainder over the background. Analysis of a large number of karyotypes (over 100 in total) indicated that the labelling of chromosomes was reproducible (Table 1). Those sites on A chromosomes that have highest template activity also show a high affinity for ^{3}H-PL. Although structural organisation of chromosomes necessary for both functions is not known in detail[2,4], it is likely that relatively exposed segments of

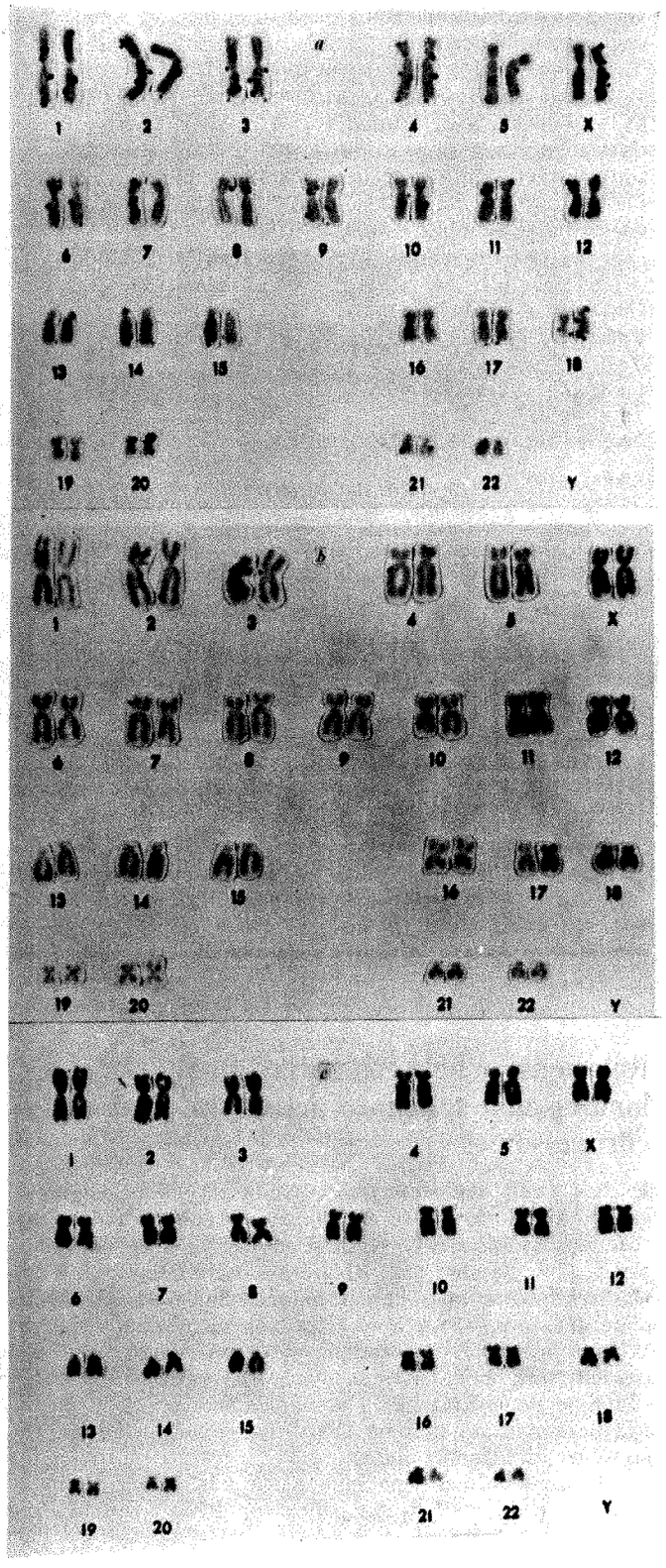


Fig. 1 Typical karyotypes of treated and control human metaphase chromosomes. *a*, Chromosomes treated with tritiated poly-L-lysine (^{3}H-PL); *b*, chromosomes used as templates in RNA polymerase reaction; *c*, chromosomes subjected to conditions of RNA polymerase reaction without the polymerising enzyme. Tissue culture of human circulating lymphocytes and the preparation of metaphase spreads was done according to a standard procedure[2]. Slides containing chromosomes were treated with ^{3}H-PL (molecular weight 50,000–100,000, specific activity 3.32 x 10^3 c.p.m. mg^{-1}) as follows. 0.1 ml of 0.1% ^{3}H-PL solution in 0.1. N acetic acid was applied and the slide was covered with a cover slip. After 20 min at room temperature the cover slip was removed, the slide washed with water and dried. After exposure to NTB-2 emulsion for 7 d, the slides were examined under a microscope. The grouping of metaphase chromosomes according to the degree of contraction was based on the measurement of A_1 chromosome (short, 8.3 μm; medium, 10.0 μm; long, 12.5 μm). Identification of individual chromosomes was aided by the application of a Giemsa banding procedure after treatment of chromosomes with trypsin[7,8]. RNA polymerase reaction was done on slides containing metaphase spreads (without any pretreatment) essentially according to the published procedure[1]. ^{3}H-UTP labelled at C5 of the uracil base (specific activity 41.7 Ci mmol^{-1}) was used for RNA labelling. In the control experiments, all the ingredients of the reaction, except the *E. coli* RNA polymerase (specific activity 240 units mg^{-1} assayed in standard conditions), were present. The slides were analysed for grains as described for ^{3}H-PL binding.

Table 1 A comparison of ^{3}H-poly-L-lysine binding, RNA synthesis and quinacrine fluorescence of human A group chromosomes

Chromosome segment	^{3}H-PL binding G	ΔG	RNA synthesis G	ΔG	Quinacrine fluorescence
A_1e	9.33	−8.87	0.48	1.30	3.20
A_1m	4.12	1.40	0.40	0.20	40.00
A_1c	13.22	−9.43	—	—	3.20
A_2es	15.00	−10.87	—	—	1.20
A_2ms	8.34	6.80	1.00	−1.00	16.40
A_2c	12.29	14.80	—	—	4.10
A_2ml	9.57	−0.50	0.37	—	32.7
A_2el	24.50	−42.87	3.20	−12.00	1.20
A_3e	11.38	−8.33	0.25	—	4.4
A_3m	4.18	1.93	0.13	0.40	53.9
A_3c	9.42	2.87	1.95	—	(7.2)

Average number of grains per unit weight (g × 10^{-15}) of a given chromosome segment[6] (G) was determined separately for karyotypes containing short, medium or long metaphase chromosomes (Fig. 1). For A_1 and A_3 chromosomes in which short and long arms could not be distinguished, the data are given for the combined two end and two middle segments. Forty-six karyotypes (16 short, 16 medium, 14 long) were analysed for ^{3}H-PL binding and 34 (11 short, 12 medium, 11 long) for RNA synthesis. The data are averages obtained with the three classes of chromosomes. The probable error computed for several groups of karyotypes belonging to the same class ranged from 15–30% of the mean value. The differences in G for each chromosomal segment between chromosomes of different length (ΔG) was also computed ($\Delta G = (G_{long} - G_{medium}) + (G_{long} - G_{short}) + (G_{medium} - G_{short})$) to show the effect of chromosomal contraction on the content and distribution of grains. The integrated intensity of quinacrine mustard fluorescence for different chromosomal segments (given in arbitrary units) was determined by measuring the corresponding areas under the fluorescence curves[5]. The value assigned to A_3c segment may vary since this is a polymorphic region[4] of A_3 chromosome.

nucleic acids are required. On the other hand, the chromosomal segments with high template activity show little fluorescence after treatment with quinacrine mustard[5]. We also observed that both functions depend on the degree of chromosomal contraction. This effect was especially pronounced for the long arm end segment of A_2 chromosome. It did bind more ^{3}H-PL and functioned better as a template when the chromosome was in a more contracted form.

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1 Sederoff, R., Clynes, R., Poncz, M., and Hachtel, S., *J. Cell Biol.*, **57**, 538 (1973).
2 Umiel, N., and Plaut, W., *J. Cell Biol.*, **56**, 139 (1973).
3 Moorhead, P. S., Nowell, P. C., Mellman, W. J., Battips, D. M., and Hungerford, D. A., *Exp Cell Res.*, **20**, 613 (1960).

[4] Evans, H. J., *Br. med. Bull.*, **29**, 196 (1973).
[5] Caspersson, T., Zech, L., Johansson, C., and Modest, E. J., *Chromosoma*, **30**, 215 (1970).
[6] Du Praw, E. J., *DNA and Chromosomes*, 135 (Holt, Rinehart and Winston, New York, 1970).
[7] Hsu, T. C., *Mammal. Chromosome Newsl.*, **13**, 43 (1972).
[8] Seabright, M., *Lancet*, ii, 971 (1971).

Lac repressor can be fused to β-galactosidase

GENETIC analysis of *i* gene mutants which had lost the capacity to bind operator but not inducer suggested that *lac* repressor binds with its amino terminus to *lac* operator[1]. Here we will show that the carboxy terminus of *lac* repressor is dispensable. We found fusions between the *i* (*lac* repressor) and *z* (β-galactosidase) genes (Fig. 1) which produce fully active *lac* repressor covalently linked to active β-galactosidase.

Such fusions are found among spontaneous *lac*$^+$ revertants of an *Escherichia coli* strain carrying the mutations i^q and z^-_{u118}. i^q stands for a mutation in the *i* promoter which leads to a ten-fold overproduction of *lac* repressor[2]; z_{u118} is a strongly polar nonsense mutation located near the *lac* operator[3]. The *lac*$^+$ revertants of $i^q z_{u118}$ fall into two classes: inducible revertants (due to direct reversion or unlinked suppression) and mutants that produce β-galactosidase at a low constitutive level. These last mentioned mutants have all the characteristics of *i z* fusion: They can be neither induced by inducer nor repressed by a functioning *i* gene in trans. The amount of β-galactosidase made depends on the nature of the *i* gene promoter. Most of these mutants (101 out of 111) no longer produce functioning *lac* repressor. In these mutants part of the structural *i* gene is deleted. In the extracts of ten mutants, however, we found *in vitro* inducer and operator binding. We concentrated our interest on these mutants: Mapping proved that only two of these ten deletions definitely entered the structural part of the *i* gene and that eight others might end in a region of the *i* gene which is still transcribed but not translated (Fig. 1). The β-galactosidase of most of the strains was more heat labile than β-galactosidase from a wild type strain.

We used antibodies against *lac* repressor (anti-repressor) and against β-galactosidase (anti-galactosidase) to decide whether *lac* repressor was fused to β-galactosidase. There is no cross reaction between these antibodies and antigens; however, both anti-repressor and anti-galactosidase precipitate β-galactosidase and *lac* repressor from extracts of fusion 71–56–14 (Table 1). This result strongly suggests, but does not prove, covalent fusion between *lac* repressor and β-galactosidase.

The pure material we needed to settle the question was obtained in the following way: We used extracts of promoter mutant i^{q1} (Müller-Hill, unpublished) from one of the strains (71–56–14) as starting material; ammonium sulphate, phosphocellulose and Sepharose 6B were used for purification. We followed both β-galactosidase and repressor activity throughout the purification steps. There is a good correlation, although not a precise one, between the specific activities of *lac* repressor and β-galactosidase throughout the purification (Table 2). The elution profile of the phosphocellulose column shows several fractions which have simultaneously β-galactosidase and repressor activity. The main fraction was further purified on Sepharose 6B. SDS-gels showed that it was at least 90% pure (Fig. 2). The subunit molecular weight is about 155,000 since our protein runs together with the β-subunit of RNA polymerase from *E. coli*. If we assume β-galactosidase to have the most plausible subunit molecular weight of 125,000 (ref. 9) and *lac* repressor a molecular weight of 37,000 (ref. 10), not much has been lost from β-galactosidase and repressor through the fusion. The repressor-galactosidase was derived from a strain that carried a new strong promoter mutation i^{q1}. We sequenced the N terminus, using the SDS-dansyl-Edman procedure[11] and found it to be identical to the N terminus of lac repressor[1,12]: H_2N-Met-Lys-Pro-Val-Thr-Leu, thereby proving again the covalent fusion and excluding an effect of the promotor mutation i^{q1} on the aminoterminal sequence.

Sequence analysis of *lac* repressor[10] had shown that older results[1] with carboxypeptidase B, which seemed to indicate the necessity of an intact C terminus for operator binding activity, were wrong. Our present results show clearly that

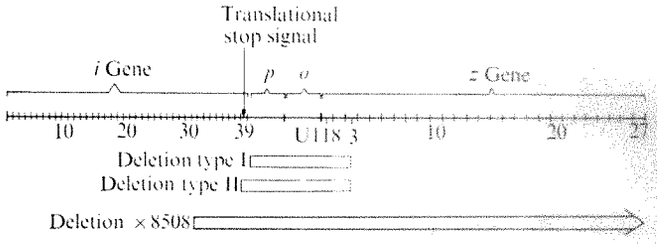


Fig. 1 Genetic map of the i gene, the promoter (*p*), operator (*o*), and the *z* gene. The *i* gene is divided in 39 deletion groups[4], the *z* gene in 27 deletion groups[5]. They are not drawn to scale. Deletion x8630 defines deletion group 39, deletion x8636 deletion group 38 of the *i* gene. Point mutants have been found which recombine with x8630 (Gho, personal communication), indicating that the translational stop signal of the *i* gene falls in deletion group 39. The fusions we found still to have *lac* repressor activity, are either of type I (71–56–9; 71–68–14; 28; 32; 34; 47; 48; 51), or of type II (71–56–14; 71–56–17). Mapping was done as in ref. 4. In some fusions (71–56–14; 17; 71–68–14; 28; 32) i^q was crossed out and i^+ back in using deletion x8508 (ref. 6). These i^+ derivatives of the fusion strains were used to see how deep the deletions entered the *z* gene. Those fusions which were tested (71–56–14 and 71–68–14; 28; 32) did recombine with a nonsense mutant mapping in deletion No. 3 but not with another mapping in deletion No. 1.

Table 1 Results of adding antibodies against *lac* repressor and β-galactosidase in two strains of *E. coli*

strain	anti-repressor added		anti-galactosidase added	
	% *lac* repressor precipitated	% β-galactosidase precipitated	% *lac* repressor precipitated	% β-galactosidase precipitated
Φ 71-56-14	35	50	20	55
i^{q1} z^+ (+IPTG)	24	≦0.2	≦1	50

The fusion 71–56–14 was crossed on the phage $\lambda 80c_{1857}dlacS_7$ (Φ71–56–14) to increase the yield of *lac* product after heat induction of a bacterial strain lysogenic for the phage[2]. As comparison, strain $i^{q1}z^+$ grown in the presence of inducer IPTG was used. The heat induction of the fusion strain was carried out as in ref. 2. 0.2 ml of extract were mixed with 0.2 ml of antiserum. The mixture was left overnight at 4° C. The visible precipitates were centrifuged for one minute in an Eppendorf microcentrifuge. The supernatants were discarded. The precipitates were resuspended in 1 ml of TMS buffer[7] and spun again. The washing procedure was repeated twice. Then repressor and β-galactosidase activity were determined[7,8]. The values obtained were expressed as percentages of the activities found before precipitation with antiserum.

Table 2 Purification of repressor-galactosidase from strain i^{q1} (71-56-14)

Fraction	Total volume (ml)	Total Protein (mg)	*lac* repressor activity: Specific activity (% mg^{-1} ml)	Recovery (%)	Purification	β-galactosidase activity: Specific activity (Units mg^{-1})	Recovery (%)	Purification
Crude extract	2,200	40,500	4.4	100	1	3,180	100	1
Dialysed ammonium sulphate precipitate (0-35%)	330	5,700	32	100	7	21,600	96	7
Phosphocellulose (main peak)	260	333	146	27	33	29,900	8	9
Sepharose 6B	84	118	266	17	60	98,000	9	31

400 g of frozen cells were suspended in 600 ml of TMS buffer[7] and disrupted in a Gaulin homogeniser at 600 kp/cm^2. The precipitate of a 0–35% ammonium sulphate cut was dissolved in 0.011 M PC buffer (potassium phosphate *p*H 7.27, 0.5 mM dithioerithrol, 5% glycerol) and dialysed against the same buffer. The dialysed sample was applied to a 500 ml phosphocellulose column (Whatman P 11) equilibrated with 0.011 M PC buffer. The protein was eluted with a linear gradient starting with 1.1 l 0.011 M PC buffer in the mixing chamber and 1.1 l 0.19 M PC buffer in the reservoir. After the gradient the column was first washed with 250 ml of the reservoir buffer, then with 1 l of 0.24 M PC buffer. The main portion of repressor-galactosidase was eluted at a concentration of 0.11 to 0.13 M potassium phosphate. The pooled material from the main peak was concentrated by ammonium sulphate and then passed through Sepharose 6B (column 2.5 × 100 cm) equilibrated with 0.11 M PC buffer. The protein is eluted in a single peak, containing both *lac* repressor and β-galactosidase activities. The specific *lac* repressor activity of the pure protein was 266: this almost reaches the specific activity of pure *lac* repressor (1,500), if the fourfold increase of subunit molecular weight is taken into consideration.

the C terminus of *lac* repressor is dispensible for activity. Furthermore *lac* repressor functions even with β-galactosidase pinned to its tail.

Both *lac* repressor and β-galactosidase are tetramers. How does the fused protein aggregate? To determine its state of aggregation we analysed the pure material obtained from the sepharose step on a glycerol gradient. Both β-galactosidase and *lac* repressor activity sediment together at the position of normal β-galactosidase (S 16). We also analysed some of the minor fractions from the phosphocellulose column and found them to contain several species of faster sedimenting material. All fractions bind *lac* operator *in vitro*, but only the S 16 fraction binds operator DNA like normal *lac* repressor (Fig. 3).The plot shows that the DNA was 20% active. From the molar ratios of the protein and DNA we calculate that 20% of the protein is active in DNA binding. The higher aggregates seem also to bind, in a way not understood, to DNA pieces which do not contain *lac* operator.

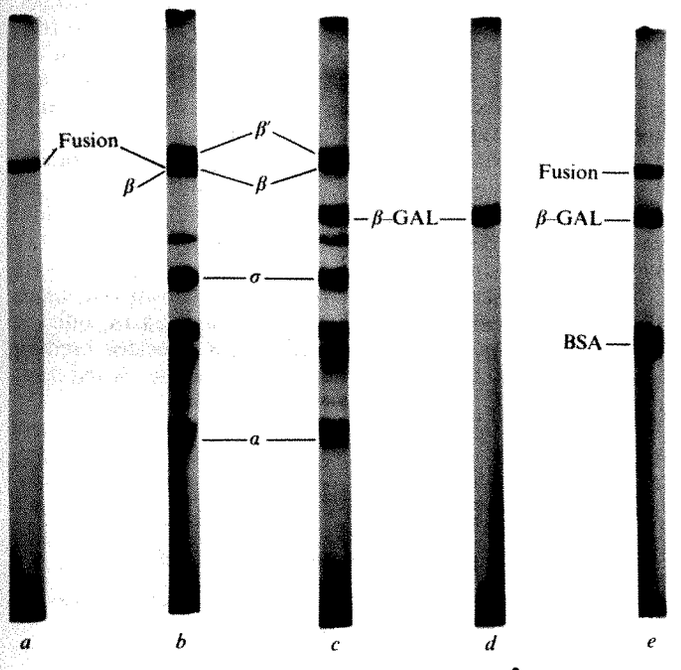


Fig. 2 Determination of the subunit molecular weight of repressor-galactosidase (fusion) by the SDS-polyacrylamide gel electrophoresis. 10 cm 5% acrylamide gels containing 0.1% SDS were prepared according to Weber and Osborn[13]. *a*, Repressor-galactosidase; *b*, repressor-galactosidase and *E. coli* RNA-polymerase (Miles-Seravac); *c*, RNA-polymerase and β-galactosidase; *d*, β-galactosidase; *e*, repressor-galactosidase, β-galactosidase, and bovine serum albumin.

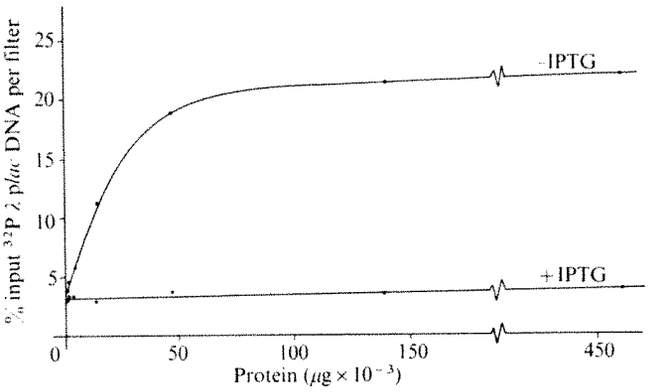


Fig. 3 Bound ^{32}P–λ*plac* DNA as a function of the amount of pure repressor-galactosidase in the assay. 3 ml of binding buffer (BB)[14] (without dimethyl sulphoxide), containing the protein, was divided into equal parts and 20 μl of a 0.1 M IPTG stock was added to one half. Then 0.1 ml of a ^{32}P–λ*plac* DNA solution was added and incubated for 8 min at room temperature. The constant amount of DNA in the 1.6 ml assay is 0.43 μg, with the activity of 23,000 c.p.m. per μg DNA. Each filter (Sartorius, 0.45 μm, 25 mm) received 0.5 ml, containing a total input of 3,000 c.p.m. The filters are washed with 1 ml BB each and counted in 10 ml Bray's Solution[15].

Finally, we analysed whether homology between *lac* repressor and the N terminus of β-galactosidase is responsible for the relative high frequency of the *i z* fusions. To test this we measured the frequency of *i z* fusions in a *Rec A* strain in which recombination is greatly reduced. We found the same number of fusions as in wild type. This excludes sequence homology. Sequence analysis which we have begun should define the unnecessary parts of β-galactosidase and *lac* repressor.

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1 Adler, K., *et al.*, *Nature*, **237**, 322–327 (1972).
2 Müller-Hill, B., Crapo, L., and Gilbert, W., *Proc. natn. Acad. Sci. U.S.A.*, **59**, 1259–1264 (1968).

[3] Jacob, F., and Monod, J., *J. molec. Biol.*, **3**, 318–356 (1961).
[4] Pfahl, M., *Genetics*, **72**, 393–410 (1972).
[5] Zipser, D., Zabell, S., Rothman, J., Grodzicker, T., Wenk, M., and Novitski, M., *J. molec. Biol.*, **49**, 251–254 (1970).
[6] Miller, J. H., Reznikoff, W. S., Silverstone, A. E., Ippen, K., Signer, E. R., and Beckwith, J. R., *J. Bact.*, **104**, 1273–1279 (1970).
[7] Gilbert, W., and Müller-Hill, B., *Proc. natn. Acad. Sci. U.S.A.*, **56**, 1891–1898 (1966).
[8] Platt, T., in *Experiments in Molecular Genetics* (edit. by Miller, J. H.), 403 (Cold Spring Harbor Laboratory, New York, 1972).
[9] Fowler, A. V., and Zabin, I., *J. biol. Chem.*, **245**, 5032–5041 (1970).
[10] Beyreuther, K., Adler, K., Geisler, N., and Klemm, A., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3576–3580 (1973).
[11] Weiner, A. M., Platt, T., and Weber, K., *J. biol. Chem.*, **247**, 3242–3251 (1972).
[12] Platt, T., Files, J. G., and Weber, K., *J. biol. Chem.*, **248**, 110–121 (1973).
[13] Weber, K., and Osborn, M., *J. biol. Chem.*, **244**, 4406–4412 (1969).
[14] Riggs, A. D., Suzuki, H., and Bourgeois, S., *J. molec. Biol.*, **48**, 67–83 (1970).
[15] Bray, G. A., *Analyt. Biochem.*, **1**, 279–285 (1960).

Mutagenicity of amino acid analogues in eukaryotes

A LOSS of fidelity in DNA replication and repair enzymes in both pro and eukaryotes, is often expressed as a mutation. Examples of this are shown in the mutator gene activity of a DNA polymerase mutant of T4 (ref. 1) and mutants with increased ultraviolet sensitivity or decreased recombination[2–4]. Springgate and Loeb[5] have reported the altered fidelity of the DNA polymerase of acute lymphatic leukaemia cells.

Amino acid analogue incorporation into protein has a varying effect on enzyme stability and activity[6–9] and it seems that the position of the amino acid in the polypeptide is the crucial factor[10].

We have recently reported[11,12] that the analogues *p*-fluorophenylalanine (PFP) and ethionine (ETH) are strongly mutagenic to reverse mutations in the basidiomycete *Coprinus lagopus*, inducing single base changes with a maximum of a 500-fold increase over the spontaneous mutation frequency shown by 2.5×10^{-4} M DL-PFP which induces 64% growth inhibition. PFP is mutagenic by incorporation into protein, while ETH mutagenesis is a function of transethylation of nucleic acids[12]. The mutagenicity of a number of amino acid analogues has been reported in *Chlamydamonas eugametos*[13] and in *Ustilago maydis*[14].

To gain a better understanding of the mutagenicity of amino acid analgues and because of the availability of defined replication and repair mutants in *Escherichia coli*, we tested the mutagenicity of PFP on reverse mutations in the bacterial system. Two point mutations were studied, the leucine requirement in strain C600 and the tryptophan requirement in strain A58. As with *Coprinus*, PFP was incorporated into the growth medium, in this case liquid Davis minimal medium, at concentrations ranging from 10^{-5} M DL-PFP to 5×10^{-4} M DL-PFP, a concentration which gave linear growth. At time zero, flasks were inoculated with exponentially growing cells and grown to stationary phase or for 5 h in the case of high analogue concentrations. The cells were washed and plated onto minimal medium reversion plates and viability plates supplemented with the auxotrophic requirement. In both cases, however, the analogue PFP had no effect on the reversion frequency of either the *leu* or *tryp* loci. The incorporation of ^{14}C-PFP confirmed that PFP was being incorporated into the protein fraction. Similarly, 10^{-3} M DL-PFP had no mutagenic effect on the reversion frequency of *Pseudomonas aeruginosa* PAO 381 (*leu*$^-$).

The mutagenicity of PFP was then tested on bacteriophage T4, chosen for its independent DNA polymerase synthesis. The effect of PFP on the plaque-forming ability of T4rII 271 on *E. coli* K and the plaque-forming ability of the amber mutant T4*am*122 on the nonsuppressive strain *E.coli* B/r/t$^-$, was studied. In both cases phage was inoculated at time zero into a permissive host grown previously for 2 h in the presence of 10^{-4} M DL-PFP. After a further 3 h, growth was stopped and the plaque-forming ability assayed. PFP had no mutagenic effect on either system.

Analogues norleucine and canavanine showed similar negative effects on *E. coli* reversion frequencies. The mutagenicity of ETH was not tested as the strains studied showed resistance to the analogue and ETH is not activated to S-adenosyl-ethionine in *E. coli* (ref. 15).

In *Coprinus*, PFP treatment followed by a 6 min dose from a 6 W Philips TUV mercury vapour lamp (8.54 erg cm^{-2}), showed a maximum mutational synergism of 9.6 times over the expected additive reversion frequencies (P. J. T., and D. L., unpublished). Mutational synergism is indicative of an interaction between mutational pathways; we have interpreted these results as the effect of PFP incorporation into ultraviolet repair enzymes. As PFP showed no mutagenicity in *E. coli* it might be possible to detect its effect by looking for mutational synergism between PFP and ultraviolet treatments; 5×10^{-5} M DL-PFP was chosen as a suitable concentration for pre-ultraviolet treatment, so as not to induce starvation-induced resistance enhancement[16]. Log phase cells of *E. coli* C600, grown in the presence of the analogue for 3 h, were irradiated for varying times. Analogue treatment did not affect ultraviolet survival or reversion frequencies of the *leu* locus.

To clarify the difference between pro and eukaryotic responses to analogue mutagenesis, we chose, as a system of comparison, mutations in the mitochondrial and nuclear genes of *Saccharomyces cerevisiae*. The existence of an independent mitochondrial DNA polymerase[17], the selective depression of mitochondrial protein synthesis in the presence of a fermentable substrate, and the differential sensitivity of certain strains to analogues in conditions of growth on nonfermentable or fermentable substrates[18], make the system advantageous for this study.

Two strains of *S. cerevisiae*, D26 (adenine requiring, erythromycin sensitive) and D6 (methionine requiring, erythromycin sensitive) were chosen. D26 showed a three-fold and D6 a two-fold increased sensitivity to PFP on nonfermentable substrate. The effect of PFP on the nuclear markers *ad* and *met* and mutation to erythromycin (1 mg ml^{-1}) resistance—the mitochondrial marker—were studied. Liquid glucose yeast extract and glycerol yeast extract media, supplemented with the analogue at concentrations giving up to 50% growth inhibition were inoculated with 10^6 yeast cells and grown to stationary phase. The cells were washed twice and tested on Wickerams synthetic medium, fully supplemented in the case of viability plates, but without the auxotrophic requirement in the reversion plates. Resistance to erythromycin was tested on glycerol yeast extract with 1 mg ml^{-1} erythromycin added (Table 1).

The positive effect of the analogue on the nuclear genes in yeast, confirms the results in *Coprinus*. The entirely negative effect of the analogue on mitochondrial mutation, if we can

Table 1 The mutagenic effect of PFP on mitochondrial and nuclear markers in *Saccharomyces cerevisiae* strains D26 (*ad*$^-$ *eryth*^{s}) and D6 (*met*$^-$ *eryth*^{s}) when supplemented to both yeast extract glucose (YES) and yeast extract glycerol (YEG) media

		Mutation frequency per 10^6 viable cells			
		Control	1.5×10^{-3} MPFP	3.0×10^{-3} MPFP	4.5×10^{-3} MPFP
		YEG and YES	YEG	YES	YES
D26	*ad*$^-$	3.5	3.3	9.2	23.2
	eryth^s	24.6	25.8	13.5	17.6
D6	*met*$^-$	3.0	5.9	76	—
	eryth^s	24.3	10.4	13.1	—

compare the mitochondrial chromosome with the chromosome of prokaryotes, confirms the negative results in prokaryotes.

It might be possible to explain the differential response to PFP on the basis of amino acid composition of DNA replication and repair enzymes in pro and eukaryotes, that is, while phenylalanine might be strategically placed in fungal replication and repair enzymes, such as at the active site, phenylalanine differently placed in bacterial and mitochondrial replication and repair enzymes, could account for the difference. From Table 1, the maximum mutagenic effect obtained in yeast is considerably lower than in *Coprinus*, at comparable growth inhibitory concentrations. This can also be explained on the differential importance of phenylalanine in the active centres in the DNA replication and repair enzymes in yeast and *Coprinus*. If this is so, then certain analogues should show mutagenicity in prokaryotes. The few we tested did not. Pardee and Prestide[19] reported that no mutagenic action of 7-azatryptophan was noted in mutation to streptomycin resistance in *E. coli* or the plaque-forming ability of T2.

The differential response might implicate the presence of histone and nonhistone proteins in eukaryotic chromosomes. PFP induces haploidisation inversions and transversions in certain fungal species by affecting chromosomal division[20] and integrity[21]. In *Chlamydamonas* the phenylalanine analogue 2-amino-3-phenylbutanoic acid, as well as being mutagenic, causes chromosomal abberations, suggested by McBride and Gowan[13] to be the result of incorporation of the analogue into chromosomal associated proteins. Hare[22] reported that canavanine incorporation into histone and nonhistone proteins in mice, mammalian and hamster tumour cells, inhibited DNA synthesis. Chromosomal abberations and an exaggerated response to mutagens have been found in children suffering from severe protein starvation[23].

Several reports have been made of the differential response of bacteria and higher organisms to various mutagens[24-27]. Most of these effects have been attributed to physiological differences between pro and eukaryotes. The differential response to amino acid analogues indicates a difference on the basis of the replication and repair of DNA.

We thank Dr B. A. Bridges for pointing out to us the work of George and Witkin[28] suggesting that some ultraviolet repair enzymes might be inducible by ultraviolet. If this is true then the interpretation of our experiments with PFP and ultraviolet light is more uncertain. This does not, however, alter the validity of the comparison between *C. lagopus* and *E. coli*.

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[1] Speyer, J. F., *Biochem. biophys. Res. Commun.*, **21**, 6 (1965).
[2] Böhme, H., *Biochem. biophys. Res. Commun.*, **28**, 191 (1967).
[3] Jyssum, K., *J. Bact.*, **95**, 165 (1968).
[4] von Borstel, R. C., Graham, D. E., La Brot, K. J., and Resnick, M. A., *Genetics*, **60**, 233 (1968).
[5] Springgate, C. F., and Loeb, L. A., *Proc. nat. Acad. Sci., U.S.A.*, **70**, 245 (1973).
[6] Janacek, J., and Rickenberg, H. V., *Biochim. biophys. Acta.*, **81**, 108 (1964).
[7] Janacek, J., *Folia microbiol., Praha* **12**, 132 (1967).
[8] Spizek, J., and Janacek, J., *Folia microbiol., Praha.* **14**, 521 (1969).
[9] Janacek, J., and Spizek, J., *Folia microbiol. Praha.* **14**, 529 (1969).
[10] Langridge, J., *J. Bact.*, **96**, 1711 (1968).
[11] Talmud, P. J., and Lewis, D., *Heredity*, **31**, 139 (1973).
[12] Talmud, P. J., and Lewis, D., *Genet. Res.*, (in the press).
[13] McBride, A. C., and Gowan, C. S., *Genet. Res.*, **14**, 121 (1969).
[14] Lewis, C. M., and Tarrant, G. M., *Mutat. Res.*, **12**, 349 (1971).
[15] Peterkovsky, A., in *Transmethylation and methionine biosynthesis* (edit. by Shapiro, S. K., and Shlenk, F.) 137, (University of Chicago Press, Chicago, 1965).
[16] Rowbury, R. J., *Int. J. radiat. Biol.*, **21**, 297 (1972).
[17] Wintersberger, E., *Biochem. biophys. Res. Commun.*, **25**, 1 (1966).
[18] Wilkie, D., *J. molec. Biol.*, **47**, 107 (1969).
[19] Pardee, A. B., and Prestidge, L. S., *J. Bact.*, **71**, 677 (1956).
[20] Day, A. W., and Jones, J. K., *Genet. Res.*, **18**, 299 (1971).
[21] Strømnaes, O., *Hereditas*, **59**, 197 (1968).
[22] Hare, J. D., *J. cell. Physiol.*, **75**, 129 (1970).
[23] Armendares, S., Salamanca, F., and Frenk, S., *Nature*, **232**, 271 (1971).
[24] Kondo, S., *Jap. J. Genet.*, **39**, 179 (1964).
[25] Zettenberg, G., *Mutat. Res.*, **3**, 393 (1966).
[26] Zettenberg, G., *Hereditas*, **68**, 245 (1971).
[27] Vaharu, T., *Genetics*, **46**, 247 (1961).
[28] George, D. L., and Wilkin, E. M., *Genetics*, **74**, 91 (1973).

Naturally occurring cytotoxic anti-tumour antibodies in sera of congenitally athymic (nude) mice

IT has been suggested that a natural function of the immune system is the detection and elimination of nascent autochthonous tumours and that clinical tumour development reflects a failure of this immune surveillance function[1]. Evidence for the involvement of thymus dependent immune processes in the prevention of spontaneous malignant disease is lacking. Thus, no significant increase in tumour incidence has been observed in either neonatally thymectomised (R. T. Prehn, personal communication) or congenitally athymic (nude) mice[2,3]. If, as suggested by other studies, immune surveillance does, in fact, operate[4], it is likely that it will involve a thymus-independent immune process. We have detected in sera of many normal mice IgM antibodies which are cytotoxic for several spontaneous and induced tumour cell lines (unpublished). The possible role of these antibodies in immune surveillance is uncertain, but if they are a manifestation of active immune surveillance, they would be expected also to occur in the sera of nude mice.

Nude mice (from Olac Ltd, Southern Bicester, England) have been studied in this laboratory and shown to have a near complete absence of θ-positive thymus-derived lymphoid cells[5]. Sera were obtained from these mice and were stored in aliquots at $-20°C$. The sera were tested for complement dependent cytotoxic activity against several tumour lines maintained in this laboratory. Two tumours which recently arose spontaneously in normal C3H mice were included in this study. One is a lymphosarcoma with surface bound IgG_2 immunoglobulin and is referred to as γG2LS. The other is an *in vitro* cell line derived from an ovarian teratoma. The cells are relatively undifferentiated epitheloid cells and are designated Ter-B. The other tumours tested are maintained in ascites form and are used extensively in several laboratories. They are C1300, a neuroblastoma of strain A mice; and EL-4, L1210, and PU-5, leukaemias of C57BL, DBA/2, and BALB/c mice, respectively.

Individual sera obtained from six, ten-week-old nude mice were tested undiluted against various tumour cell lines and were found to possess significant complement dependent lytic activity against all but the PU-5 target cells. A pool of the sera from the individual nude mice was also tested at several dilutions against the different tumour cell lines. The results of these cytotoxic assays are depicted in Fig. 1. The observed levels of cytotoxic activity are within the range of variation obtained when different sera of normal mice are similarly tested (unpublished). Sera from nude mice show no cytotoxic activity when tested on normal allogeneic spleen cells.

The presence of naturally occurring cytotoxic antibody in the sera of nude mice indicates the capacity of the thymus-independent immune system to respond immunologically to

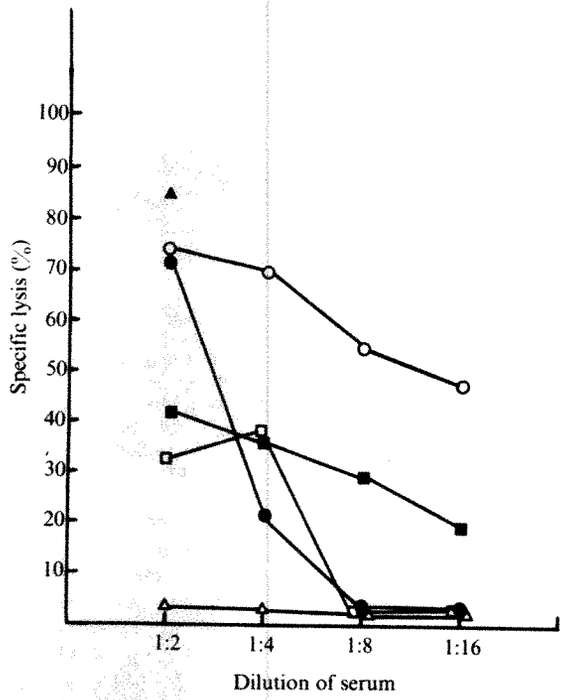


Fig. 1 Complement dependent antitumour cytotoxic activity of serum obtained from nude mice. Target cells were obtained from the following tumours: Ter-B (▲); γG2LS (○); L1210(●); C1300 (■); E1–4 (□), and PU–5 (△). See text for description of tumours. In each assay, 50 μl of a suspension containing 3×10^6 tumour cells ml^{-1} in Dulbecco's modified minimal essential medium with 10% foetal calf serum were added to 50 μl of a dilution of pooled serum obtained from nude mice. The mixture was incubated for 45 min in a humidified incubator at 37° C with a 10% CO_2:90% air atmosphere. The cells were washed with 300 μl of medium and resuspended in 50 μl of a 1:6 dilution of rabbit serum as a source of complement. The rabbit serum had been previously absorbed at 2° C with a one-fifth volume of mouse spleen cells to reduce its toxicity. After 45 min incubation in the presence of complement, the tubes were placed in an ice bath. A volume of 50 μl of trypan blue solution was added to the cell suspensions and aliquots removed for microscopic enumeration of % nonviable (stained) cells. Two groups of controls were included in each experiment: first, incubation of tumour cells with serum of nude mice, but followed by the addition of medium rather than complement and second, initial incubation of the cells in medium followed by incubation in complement. Less than 5% nonviable cells were observed with the first group of controls for each tumour cell line tested. Less than 15% nonviable cells were observed with the complement control (C) for each of the tumour cell lines tested. Specific lysis was calculated by subtracting C from the % nonviable cells observed with serum plus complement, dividing by (100–C) and multiplying by 100. Duplicate determinations of cytotoxic activity of nude mouse sera were made for each tumour cell line.

tumour-associated antigens. The nature of the antigens recognised by naturally occuring antibodies in sera of normal mice is currently being investigated. Some of these antigens are expressed in appreciable amounts during foetal development, while others are expressed in certain allogeneic strains[6,7]. It is possible that cytotoxic anti-tumour antibody production is partly maintained in normal and nude mice by the derepression of these foetal and alloantigenic components on nascent autochethonous tumours.

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1 *Immune Surveillance* (edit. by Smith, R. T., and Landy, M.) (Academic Press, New York, 1970).
2 Pantelouris, E. M., *Nature*, **217**, 370 (1968).
3 Stuttman, O., *Science*, (in the press).
4 Martin, W. J., Esber, E., Cotton, W. G., and Rice, J., *Br. J. Cancer*, **28**, supplement. 1, 48 (1973).
5 Raff, M. C., *Nature*, **246**, 350 (1973).
6 Martin, W. J., in *Immunology of the Tumour Host Relationship* (edit. by Smith, R. T., and Landy, M.) (Academic Press, New York, in the press).
7 Sato, H., Boyse, E. A., Aoki, T., Iritani, C. and Old, L. J., *J. exp. Med.*, **138**, 593 (1973).

Homology between human breast tumour RNA and mouse mammary tumour virus genome

Several studies have provided indirect evidence linking particles related to mouse mammary tumour virus (MuMTV) with human breast cancer[1–3]. In addition to morphological and biochemical similarities between human milk particles and known oncornaviruses shown in several laboratories[4], Axel *et al.*[5] demonstrated partial homology between MuMTV genome and polysomal RNA isolated from human breast tumour by molecular hybridisation experiments. Using Cs_2SO_4 density gradient analysis they showed that 67% out of 29 tumours tested had detectable amounts of MuMTV-related RNA. But because of intrinsic problems involved in Cs_2SO_4 gradient analysis it is not possible to determine hybridisation quantitatively. In this paper we describe hybridisation experiments using MuMTV-specific DNA probes and human tumour RNA under stringent conditions.

We have used single-strand specific nuclease (S_1) from *Aspergillus oryzae*[6] for the detection of hybrid formation. This enzyme specifically degrades single-stranded nucleic acids but not double stranded nucleic acids or DNA–RNA hybrids. Highly radioactive, complementary and predominantly single-stranded DNA synthesised by endogenous RNA-directed DNA polymerase of MuMTV in the presence of actinomycin D was used as a probe to detect MuMTV-related sequences in whole cell RNA isolated from human tumours. Most of the human lesions were carefully selected by examining representative frozen sections histologically to give maximum ratio of malignant cells to stromal cells. Detailed data on the histopathology and antigenicity of the tumour tissues in relation to hybridisation will be published elsewhere.

Optimum hybridisation conditions described by Varmus *et al.*[7] were used (see Table 1 for details). The experiments were performed under conditions of excess RNA and limiting DNA probes. Under these conditions the rate of hybridisation is determined by the concentration of homologous RNA and time of incubation. The product of RNA (mol l^{-1}) and time of incubation (s) is designated C_rt which is similar to the C_0t value described by Britten and Kohne[8]. The C_rt values were computed from the A_{260} of the RNA samples[8] and corrected to 0.18 M[Na^+] according to Britten and Smith[9].

To provide a rapid determination of hybridisation, tumour RNAs were incubated with MuMTV DNA probe at C_rt values greater than 10^4. Table 1 summarises the results obtained. Any tumour RNA that hybridised more than 10% of the DNA probe was considered significantly positive. It can be seen that out of 17 tumours tested 5 had RNA sequences homologous to MuMTV genome. In one case (HuT 11) the tumour tissue itself revealed some homologous RNA but surrounding 'control' tissue was negative for MuMTV-related RNA sequences. When the DNA probe synthesised by Mason-Pfizer monkey virus

Table 1 Hybridisation of human tumour RNA with MuMTV DNA probes

Tissue	Histopathology	Hybridisation with MuMTV probe	
Human tumour no.		% DNA hybridised	C_rt
Invasive carcinoma of breast			
HuT–3	Mucinous type	0.89	2.87×10^4
HuT–4	NST*	0.0	2.32×10^4
HuT–5	NST	1.56	3.73×10^4
HuT–6	NST	0.0	2.72×10^4
HuT–8	NST	0.67	3.35×10^4
HuT–9	Medulary type with *in situ* foci	42.0	2.21×10^4
HuT–10	Medulary type	6.9	1.4×10^4
HuT–12	NST	76.7	6.73×10^4
HuT–13	NST	54.4	3.56×10^4
HuT–16	NST	0.0	3.96×10^4
HuT–17	NST	0.0	2.3×10^4
In situ, carcinoma of breast			
HuT–7	Lobular type	68.0	2.16×10^4
HuT–11	Lobular type	18.0	2.72×10^4
HuT–14	Ductular type	1.66	2.16×10^4
HuT–15	Ductular type	0.0	3.93×10^4
Other human tumours			
HuT–1	Adenocarcinoma of colon	0.89	3.07×10^4
HuT–2	Carcinoma of cecum	4.58	3.34×10^4
Benign breast lesions			
'Control' of HuT–11	Lobular & ductal hyperplasia	0.0	1.19×10^4
'Control' of HuT–14	Lobular & ductal hyperplasia	3.54	1.92×10^4
Controls			
Rat liver	—	0.0	3.0×10^4
MuMTV 70S RNA	—	100	4.12×10^0
RIII lactating mammary gland	—	100	5.00×10^3
C57 transplanted mammary tumour	—	100	2.22×10^4

*NST = No special type

RNA from tissues was isolated by SDS-phenol extractions, DNase treatment and ethanol precipitation as described by Varmus *et al.*[7] and resuspended in 0.02M Tris HCl *p*H 7.2 to give a concentration of about 10 mg ml^{-1}. The DNA probes were synthesised from ~1.5 mg purified MuMTV from RIII mouse milk. The following solutions were added to the virus suspended in 200 μl 0.01 M Tris HCl *p*H 8.3: 24 μl of 1 M DTT; 20 μl of 1% bovine serum albumin; 12 μl of 5% Nonidet–P40; 16 μl of 2.5 mM ATP; 50 μl of 1 mg ml^{-1} actinomycin D; 100 μl of a mixture containing 0.25 M Tris–HCl *p*H 8.3, 0.05 M NaCl, 0.04 M $MgCl_2$; 60 μl of a mixture containing 0.01 M each of dCTP, dATP and dGTP; and ^{3}H–TTP (specific activity 50 Ci mmol^{-1}) was added to give a concentration of 10 μM. After the incubation at 37° C for 18 h the reaction was stopped by adding 1/10 volumes of 10% SDS and 4M NaCl. The reaction mixture was diluted to 2 ml with a buffer containing 0.01 M Tris HCl *p*H 8.3, 0.01 M EDTA and 0.1 M NaCl and extracted three times with phenol-cresol (7:1 *p*H 7.5). Following overnight ethanol precipitation the template RNA molecules were digested by treating the probes in 0.003 M EDTA with 100 μg ml^{-1} RNase A at 37° C for 1 h. The RNase was destroyed by the treatment with 0.6 M NaOH at 37° C for 1 h (ref. 12). After neutralisation with HCl the probes were precipitated in ethanol and resuspended in 0.02 M Tris HCl *p*H 7.2 The hybridisation reactions were carried out at high C_rt values (see text) in duplicate 5 μl mixture sealed in capillary tubes and incubated at 68° C for 66 h. The mixture contained 30 to 50 μg RNA and 300–500 c.p.m. of DNA probe in 0.04 M Tris *p*H 7.2 0.002 M EDTA and 0.6 M NaCl. After the incubation hybridisation mixture was treated with single-strand specific nuclease (S_1) from *Aspergillus oryzae*[6] according to the method described by Varmus *et al.*[7]. The probes annealed with heterologous RNA (rat liver) were also treated with S_1 to give self-hybridisation values which were used to correct the results.

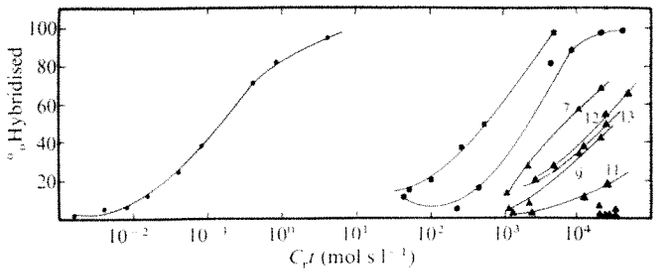


Fig. 1 C_rt curve analyses of hybridisation between MuMTV DNA probes and RNAs from various sources. The conditions of hybridisation were the same as in Table 1. Different C_rt values were obtained by diluting the test RNAs with heterologous (rat liver) RNA. ●, MuMTV 70S RNA; ■, RIII lactating mammary glands; ♦, C57 transplanted mammary tumour; ▲, various human tumours including positive tumours (Table 1) HuT 7, 9, 11, 12 and 13.

(MPMV, a virus isolated from the breast tumour of a rhesus monkey) was used for hybridisation under the same conditions, none of the tumours tested showed any RNA sequences homologous to MPMV genome (data not shown).

Hybridisation kinetics for the positive tumour RNAs were observed by C_rt curve analysis shown in Fig 1. For comparison, C_rt curves obtained with MuMTV 70S RNA, RNA from RIII lactating mammary glands and from C57 transplanted mammary tumours are also illustrated. All five positive human tumour RNAs showed varied rates of hybridisation. By comparing $C_rt_{1/2}$ values of various human tumour RNAs with MuMTV 70S RNA it is possible to arrive at an estimation of virus-related RNA molecules in these tumours. As seen in Table 2, the numbers of MuMTV related RNA molecules per cell in these tumours was low ranging from 1.5 to 8. These estimates are based on the hybridisation of a nonuniformly represented DNA probe and are likely to vary depending upon the representation of viral genome in the synthesised DNA probes.

Thermal stability of hybrids is an important criterion for establishing their true nature. Figure 2 shows a thermal stability experiment performed on the hybrids formed between MuMTV DNA and human tumour RNA. The T_m for the hybrids of MuMTV DNA and RNA from RIII lactating mammary gland (a tissue which produces MuMTV) was 69° C compared with 62° C for the hybrid of MuMTV DNA and human tumour RNA. This difference in thermal stability would indicate about 5% mismatching between the MuMTV DNA and the tumour RNA (ref. 10). Considering the species differences between the sources of two nucleic acids, the hybrids were remarkably well matched.

Extensive electron microscopic searches have failed to reveal unequivocally the presence of complete MuMTV-like

Table 2 Estimations of MuMTV 70S RNA equivalents per cell

Sample	$C_rt_{1/2}$*	% Viral RNA	MuMTV 70S RNA equivalents per cell
MuMTV 70S RNA	1.5×10^{-1}	100	—
HuT 7	7.5×10^3	0.002	8
HuT 9	3.3×10^4	0.00045	1.5
HuT 12	2.1×10^4	0.00072	2.2
HuT 13	2.75×10^4	0.00054	1.68
RIII lactating mammary glands	5.2×10^2	0.029	90
C57 transplanted mammary tumour	1.9×10^3	0.0078	25

*C_rt at which 50% of the input DNA probe was hybridised
By comparing $C_rt_{1/2}$ of MuMTV 70S RNA and of the test RNAs, percentage of MuMTV-related RNA in a given sample was determined. Based on assumptions that each MuMTV 70S RNA weighs 1.6×10^{-11} μg and total cellular RNA weighs 5×10^{-6} μg, the number of MuMTV 70S-related RNA molecules per cell was estimated.

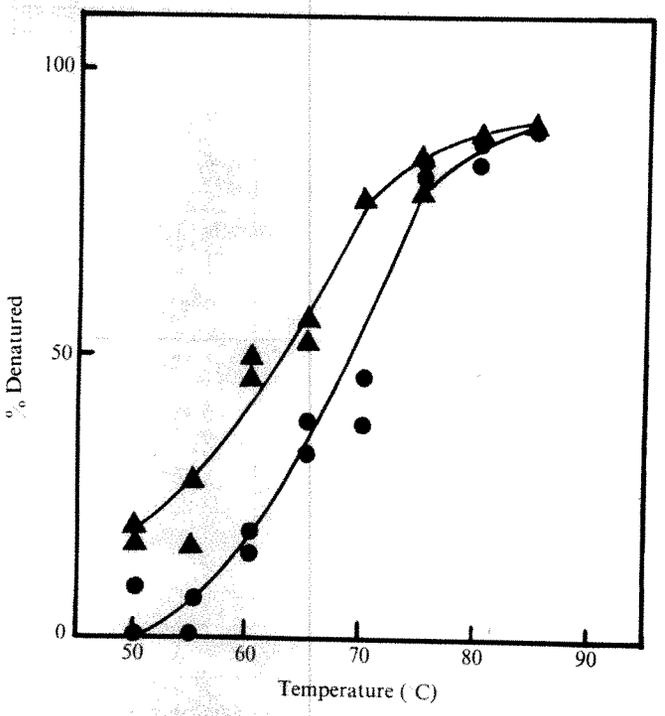


Fig. 2 Thermal stability experiment. Approximately 7,000 c.p.m. of MuMTV probe DNA were annealed with RNAs from human breast tumour (▲) and RIII lactating mammary gland (●). Following ethanol precipitation the hybrids were resuspended in 0.01 M Tris HCl *p*H 8.3 and aliquots were heated at various temperatures for 15 min, chilled quickly and treated with S_1 nuclease as described in Table 1.

particles in human breast tumours. (A. B. V., unpublished work, and N. H. Sarkar, personal communication.) But the results shown in this communication are very similar to those reported[7] for murine tissues apparently negative for MuMTV production. Particularly, in spontaneous mammary tumours of BALB/c mice, B-particles of MuMTV are not seen (N. H. Sarkar, personal communication), but a small amount of MuMTV-related RNA is present[7,11].

According to current theories of viral oncogenesis, the genome of the virus should be integrated with the host genome. If a MuMTV-related virus is involved in the aetiology of human breast cancer, one should expect MuMTV-related genomic information integrated into the DNA of the tumour cells. We plan to investigate this possibility using molecular hybridisation techniques.

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[1] Moore, D. H., *et al.*, *Nature*, **229**, 611–615 (1971).
[2] Schlom, J., Spiegelman, S., and Moore, D. H., *Nature*, **231**, 97–100 (1971).
[3] Das, M. R., Sadasivan, E., Koshy, R., Vaidya, A. B., and Sirsat, S. M. *Nature new Biol.*, **239**, 92–95 (1972).
[4] Vaidya, A. B., *Science*, **180**, 776–779 (1973).
[5] Axel, R., Schlom, J., and Spiegelman, S., *Nature*, **235**, 32–36 (1972).
[6] Ando, T., *Biochim. biophys. Acta*, **114**, 158–168 (1966).
[7] Varmus, H. E., Quintrell, N., Medeiros, E., Bishop, J. M., Nowinski, R. C., and Sarkar, N. H., *J. molec. Biol.*, **79**, 663–679 (1973).
[8] Britten, R. J., and Kohne, D. E., *Science*, **161**, 529–540 (1969).
[9] Britten, R. J., and Smith, J., *Carnegie Inst. Wash. Yearbook*, **68**, 378–386 (1970).
[10] Laird, C. D., McConaughy, B. L., and McCarthy, B. J., *Nature*, **224**, 149–154 (1969).
[11] Schlom, J., Michalides, R., Kufe, D., Hehlmann, R., Spiegelman, S., Bentvelzen, P., and Hageman, P., *J. natn. Cancer Inst.*, **51**, 541–551 (1973).
[12] Garapin, A-C., Varmus, H. E., Faras, A., Levinson, W. E., and Bishop, J. M., *Virology*, **52**, 264–274 (1973).

Levamisole inactive in treatment of four animal tumours

LEVAMISOLE (2,3,5,6-tetrahydro-6-phenylimidazo (2, 1-b) thiazole) a compound known to be active against a wide range of nematodes[1,2], has been reported to enhance the humoral and cellular immune responses of animals and to reduce the incidence of both primary tumours and lung metastases in the Lewis lung (3a) tumour system in C57BL mice[3]. We report here studies on the effect of levamisole on the growth of four transplantable tumours, all known to be immunogenic. They were: a transplantable adenovirus 12-induced tumour of CBA mice[4]; a chick embryo lethal orphan (CELO) virus-induced tumour of hamsters[5]; a Moloney leukaemia virus-induced lymphoma of BALB/c mice[6]; and a chemically-induced metastasising anaplastic neoplasm of inbred rats[7]. We also studied the effect of levamisole on the humoral antibody response to influenza virus vaccine in hamsters.

The tumours were maintained by serial, subcutaneous inoculation of tumour fragments at 2–3 week intervals. For inoculation of experimental animals, small tumours of 10–15 mm diameter were removed from the animals, freed from normal and necrotic tissue and washed with Hank's saline. They were then minced to small fragments and treated with 0.25% trypsin; the resulting single cell suspension was washed three times in Hank's saline, and suspended to the required concentration in Eagle's minimal essential medium containing 2% inactivated calf serum. The cells were used immediately to inoculate animals. A 10 mg ml^{-1} solution of levamisole was made in pyrogen-free saline, and stored at −20°C. Immediately before use, aliquots of the stock solution were thawed, and diluted to the required concentration in pyrogen-free saline.

Hamsters were inoculated subcutaneously with 5×10^6 viable adenovirus 12-induced tumour cells in an 0.2 ml volume. One group of these animals was inoculated subcutaneously with 0.5 mg kg^{-1} of levamisole from 4 d before inoculation with tumour cells, a second group was inoculated with levamisole from the day of tumour cell inoculation, and a third group was untreated. Levamisole was given every 48 h from the start of treatment to the end of the study. The hamsters were examined regularly for tumours, and the diameter of tumours were measured with calipers. The results are shown in Table 1. Of the 10 hamsters which were not treated with levamisole, a single tumour was observed 17 d after inoculation, and three animals had large tumours (> 2.5 cm in diameter) 35 d after inoculation. In contrast, the incidence of tumours for hamsters which had been treated with levamisole from 4 d before tumour

Table 1 Effect of levamisole on transplanted adenovirus 12-induced tumours of CBA mice

Levamisole from day (0.5mg kg^{-1})	No. of tumours (day after inoculation) 10	14	17	24	31	35
—4*	0/10	1/10†	4/10 (0.25)	5/10 (1.1)	7/10 (1.8)	7/10 (>2.5)
0	0/10	3/10 (0.1)	5/10 (0.5)	6/10 (1.3)	9/10 (1.9)	9/10 (>2.5)
Nil	0/10	0/10	1/10 (0.5)	3/10 (1.0)	3/10 (2.1)	3/10 (>2.5)

* Treatment (0.5 mg kg^{-1}) subcutaneously on alternate days from 4 d before tumour cell inoculation to end of study.
† $\frac{\text{No. animals with tumours}}{\text{No. animals inoculated}}$ (mean tumour diameter in cm)

Table 2 Effect of levamisole on transplanted Rd/3 tumour cells of rats

Experiment no.	Levamisole from day (0.5mg kg^{-1})	Incidence of tumours at 35 d after inoculation foot pad (grade)¶	popliteal lymph node (grade)	paraaortic lymph node (grade)
1	—5*	3/3 (3, 3, 3)†	3/3 (2, 2, 1)	1/3 (1)
	Nil	3/3 (3, 3, 3)	3/3 (3, 2, 2)	2/3 (1, 1)
2	0	3/3 (3, 3, 3)	1/3 (3)	1/3 (2)
	Nil	2/3 (3, 3)	2/3 (2, 1)	0/3
3	14	3/3 (3, 3, 3)	2/3 (2, 1)	1/3 (1)
	Nil	3/3 (3, 3, 3)	3/3 (3, 3, 2)	3/3 (3, 3, 2)

*Treatment (0.5 mg kg^{-1}) subcutaneously daily from day 5 before tumour cell inoculation to the end of the study.
† $\frac{\text{No. animals with tumours}}{\text{No. animals inoculated}}$ (grade of tumour growth).
¶ Grade 1, tumour cells present only in subcapsular sinus; grade 2, tumour cells present in subcapsular and radial sinuses, but without destruction of the lymph node; grade 3, massive replacement of the lymph node by neoplasm.

Table 3 Effect of levamisole on HI antibody response to influenza A2/Aichi/68 vaccine in hamsters

Previously infected with A1/FM/1/47	A2/Aichi/68 vaccine given (150 CCA)	Levamisole* treatment (0.1 mg kg^{-1})	Serum HI antibody to (following immunisation with 150 CCA of A2/Aichi/68 vaccine) A1/FM/1/47 21 d post infection	A2/Aichi/68 14 d post immunisation	A2/Aichi/68 21 d post immunisation
+	+	—	6/6 (240-640)†	1/6 (15)	5/6 (15)
+	+	+	6/6 (160-640)	4/6 (15-60)	5/6 (10-120)
—	+	—	0/6	0/6	0/6
—	+	+	0/6	0/6	1/6 (15)

*0.1 mg kg^{-1} inoculated subcutaneously on day of immunisation, and subsequently every 48 h for 14 d.
† $\frac{\text{No. hamsters with HI antibody}}{\text{No. hamsters tested}}$ (range of HI antibody titres).

cell inoculation was 7/10, and for animals inoculated with the drug from the day of tumour cell inoculation the incidence was 9/10, 35 d after inoculation. The incidence of tumours in the two groups of hamsters treated with levamisole, when taken together, was significantly greater than that for the untreated animals ($\kappa^2 = 4.965$; $N = 1$; $P = <0.02$). Levamisole treatment therefore increased the incidence of transplanted adenovirus 12-induced tumours in hamsters.

In a similar way, the transplantability of a CELO virus-induced tumour of hamsters and a Moloney virus-induced lymphoma of BALB/c mice was investigated in animals treated with levamisole. The incidence of both these tumours was similar in treated and untreated animals. Thus, levamisole treatment did not decrease either tumour incidence or the size of tumours produced by transplanted CELO virus-induced tumour cells of hamsters, or Moloney virus-induced tumour cells of mice.

Groups of rats were inoculated into the foot pad with 5×10^6 RD3 cells; some of these animals were given levamisole (0.5 mg kg^{-1}) daily for 5 d before tumour cell inoculation, others from the day of tumour cell inoculation, and a further group were given levamisole from 14 d after tumour cell inoculation when the tumours were well established. The incidence of tumours at the site of inoculation, and the grade of metastatic tumour growth, is shown in Table 2. For animals treated with levamisole before or from the day of, tumour inoculation, the incidence of tumours in the foot pad and the extent of metastatic tumour growth in the popliteal or para-aortic lymph glands was similar for treated and untreated control rats (Table 2, Experiments 1 and 2). In the remaining test, where levamisole treatment was started 14 d after tumour cell inoculation, the degree of metastatic spread was less than that of untreated rats (Table 2; Experiment 3). This result, however, could not be repeated in further studies. The results indicate that levamisole did not significantly affect the incidence of primary or metastatic growth of RD3 tumour cells.

Two groups of hamsters were infected intranasally with influenza virus A1/FM/1/47 (H1N1). It has been shown in previous studies that hamsters do not produce haemagglutination inhibiting (HI) antibody in response to conventional doses of influenza virus A2/Aichi/68 vaccine unless previously infected with live influenza A virus[8,9]. Three weeks after virus infection, these hamsters, which all developed HI antibody to the infecting virus, together with two groups of normal hamsters, were inoculated intramuscularly with 150 CCA of inactivated influenza virus A2/Aichi/2/68 vaccine. One group of infected and one group of uninfected animals were also given 0.1 mg kg^{-1} levamisole subcutaneously on the day of immunisation, and subsequently every 48 h for 14 d. Serum specimens were collected from all hamsters at each stage of the experiment from the retro-orbital sinus, and tested for HI antibody. The results are shown in Table 3. None of the hamsters given vaccine only produced demonstrable serum HI antibody and one of the immunised animals given levamisole produced antibody (Table 3). For hamsters infected with influenza virus A1/FM/1/47 and later immunised, one animal

developed serum HI antibody detectable at 14 d after inoculation, and four developed antibody after 21 d. In contrast, for the six virus infected and immunised hamsters which were given levamisole, four developed serum HI antibody to the vaccine virus at 14 d after immunisation (geometric mean titre (GMT) 1:22.8) and one developed HI antibody at 21 d after immunisation (GMT = 1:34.6). Thus, in hamsters primed by previous infection with influenza virus A1/FM/1/47, levamisole promoted an earlier serum HI antibody response, and higher titres of antibody were detected.

The reaction of the host to experimental chemical or virus-induced tumours has been shown to be a cell-mediated immune reaction[10,11] and this cellular immune reaction has been specifically shown for the adenovirus 12-induced tumour of CBA mice and CELO virus-induced tumours of hamsters used in the present study (ref. 12 and R.C.R., and C.W.P., unpublished). In addition, factors which promote the cellular immune reaction of the host may result in an increased immunity to transplanted tumour cells[13-15]. Levamisole has been reported to increase the immune response of mice; the drug re-established the immune reactions of aged mice, and enhanced cell-mediated immune reaction. In addition, levamisole was reported to cause a significant reduction in primary tumour formation and metastases in mice inoculated with a transplantable lung tumour[3]. In our study, however, the effect of the drug on tumour development could not be extended to four further transplanted animal tumours. Levamisole, at the same concentration as used in the earlier study, had no effect on three transplantable tumours; and the effect on an adenovirus 12-induced tumour of CBA mice was to enhance tumour development.

Sera from CBA mice bearing transplanted adenovirus 12-induced tumours has been shown to contain a serum factor which promoted the development of transplanted tumours by interfering with cell-mediated immunity[12]. Blocking factor has been described for other tumour-bearing animals[16,17] and in serum from patients with cancer. It has been suggested that the blocking factor is a serum antibody[18] or antigen-antibody complex[19]. Thus, the promotion of humoral antibody may lead to the formation of blocking factors which would enhance tumour growth.

Our study shows that levamisole enhanced the serum HI antibody response of hamsters to A2/Aichi/68 vaccine, and lack of activity to this drug may be due to the stimulation of humoral antibody and the blocking of cell-mediated immunity. In contrast, levamisole was shown to reduce the incidence of Lewis 3a tumours of mice[3]. The effect of levamisole may vary for different tumour systems, therefore, as the antigenicity of tumours and the immune response to different tumours is not identical.

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1 Corba, J., Scales, B., and Froyd, G., *Trop. Anim. Hlth Prod.*, **1**, 19 (1969).
2 Weisbroth, S. H., and Scher, S., *Lab. Anim. Sci.*, **21**, 213 (1971).
3 Renoux, G., and Renoux, M., *Nature new Biol.*, **240**, 217 (1972).
4 Potter, C. W., and Oxford, J. S., *Int. J. Cancer*, **6**, 410 (1970).
5 Schild, G. C., Oxford, J. S., and Potter, C. W., *Arch. ges. Virusforsch.*, **29**, 25 (1970).
6 Glynn, J. P., Halpern, B. L., and Fefer, A., *Cancer Res.*, **29**, 515 (1969).
7 Carr, I., and McGinty, F., *J. Path.*, (in the press).
8 Potter, C. W., Jennings, R., Marine, W. M., and McLaren, C., *Microbios*, **8**, 101 (1973).
9 Jennings, R., and Potter, C. W., *Arch. ges. Virusforsch.*, **42**, 197 (1973).
10 Klein, G., *Cancer Res.*, **28**, 625 (1968).
11 Allison, A. C., *Ann. Inst. Pasteur*, **122**, 619 (1972).
12 Rees, R. C., and Potter, C. W., *Eur. J. Cancer*, **9**, 497 (1973).
13 Zbar, B., Bernstein, I., Tanaka, T., and Rapp, H. J. *Science*, **170**, 1217 (1970).
14 Lemonde, P., Dubrevil, R., Guindon, A., and Lussier, G., *J. natn. Cancer Inst.*, **47**, 1013 (1971).
15 Ankerst, J., and Johssen, N., *Int. J. Cancer*, **10**, 351 (1972).
16 Hellstrom, I., and Sjorgren, H. O., *J. exp. Med.*, **125**, 1105 (1967).
17 Ankerst, J., *Cancer Res.*, **31**, 997 (1971).
18 Hellstrom, K. E., and Hellstrom, I., *Cancer*, **28**, 1266 (1971).
19 Baldwin, R. W., Price, M. R., and Robins, R. A., *Nature*, **238**, 185 (1972).

Embryo-derived teratocarcinomas elicit splenomegaly in syngeneic host

MOUSE egg-cylinders transplanted to extrauterine sites develop either as teratomas (benign tumours composed of adult tissues) or as teratocarcinomas (retransplantable malignant tumours containing, in addition, undifferentiated embryonic carcinoma cells)[1-4]. Rat egg-cylinders similarly transplanted, however, always develop as teratoma[5]. When transplanted to a syngeneic host, C57BL mouse egg-cylinders develop as teratomas but when transplanted to (C3H × C57BL)F_1 hybrids they develop into teratocarcinoma[3,4]. This suggests that the host reaction is important in determining the fate of a transplanted embryo. Specific embryonic and foetal antigens which are re-expressed during malignant transformation are now well established[6-8], and we thought that an immunological reaction of the host against embryonic antigens in the egg-cylinder might determine whether the transplant develops as teratoma or teratocarcinoma. Splenomegaly is considered to be a sign that a host has reacted to the antigenic stimulation of a graft or tumour[9,10], and we have found that although it is always present in mice with transplanted egg-cylinders, it is much more pronounced in those with teratocarcinoma. Splenectomy, however, reduced the growth of the transplanted embryos, suggesting that the immunological reaction of the host stimulates the growth of the graft.

Seven-day-old mouse egg-cylinders (the day when the vaginal plug was found was considered day 0) were isolated from uterine swellings of pregnant C3H/H mice and transplanted under the kidney capsules or into the testes of 6-8-week-old syngeneic or (C57BL × C3H/H)F_1 hybrid recipients as before[1,2]. To exclude reaction to antigens linked to the Y chromosome[11], and presumably present in half of the transplanted egg-cylinders, all recipients were males. Animals were killed two months later. Tumours grown from the embryos and the spleen of each tumour-bearing animal were weighed and examined histologically. Male animals of the same age kept under identical conditions served as controls for the weight of the spleen. Some of the C3H/H animals were splenectomised at the time of grafting.

As Table 1 shows, the weight of the tumours obtained from under the kidney capsule differed significantly from those obtained by grafting the embryo into the testis. Tumours from embryos grafted to hybrid recipients weighed significantly more than those in syngeneic hosts. Tumours from embryos grafted into syngeneic hosts which were splenectomised at the time of transplantation were much smaller and weighed significantly less than tumours

Table 1 Weight of tumours grafted to the testis and under the kidney capsule

Location	Strain of recipient: C3H/H	(C3H/H × C57BL) F_1 hybrid	C3H/H splenectomised
Kidney	3,212 ± 689 (31)	4,130 ± 805 (29)	1,139 ± 449 (21)
Testis	2,149 ± 529 (39)	—	—

All tumours were obtained from grafts of 7-d-old C3H/H mouse egg-cylinders. Weight is given in mg ± s.e. When the *t*-test was applied, values for each group differed significantly from all others ($P < 0.01$). The number of animals in each group is given in parentheses.

in untreated syngeneic hosts, irrespective of the site of transplantation.

All grafts induced some increase in the weight of the host's spleen (Table 2). Embryo-derived teratoid tumours were classified on a histological basis into benign teratomas and teratocarcinomas. Spleens were significantly heavier in animals bearing teratocarcinomas than those bearing tertomas. Tumours obtained from under the kidney capsule induced significantly more splenomegaly than those in the testis. The difference in the weight of spleens is not only statistically significant when all tumours in the kidney are compared with tumours in the testes, but also when teratomas are compared with teratomas and teratocarcinomas are compared with teratocarcinomas in the two locations. In spite of the larger size of tumours obtained under the kidney capsule in hybrid hosts compared with those in syngeneic hosts (Table 1), there was no difference in the weight of spleens in the two groups (Table 2).

Table 2 Weight of spleens in mice bearing embryo-derived teratomas and teracarcinomas

Location	Strain of receipient: C3H/H		(C3H/H × C57BL) F_1 hybrid	
	Teratoma	Terato-carcinoma	Teratoma	Terato-carcinoma
Kidney	194 ± 30 (16)	374 ± 37 (15)	162 ± 37 (13)	353 ± 37 (16)
Testis	132 ± 3 (23)	276 ± 23 (16)	—	—
Control	117 ± 2.45 (18)		122 ± 3.22 (15)	

Values are given in mg ± s.e. The *t*-test was used to compare means. All values differed significantly from the controls ($P < 0.01$) as well as among themselves ($P < 0.01$) except for the weight of spleens in C3H/H and F_1 hybrid mice grafted with embryos under the kidney capsule. All tumours were derived from C3H/H egg-cylinders. The number of animals in each group is given in parentheses. Tumours containing undifferentiated embryonic cells (embryonic carcinoma cells) were classified as teratocarcinomas. Spleens from normal untreated males served as controls.

Our data indicate that the weight of the spleen increases in all animals bearing grafted embryos. This splenomegaly suggests an immune type of reaction. Recent results have shown a significant increase in the total number of spleen cells, the number of lymphoid subpopulations and in T and B cell functions in tumour-bearing animals[12]. Since only the largest grafts contained necrotic tissue, splenomegaly resulting from reaction to tissue destruction could be excluded with a high degree of certainty. Histologically, the appearance of these spleens corresponds to the descriptions of graft-induced splenomegaly[9,10].

We found heavier spleens in animals with teratocarcinomas than in those with small, nonproliferating teratomas (Table 2). There are at least two possible explanations for this: one is that there is more tissue for antigenic stimulation available in the large teratocarcinomas than in the usually much smaller teratomas; second, since teratocarcinomas contain foci of undifferentiated embryonic cells, these cells are developmentally and antigenically different from the host, and therefore may exert a stronger antigenic stimulation than the cells in the teratomas.

When transplanted, all grafts contain undifferentiated embryonic cells[13], but these disappear from teratomas and persist only in teratocarcinomas. We suggest that the slight increase in the weight of the spleen of the teratoma-bearing animal represents a reaction to the presence of undifferentiated embryonic cells during the first 15 d after grafting of the egg-cylinder. Only the persistence of embryonic undifferentiated cells in the teratocarcinomas, however, could induce a three-fold or even more pronounced increase in the weight of the host's spleen.

Whether the size of testicular tumours, which differs as Table 1 shows, is determined by some anatomical factor (blood supply, lower temperatures due to the scrotal location, or tenseness of the tunica albuginea) or is related to a possibly more or less immunologically privileged site for the growth of embryonic tissues remains to be determined.

The greater weight of tumours obtained in hybrids at the same site in syngeneic hosts may be explained by minor antigenic differences between host and graft which provoked a stimulatory immune reaction[14]. The reduction in weight of embryo-derived tumours caused by splenectomy at the time of grafting might be explained if blocking antibodies prevent tumour-invoked cell-mediated immunity in intact mice. In splenectomised animals such antibodies could be synthesised in much smaller quantities, leaving embryonic carcinoma cells exposed to the cellular immune response. Observations[8] that stimulation with foetal cells protects splenectomised hamsters much better than intact hamsters against SV40-induced tumours could be explained by similar mechanisms. Enhancing antibodies[15], secreted by the spleen, and thus not present in splenectomised animals, could also explain the smaller tumour growth. Our finding[3,4] that about half of the transplanted embryos develop as teratocarcinomas and half as teratomas might mean that small changes in the host's immune response could swing development either way. Tumour cells probably produce many different classes of antibody, so that the outcome of tumour-immune system interaction would depend on many functions not easily controlled during an experiment. We are now trying to characterise the humoral and cellular response elicited by embryo-derived tumour, and to discover how different components of immune response might regulate the development of transplanted embryo.

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1 Solter, D., Škreb, N., and Damjanov, I., *Nature*, **227**, 503 (1970).
2 Damjanov, I., Solter, D., Belicza, M., and Škrep, N., *J. natn. Cancer Inst.*, **46**, 471 (1971).
3 Škreb, N., Damjanov, I., and Solter, D., in *Cell differentiation* (edit. by Harris R., Alin, P. and Viza, D.) 151 (Munksgaard, Copenhagen, 1972).
4 Damjanov, I., and Solter, D., *Z. Krebsforsch*, **81**, 63 (1974).

[5] Škreb, N., Švajger, A., and Levak-Švajger, B., *J. embryol. exp. Morph.*, **25**, 47 (1971).
[6] Anderson, N. G., and Coggin, jun., J. H., in *Proc. first Conf. and Workshop on embryonic and fetal antigens in cancer* (edit. by Anderson, N. G., and Coggin, J. H., jun.), 7 (National Technical Information Service, US Department of Commerce, Springfield, Virginia, 1971).
[7] Alexander, P., *Nature*, **235**, 137 (1972).
[8] Coggin, jun., J. H., and Anderson, N. G., in *Embryonic and fetal antigens in cancer* (edit. by Anderson, N. G., Coggin, J. H., jun., Cole, E., and Holleman, J. W.), **2**, 91 (National Technical Information Service, US Department of Commerce, Springfield, Virginia, 1972).
[9] Woodruff, M. F. A., and Symes, M. O., *Br. J. Cancer*, **16**, 120 (1962).
[10] Blamey, R. W., and Evans, D. M. D., *Br. J. Cancer*, **25**, 527 (1971).
[11] Eichwald, E. J., and Silmser, C. R., *Transplant. Bull.*, **2**, 148 (1955).
[12] Kondå, S., Nakao, Y., and Smith, R. T., *Cancer Res.*, **33**, 2247 (1973).
[13] Stevens, L. C., *Devl Biol.*, **21**, 364 (1970).
[14] Prehn, R. T., *Science*, **176**, 170 (1972).
[15] Hellström, K. E., Hellström, I., and Braun, J., *Nature*, **224**, 914 (1969).

Immunisation of marmoset monkeys with a killed oncogenic herpesvirus

HERPESVIRUSES are suspected to be causal agents in human malignant neoplasia. At the present level of knowledge, clinical trials in man with viral vaccines against oncogenic herpesviruses cannot be initiated, but the oncogenic herpesviruses derived from monkeys[1,2] can be used *in vivo* to study the problems of herpesvirus cancer vaccines. The oncogenic herpesvirus saimiri (HVS) regularly induces malignant lymphoma in cotton topped (CT) marmoset monkeys (*S. oedipus*). A killed vaccine from HVS was developed in our laboratory and tested for safety and efficacy in this primate model system.

For the production of the killed vaccine HVS isolate No. 810 (ref. 1) was cultivated on owl monkey kidney (OMK) cells[3].

Table 1 Immunisation of six CT marmoset monkeys with killed HVS

Animal	Time post vaccination (d)	Infectivity titre of whole blood ($TCID_{50}$ ml^{-1})*	Serum antibodies against HVS NI†	CF‡
174 A	267§	$< 10^{0.0}$	> 3.3	1: 8
175 A	253§	$< 10^{0.0}$	2.5	¶
191 A	211§	$< 10^{0.0}$	3.3	¶
214	122§	$< 10^{0.0}$	> 3.0	1:64
215	122§	$< 10^{0.0}$	3.0	1:64
216	122§	$< 10^{0.0}$	> 3.0	¶

* Determined in OMK cells.
† Neutralisation indices.
‡ Titres of HVS specific complement fixing antibodies.
§ Monkey still alive.
¶ Sera had anticomplementary activity.

Three days before collection, when the cytopathic effect of HVS appeared, the OMK cell cultures were washed and fed with fresh medium, free of serum. The stock virus, stored at 4° C, was centrifuged twice at 2,000*g* for 10 min and the clarified fluid containing virus was passed through Millipore membrane filters (450 μm). The filtrate was sonicated for 10 s (Branson Sonifier B-12, micro tip, maximal output). The infectivity titre of this preparation, measured in OMK cells, was $10^{5.0}$–$10^{5.5}$ $TCID_{50}$ ml^{-1}. The virus was distributed into 100 ml glass vials and heated at 56° C for 4 h. This heat-treatment completely destroyed the infectivity which was tested in OMK cell cultures. The heat inactivation did not significantly reduce the titre of the HVS specific complement fixing (CF) antigens. CF titres were determined according to standard techniques[4] using an HVS antiserum from an owl monkey[3]. In addition to the heat treatment the virus preparation was incubated with formaldehyde (100 μg HCHO ml^{-1}, *p*H adjusted to 6.5 at 37° C). The formaldehyde treatment was stopped after 6 d by neutralisation with equimolar sodium-disulphite ($Na_2S_2O_5$). The formaldehyde had inactivated 50–75% of the HVS specific CF antigens. The resulting suspension was concentrated 100-fold by dialysis against Aquacide (Calbiochem, Los Angeles). The concentrate was centrifuged at 12,000*g* for 10 min at 4° C (B 20 centrifuge, International Equipment, Boston) and the supernatant was stored at −60° C and used for vaccination. From each vaccine preparation (10 ml), 30% were tested for cytopathogenicity in OMK cells. No CPE appeared in these cultures during an observation period of 36 d.

In contrast to previously reported findings[5] small amounts of infectious HVS particles were found in our laboratory when the heat treatment of HVS at 56° C was stopped after 30 min. The survival curve of HVS is multicomponential after treatment with heat as well as after treatment with formaldehyde. For vaccine production this problem could be solved by prolonged incubation periods.

The killed vaccines were tested for infectivity, oncogenicity and antigenicity in CT marmoset monkeys. A distinction has to be drawn between infectivity and oncogenicity of herpesviruses since killed herpesvirus hominis induced neoplastic transformation *in vitro* without productive infection[6,7]. After being absorbed with Aluminiumhydroxygel (10% v/v of a 3% solution, Behringwerke, Marburg, West Germany) as adjuvant, nine CT marmosets were immunised. Four or five intramuscular inoculations of 1.0 ml each of the killed HVS concentrate were given to each animal within 4–6 weeks. The killed HVS vaccine in all these monkeys induced high titres of neutralising and CF antibodies against HVS (Table 1). The neutralisation tests were performed in OMK cell cultures with a constant serum dilution (1:5) and varying ten-fold virus dilution according to standard techniques[4]. The serum antibodies appeared about 3 weeks after the first inoculation and reached titres as high as those found in the sera of the three non-immunised control monkeys, who developed malignant lymphoma after inoculation of infectious HVS (Table 2). In contrast to the tumour-bearing monkeys (Table 2), HVS could

Table 2 Challenge of three immunised CT marmoset monkeys and infection of three control animals with $10^{5.5}$ $TCID_{50}$ of live HVS

Animal	Time interval between immunisation and challenge (d)	Survival time after inoculation of HVS (d)	Infectivity titre of whole blood ($TCID_{50}$ ml^{-1})*	Serum antibodies against HVS NI†	CF‡
174 B§	53	61¶	$< 10^{0.0}$	> 3.5	1: 8
175 B§	60	64**	$10^{5.5}$	> 3.5	1: 16
191 B§	56	94**	$10^{6.5}$	3.5	1:128
184	Not immunised	33**	$> 10^{5.5}$	3.0	
185	Not immunised	30**	$> 10^{5.5}$	> 3.3	1: 64
186	Not immunised	28**	$10^{5.0}$	3.0	

* Determined in OMK cells at the time of death.
† Neutralisation indices at the time of death.
‡ Titres of HVS specific complement fixing antibodies at the time of death.
§ Animals immunised in the same way as animals 174 A, 175 A and 191 A (Table 1).
¶ Died without signs of a tumour.
** Died of malignant lymphoma.

not be isolated from the immunised animals by cocultivation of fresh peripheral blood with OMK cells (Table 1). The vaccines proved not only to be free of infectious HVS, but also to be non-oncogenic. All of the immunised monkeys remained clinically well; two of them (174 A and 175 A) have been now under observation for 9 months (Table 1).

The immunised monkeys 174 B, 175 B and 191 B were challenged with $10^{3.8}$ $TCID_{50}$ of cell-free HVS (passed through a 450 μm Millipore filter) by intramuscular inoculation. Monkey 174 B died 61 d after infection, of an accident, without signs of malignant lymphoma. Animals 175 B and 191 B died 64 and 94 d post infection of a tumour which was characteristic for HVS in gross pathology and histopathology (Table 2). Three control monkeys who received the same HVS inoculation had already died 28, 30 and 33 d post infection because of malignant lymphoma (Table 2). This result indicates that the immunisation delayed the development of malignancy. Experiments are in progress to examine whether CT marmoset monkeys, actively immunised against HVS, are resistant to challenge with smaller doses of HVS than the very high challenge dosage already examined here.

These findings clearly demonstrate that a killed vaccine from oncogenic HVS can be prepared which is safe in respect to infectivity and oncogenicity. The immunisation of CT marmoset monkeys with the killed vaccine induced high titres of HVS specific serum antibodies and delayed tumour development after challenge with a very large dose of infectious HVS. The recent demonstration of induction of lymphomas in CT marmoset monkeys[8] and an owl monkey[9] by EB virus may aid in the extrapolation of our data in regard to the wanted vaccine against Burkitt's lymphoma in man.

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1 Meléndez, L. V., Hunt, R. D., Daniel, M. D., Garcia, F. G., and Fraser, C. E. O., *Lab. Anim. Care*, **19**, 378 (1969).
2 Laufs, R., and Meléndez, L. V., *J. natn. Cancer Inst.*, **51**, 599 (1973).
3 Laufs, R., and Meléndez, L. V., *Med. Microbiol. Immun.*, **158**, 299 (1973).
4 Lennette, E. H., and Schmidt, N. J., *Diagnostic Procedures for Viral and Rickettsial Diseases* (American Public Health Association, New York, 1964).
5 Ablashi, D. V., Loeb, W. F., Pearson, G., Valerio, M. G., Armstrong, G. R., Rabin, H., Kingsbury, E. W., and Heine, U., *Nature new Biol.*, **242**, 28 (1973).
6 Duff, R., and Rapp, F., *J. Virol.*, **8**, 469 (1971).
7 Rapp, F., Li, J. H., and Jerkofsky, M., *Virology*, **55**, 339 (1973).
8 Shope, T., Dechairo, D., and Miller, G., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2487 (1973).
9 Epstein, M. A., Hunt, R. D., and Rabin, H., *Int. J. Cancer*, **12**, 309 (1973).

Involvement of IgE in con A-induced histamine release from human basophils *in vitro*

CONCANAVALIN A (con A) induces the release of histamine from rat[1] and hamster mast cells[2] and human basophils. Time course, cationic requirements, enzymatic sequences and dose-response seem to be the same for con A-induced release as for antigen-induced release, which is mediated by reaginic antibody (reagin), believed to be bound by its Fc portion to a receptor in the membrane of the histamine-releasing cell. Human reagin is an antibody of the IgE class[3]. Antibodies[4] specific for IgE can induce histamine release whereas the papain digest[5] of those antibodies (which produces monomer anti-IgE) fails to elicit a response and can inhibit the intact anti-IgE-induced release[6]. The requirement for bridging to initiate histamine release has been established[7–13]. The types of sugar moieties to which con A binds have been studied[14–17], and the physicochemical properties of con A[15,18–22] indicate that at physiological *p*H it can bridge two or more of those sugar moieties. The question is raised as to whether con A is bridging sugar moieties at the receptor for reagin, or whether it is bridging sugar moieties which are directly attached to the reagin. The nature of the receptor remains to be established and the observation that con A can induce histamine release raised expectations that this interaction could elucidate some of the properties of this receptor.

The premise that con A is interacting with a receptor has been weakened by the report[1] that mast cells from rats immunised to produce high reaginic antibody released to con A whereas mast cells from nonimmunised rats did not. I report here that con A release from human basophils involves IgE. Antibodies specific for the Fc portion of IgE (Pharmacia) and antibodies specific for the Fab portion of human immunoglobulins (Behring) were used. They were papain-digested to a monomer form, and lost the ability to induce histamine release but not the ability to bind the IgE. The data show that release induced by con A (Pharmacia) can be inhibited by Fc-specific monomer anti-IgE but not by monomer anti-Fab (Fig. 1). This indicates that con A-induced release in human basophils is mediated by the Fc, but not the Fab, portion of the membrane-bound IgE. The fact that Fc-specific monomer anti-IgE-inhibited release is not conclusive evidence that con A is not bridging sugar moieties at the receptor for IgE. If the IgE receptor has sugar groups close to the Fc portion of IgE, then a steric hindrance of the con A by the monomer is a possibility. However, human basophils can be passively sensitised[23], which implies that not all the receptors for IgE are occupied. It is not likely that the monomer would sterically hinder the binding of con A to an unoccupied receptor. Since inhibition by the monomer is nearly complete, it indicates that either the receptor has no sugar moieties to which con A binds, or that the receptor must have an IgE molecule in place before an activation of the basophil can be mediated by it.

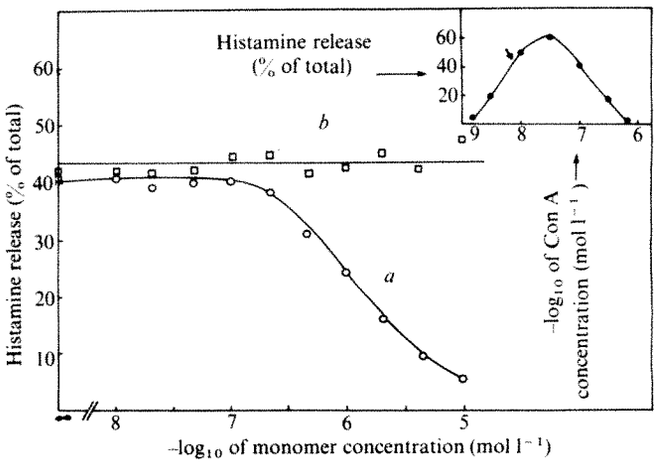


Fig. 1 Histamine release from the basophils of a nonallergic individual (A.A.). Cells were obtained by sedimentation of whole blood. The reaction was carried out at 37° C for 50 min in a Tris-buffered solution (*p*H 7.4) containing 0.03% human albumin, 1×10^{-3} M Ca^{2+}, 1×10^{-3} M Mg^{2+} and 2×10^{6} leukocytes per ml corresponding to 20–30 ng histamine base per ml (ref. 24). Supernatants were assayed for histamine by a spectrofluorometric technique[25,26]. Blanks were less than 5% of the total histamine content. In estimating the molar concentrations, 71,000 daltons[18] was assumed for con A, and 50,000 daltons[5] for the monomer preparations. *Inset*, histamine release induced by con A. The arrow on the dose-response curve indicates the concentration of con A (6×10^{-9} M) used to obtain the data in the other two curves. ○, The effect of increasing concentrations of monomer Fc specific anti-IgE (abscissa) on the release of a fixed amount of con A (6×10^{-9} M). □, The effect of increasing concentrations of monomer anti-Fab (abscissa) upon the release of a fixed amount of con A (6×10^{-9} M).

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1 Keller, R., *Clin. exp. Immun.*, **13**, 139 (1973).
2 Hook, W. A., Dougherty, S. F., Oppenheim, J. J., *Fedn. Proc. Abs.*, **33**, 1000 (1973).
3 Ishizaka, T., Ishizaka, K., Johansson, S. G. O., and Bennich, H., *J. Immun.*, **102**, 884 (1969).
4 Johansson S. G. O., and Bennich, H., *Immunology*, **13**, 381 (1967).
5 Porter, R. R., *Biochem. J.*, **73**, 119 (1959).
6 Ishizaka, K., and Ishizaka, T., *J. Immun.*, **103**, 588 (1969).
7 Landsteiner, K., *J. exp. Med.*, **39**, 631 (1924).
8 Tillett, W. S., Avery, O. T., and Geobel, W. F., *J. exp. Med.*, **50**, 551 (1929).
9 Landsteiner, K., and Levine, P., *J. exp. Med.*, **52**, 347 (1930).
10 Landsteiner, K., and Van der Scher, J., *J. exp. Med.*, **56**, 399 (1932).
11 Overy, Z., *C. r. hebd. Séane. Acad. Sci.*, **253**, 582 (1961).
12 Levine, B. B., *J. Immun.*, **94**, 111 (1965).
13 Magro, A., and Alexander, A., *J. Immun.* (in the press).
14 Sumner, J. B., and O'Kane, D. J., *Enzymologia*, **12**, 251 (1948).
15 Goldstein, I. J., Hollerman, C. E., and Merrick, J. M., *Biochem. Biophys. Acta*, **97**, 68 (1965).
16 Goldstein, I. J., Hollerman, C. E., and Smith, E. E., *Biochemistry*, **4**, 876 (1965).
17 Portez, R. D., and Goldstein, I. J., *Biochemistry*, **9**, 2890 (1970).
18 Olson, M. O. J., and Liener, I. E., *Biochemistry*, **6**, 105 (1971).
19 Olson, M. O. J., and Liener, I. E., *Biochemistry*, **6**, 3801 (1967).
20 Agrawal, B. B. L., and Goldstein, I. J., *Archs Biochem. Biophys.*, **124**, 218 (1968).
21 McCubbin, W. B., and Kay, C. M., *Biochem. biophys. Res. Commun.*, **44**, 101 (1971).
22 Becker, J. W., Reeke, G. N., and Edelman, G. M., *J. biol. Chem.*, **246**, 6123 (1971).
23 Levy, D. A., and Osler, A. G., *J. Immun.*, **97**, 203 (1966).
24 Lichtenstein, L. M., and Osler, A. G., *J. exp. Med.*, **120**, 507 (1964).
25 Siraganian, R. P., *Analyt. Biochem.*, **57**, 383 (1974).
26 Shore, P. A., Burkhalter, A., and Cohn, jun., V. H., *J. Pharmac. exp Ther.*, **127**, 182 (1959).

Possible role for the Fc receptor on B lymphocytes

B LYMPHOCYTES[1], polymorphonuclear cells[2], mast cells[3] and cells of the macrophage–monocyte[2] series possess surface receptors for the Fc portion of the immunoglobulin molecule. In some cases the functions of this receptor is clearly understood but this is not true for B lymphocytes. It has been suggested that the Fc receptor on B cells plays a role in the localisation of antigen in the form of antigen–antibody complexes[4]. We propose an alternative hypothesis that the Fc receptor on B cells is a membrane receptor (termed the proreceptor) for the immunoglobulin which functions as antigen receptor on these cells and present some evidence compatible with this hypothesis.

The plasma cell tumour MOPC 21 (P3) which secretes IgG1 kappa molecules in tissue culture[5] has surface immunoglobulins but lacks Fc receptors[6]. Mutants which secrete altered immunoglobulin molecules or no immunoglobulin can be obtained[7,8], and the nonsecretors lack surface immunoglobulin[6]. According to our hypothesis the apparent absence of Fc receptors on P3 cells would be due to the occupation of Fc receptors by surface immunoglobulin. If this was the case it would be expected that nonsecretors lacking surface immunoglobulin would show the presence of Fc receptors. The results of our investigations on several cell lines are summarised in Table 1.

This shows that in accordance with the hypothesis there is in general an inverse correlation between the presence of surface immunoglobulin and Fc receptors on the cell lines. However there is a variation in the intensity of the Fc receptor reaction on the different nonsecretor lines with X60 showing no reaction. The mutations leading to nonsecretion are unlikely to be simple and could also affect other aspects of cell function. The variation in the Fc receptor reaction could be a consequence of this. The cell lines IF1 and IF2 are more relevant as the mutations are likely to be more restricted to the structure of the immunoglobulin molecules. The weaker reaction on IF1 for surface immunoglobulin is accompanied by the appearance of Fc receptors. IF2 shows a weak Fc receptor reaction but there is no detectable decrease in surface immunoglobulin. This indicates that the C_H3 region is important in the binding of IgG to the Fc receptors. This was confirmed by using purified protein to inhibit Fc rosette formation. In the experimental conditions used, the wild type and IF2 protein are inhibitory while IF1 protein is not (Table 2).

Table 1 Properties of the cell lines

Cell line	Secreted molecules	Fc receptors	s Ig
P3	IgG	—	+ +
X27	IgG	—	+ +
X63	IgG	—	+ +
IF2	IgG with C_H1 region deleted	+	+ +
IF1	IgG with C_H3 region deleted	+ +	+
D1-20	Nonsecretor	+	—
Pr4	Nonsecretor	+	—
289-16	Nonsecretor	+ + +	—
X60	Nonsecretor	—	—

Surface immunoglobulin was detected with a fluorescein-conjugated IgG fraction of a sheep anti-mouse Fab and a rabbit anti-mouse IgG antiserum[6]. Fc receptors were detected by a rosette technique using bovine erythrocytes coated with mouse antibody as indicator cells[9–11]. Tumour cells were grown in RPMI 1640 medium (Flow Laboratories, Scotland) containing 15% foetal calf serum. X27 was a cloned cell line derived from a P3 culture. X63, IF1, Pr4 and 289-16 were cloned from X27 cells, while X60, D1-20 and IF2 were isolated from a P3 culture.

Because of variability from test to test, a four point scale is used to describe the Fc receptor reaction on different lines. + + + denotes in general a reaction on greater than 50% of live cells from a culture; + + a reaction on 10–50%; + a reaction on up to 10% of cells while negative is not significantly above controls with unsensitised indicator cells. The surface immunoglobulin staining on the secretor cells is weaker than on B lymphocytes and a two point scale is used to describe the intensity of staining.

The relation between surface immunoglobulin and Fc receptors found on the cell lines is consistent with our hypothesis provided that the surface immunoglobulin on secretor cells is not secreted immunoglobulin passively absorbed from the culture medium. Experiments where 289–16 cells were incubated with P3 IgG and then washed and stained with the rabbit anti-globulin showed that immunoglobulin could be passively absorbed. Taking advantage of the observation that rabbit IgG is recognised by mouse Fc receptors[4] we find that the presence of 10% rabbit serum can prevent absorbtion of mouse IgG from medium containing 50 mg ml^{-1} IgG. In contrast, the surface immunoglobulin on secretor cells is not lost from cells grown in 10% rabbit serum for up to 7 d. This shows that the surface immunoglobulin on the secretor cells is not passively absorbed.

The majority of B lymphocytes in mouse possess monomeric IgM receptors[9–11] but secreted IgM is found to only weakly inhibit Fc rosettes on B cells[1,12]. One explanation is that receptor IgM may have a higher affinity for Fc receptors than secreted IgM. This possibility is being investigated at present.

Reports in the literature indicate a large number of murine leukaemias of non-T-cell origin which possess Fc

Table 2 Inhibition of Fc rosettes on 289-16 cells with P3, IF1, and IF2 proteins

Inhibitor	% reacting cells ±s.e.	% inhibition
Nil	24.0 ± 4.2	—
P3(580 μg ml^{-1})	5.8 ± 1.7	75.5 $P < 0.05$
IF1(580 μg ml^{-1})	5.8 ± 1.7	1.7 (P not significant)
IF2(580 μg ml^{-1})	6.4 ± 3.5	73.4 $P < 0.05$

receptors but no surface immunoglobulin[6,14]. A light chain secreting variant of a plasmacytoma is also found to be Fc receptor positive[1,12]. In patients with X-linked agammaglobulinaemia cells are found which lack surface immunoglobulin and possess Fc receptors[15,16]. We suggest that these may also be cases where the Fc receptors are exposed in the absence of immunoglobulin.

Finally we would like to suggest that in view of the observations that antigen–antibody complexes can induce nonspecific transformation in lymphocytes[17] it is possible that the specific activation of B cells by antigen is mediated through proreceptors.

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1 Basten, A., Miller, J. F. A. P., and Sprent, J., *J. exp. Med.*, **135**, 610–624 (1972).
2 Lay, W. M., and Nussensweig, V., *J. exp. Med.*, **128**, 991–1007 (1968).
3 Bloch, K. J., *Prog. Allergy*, **10**, 84–150 (1967).
4 Paraskevas, F., Lee, S. J., Anderson, E. J., Orr, K. B., and Israels, L. G., *J. Immun.*, **108**, 1729–1732 (1972).
5 Horibata, K., and Harris, A. W., *Expl Cell. Res.*, **60**, 61–77 (1970).
6 Ramasamy, R., and Munro, A. J., *Immunology*, **26**, 563–570 (1974).
7 Cotton, R. G. H., Secher, D. S., and Milstein, C., *Eur. J. Immun.* **3**, 135–140 (1973).
8 Secher, D. S., Cotton, R. G. H., and Milstein, C., *FEBS lett.*, **37**, 311–316 (1973).
9 Hammerling V., and Rajewsky, K., *Eur. J. Immun.*, **1**, 447–452 (1971).
10 Marchalonis, J. J., Cone, R. E., and Atwell, J. L., *J. exp. Med.*, **135**, 956–971 (1972).
11 Vitteta, E. S., Bianco, C., Nussensweig, V., and Uhr, J. W., *J. exp. Med.*, **136**, 81–93 (1971).
12 Cline, M. J., Sprent, J., Warner, N. L., and Harris, A. W., *J. Immun.*, **108**, 1126–1131 (1972).
13 Hallberg, T., Gurner, B. W., and Coombs, R. R. A., *Int. Arch. Allergy*, **44**, 500–513 (1973).
14 Shevach, E. M., Jaffe, E. S., and Green, I., *Transpln Rev.*, **16**, 3–28 (1973).
15 Froland, S. S., and Natvig, J. B., *Transpln Rev.*, **16**, 114–162 (1973).
16 Bentwich, Z., and Kunkel, H. G., *Transpln Rev.*, **16**, 29–50 (1973).
17 Moller, G., *Clin. exp. Immun.*, **4**, 65–82 (1969).

Con A differentiates among grass pollens by binding specifically to wall glycoproteins and carbohydrates

AIRBORNE grass pollens are a major cause of hayfever. The allergens involved are mostly proteins and glycoproteins[1,2], which are stored in pollen walls[3] and rapidly released on moistening and which seem to play a recognition role in pollen/stigma interactions[4]. The capacity of certain plant lectins to bind specific carbohydrates and glycoproteins[5] and form precipitates with them in double diffusion tests[6,7] indicates a means of investigating these substances in pollen. We report here how concanavalin A (con A), the lectin from jack beans, *Canavalia ensiformis*, differentiates among grasses by specifically precipitating wall-held materials from pollens mainly of nonfestucoid species. Furthermore, pollen of corn (*Zea mays*) and its closest relatives is easily distinguishable from that of other grasses by its characteristic reactions with con A.

We have used double diffusion tests to screen grass pollen from 42 species (33 genera, including many known to be important sources of allergens) for materials precipitating with con A. Extracts from 9/42 species have given clear precipitation lines against con A (Table 1). The contrast between festucoids (1/23 reacting clearly) and nonfestucoids (8/19) is marked. In addition, the reacting andropogonoids and *Cynodon* give denser precipitates than the rest, while Maydeae alone (*Zea, Euchlaena, Tripsacum*) produce two definite bands against con A (Fig. 1*c*). The relatively faint precipitates with *Festuca, Phragmites, Stipa* and *Echinochloa* are consistently reproducible (Fig. 1*a* and *b*). When diffusates of the positively-reacting pollens of *Cynodon, Sorghum, Zea* and *Festuca* and of the negatively-reacting *Lolium perenne* were concentrated by ultrafiltration and partially purified by Sephadex G25 chromatography, *Lolium* continued to give a negative result, *Cynodon* now gave no reaction while the two andropogonoids and *Festuca* precipitated con A as before. The *Cynodon* reaction probably involves carbohydrate diffusates rather than glycoproteins, and other faintly-precipitating species (where shortage of pollen has precluded more detailed tests) may fall into the same category. A high molecular weight *Zea* fraction (>10,000 daltons) shows two intense precipitation bands (Fig. 1*d*), there being only slight precipitation in a lower molecular weight fraction probably containing carbohydrates.

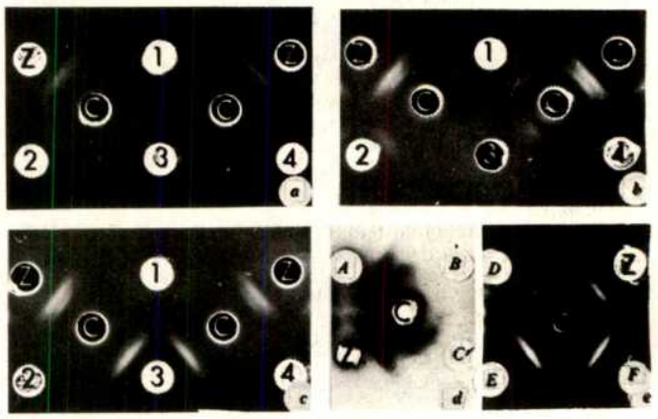


Fig. 1 Double diffusion tests of grass pollen extracts (outer wells) against con A (inner wells). Extracts prepared as in Table 1. *a*, *Festucoids*: 1, *Lolium multiflorum;* 2, *Festuca rubra;* 3, *Poa annua;* 4, *Bromus inermis. b*, Nonfestucoids: 1, *Phragmites australis;* 2, *Cynodon dactylon;* 3, *Stipa falcata;* 4, *Danthonia eriantha. c*, Nonfestucoids: 1, *Sorghum halapense;* 2, *Coix lacrymajobi;* 3, *Euchlaena mexicana;* 4, *Setaria geniculata.* Arrows indicate faint precipitin lines. *d*, Reactions of con A to various *Zea* diffusate fractions. After Sephadex G25 gel filtration, *A*, a high molecular weight fraction (>5,000 daltons) shows two clear bands; *B*, a low molecular weight fraction (<5,000 daltons) shows no precipitation band; *C'*, a fraction from ultrafiltration with a molecular weight of >10,000 daltons, showing precipitation corresponding to inner band of the pair present with whole extract. *e*, Reactions of con A to *Zea* extracts after proteolytic digestion or heat treatment. *D*, after treatment with insolubilised pronase; *E*, after treatment with papain; *F*, after incubation for 1min at 100° C. *a*, *b*, *c*, and *e*, photographed by dark field illumination of unstained gels; *d*, dried gel stained with Coomassie Blue. Z, *Zea mays*; C, con A.

The two lines against *Zea* extracts are probably con A-bound glycoproteins, as one is eliminated and the other rendered fainter by previous treatment with pronase (Fig. 1*e*). Preliminary experiments show that the con A-bound substances are antigenic and correspond in electrophoretic

mobility with two of the five antigens we have detected with high titre rabbit antiserum to *Zea* pollen diffusate[8]. Con A continues to precipitate extracts after incubation for 1 or 20 min at 100° C (Fig. 1*e*) and it is interesting that similar heat stability is also characteristic of some of the 'I' antigens, considered to be the main allergens of other grass pollens[1,9].

When pollen is sprinkled on con A gels, both *Zea* and *Euchlaena* grains show radially-aligned wisps of precipitate within 2 min (Fig. 2*a*). After 3 h, intense deposits of precipitate were evident (Fig. 2*b*). These reactions do not occur in control preparations without con A (Fig. 2*d*), and when other pollens are mixed with *Zea* as controls of specificity (Fig. 2*c*) conspicuous precipitates form only around the *Zea* grains even after 24 h incubation. We have also modified immunofluorescence procedures for pollen antigen location[10,11], to detect sites of con A binding to glycoproteins or carbohydrates in freeze-sectioned *Zea* and *Tripsacum* grains. Intensely-fluorescing precipitins appeared only in pollen wall sites (Fig. 3*a*), on the surface of the outer exine and in the inner cellulosic intine layer, and were absent from appropriate controls (Fig. 3*b*). The diffusible antigens of *Phalaris tuberosa* pollen have been located in these same sites[3], and show characteristic release kinetics. Immediately grass pollen grains are moistened, proteins and glycoproteins stored in cavities of the exine are released through surface micropores[4]. Only after about 3 min do those of the inner intine begin to be released, through the single pore or germinal aperture. While *Zea* and *Euchlaena* pollen precipitates con A within 2 min of moistening, the considerable increase in precipitation by 3 h indicates the involvement of both wall fractions. The immunofluorescence experiment seems to confirm this.

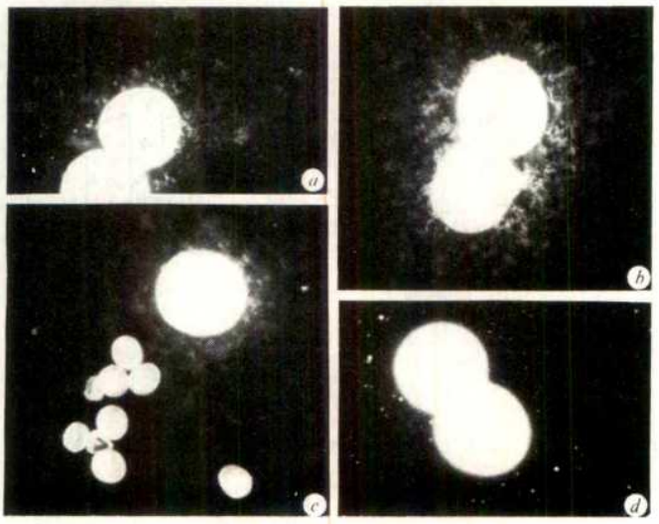


Fig. 2 *Zea* pollen sprinkled on 0.5% agarose gels containing 2 mg ml^{-1} con A and 0.2 M NaCl, viewed by dark field microscopy: *a*, After 2 min showing precipitate around grain; *b*, after 3 h showing dense radiating precipitates; *c*, mixture of *Zea* and *Lolium* pollen after 3 h with precipitation confined to the large *Zea* grains and absent from *Lolium* grains (in sharp focus); *d*, control with *Zea* grains sprinkled on gels without con A. (× 270).

It is not widely known that allergic persons often show taxonomically-patterned responses to festucoid, chloridoid, panicoid and andropogonoid grasses (refs 12, 13 and unpublished data). Methods have not yet been devised for distinguishing airborne grass pollens so it remains unclear how far this phenomenon results merely from factors affecting atmospheric availability of pollen for sensitisation. Our observations with con A, however, are consistent with the possibility that it directly reflects taxonomically-ordered variation in the allergenic components of pollen grains, as

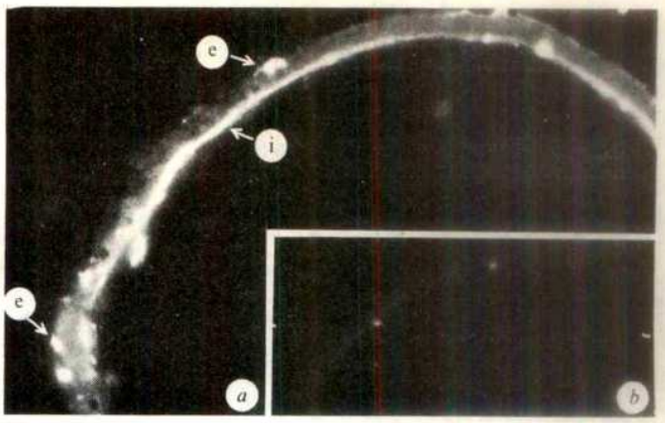


Fig. 3 Immunofluorescence localisation of con A-binding substances in freeze-sectioned *Zea* pollen, fluorescence image. *a*, Section treated with con A (40 min), saline rinse (15 min), rabbit anti-con A serum (40 min), saline rinse (15 min), FITC-labelled anti-rabbit γ-globulin serum (40 min); fluorescing precipitins present in intine (i) and surface exine cavities (e) but absent from protoplast; *b*, control section treated with con A, saline rinse, normal rabbit serum, saline rinse and FITC-labelled anti-rabbit γ-globulin serum, no fluorescing precipitins evident. (× 900)

are the findings of Augustin[13] and Marsh *et al.*[9] who, while working mostly with festucoids, have found a few non-festucoids to be immunologically very distinct. Such taxonomic predictability needs pursuing in detail, as it has medical potential for detecting cross reactions among allergenic pollens and for efficient selection of appropriate extracts or allergoids[14] for desensitisation therapy. It also points to the need in atmospheric pollen counts to try and identify the grasses, at least to major group level, and Fig. 2*c* indicates how further work with other lectins may help.

Many of the materials released when pollen is moistened, including proteins, glycoproteins and carbohydrates, function in early germination processes on the stigma. In *Phalaris tuberosa*, rapidly-released antigens bind to the stigma surface[3]. They seem to act in a variety of ways as pollen/stigma recognition substances[4], being concerned in regulating the breeding system. It is therefore intriguing that pollens of cultivated corn (*Zea mays*) and species directly involved in its evolution (*Euchlaena, Tripsacum*)[15] produce an identical and unique reaction with con A.

Table 1 List of grass pollen extracts tested with con A by double diffusion in agarose

Festucoids
Agropyron repens, *Agrostis tenuis*, *Anthoxanthum odoratum*, *Avena barbata*, *Bromus carinatus*, *B. inermis*, *B. rigidus*, *Dactylis glomerata*, *Elymus condensatus*, *E. triticoides*, **Festuca rubra**, *Holcus lanatus*, *Hordeum vulgare*, *Lolium multiflorum*, *L. perenne*, *L. temulentum*, *Phalaris arundinacea*, *Ph. minor*, *Ph. tuberosa*, *Poa annua*, *P. pratensis*, *P. scabrella*, *Secale cereale*

Chloridoids
Bouteloua sp., *Chloris truncata*, **Cynodon dactylon**, *Distichlis spicata* *Sporobolus aeroides*

Arundinoid—Danthonioids
Danthonia eriantha, **Phragmites australis, Stipa falcata**

Panicoids
Echinochloa crus-galli, *Paspalum dilatatum*, *Pennisetum villosum*, *Setaria geniculata*

Andropogonoids
(1) Andropogoneae: *Saccharum officinarum*, **Sorghum halapense,** *Themeda australis*
(2) Maydeae: *Coix lacrymajobi*, **Euchlaena mexicana, Tripsacum floridanum Zea mays**

Those showing precipitation appear in **bold face.** 0.2 g ml^{-1} dry pollen (obtained from Greer Laboratories, Lenoir, North Carolina; Commonwealth Serum Laboratories, Melbourne, Australia, and collected by us) extracted in 0.2 M NaCl in 0.05 M tris buffer, *p*H 7.5 for 20 min at room temperature. Con A (Pharmacia) used at 2 or 5 mg ml^{-1} in same buffered saline.

In *Silene* the pollen wall proteins bind to the stigma surface pellicle, a protein overlay found in most if not all angiosperms and which is the probable site of recognition reactions determining acceptance or rejection[16]. An interpretation of the situation in *Zea* and its closest relatives open to experimental testing (comparable with mammalian sperm/egg recognition[17]) is that the stigmatic surface receptors may possess specific determinants similar to those of con A.

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1 Augustin, R., and Hayward, B. J., *Immunology*, **5**, 424–460, (1962).
2 Augustin, R., O'Sullivan, S., and Davies, I., *Int. Arch. Allergy. appl. Immun.*, **41**, 144–147 (1971).
3 Knox, R. B., and Heslop-Harrison, J., *J. Cell Sci.*, **9**, 238–251 (1971).
4 Heslop-Harrison, J., Heslop-Harrison, Y., Knox, R. B., and Mattsson, O., *Biol. J. Linn. Soc.*, (in the press).
5 Lis, H., and Sharon, N., *A. Rev. Biochem.*, **42**, 541– (1973).
6 Goldstein, I. J., and So, L. L., *Archs Biochem. Biophys.*, **111**, 407– (1967).
7 So, L. L., and Goldstein, I. J., *J. Immun.*, **99**, 158–163 (1967).
8 Boreham, P., and Gill, G. S., *Acta Tropica*, **30**, 356–365 (1973).
9 Marsh, D. G., Haddad, Z. H., and Campbell, D. H., *J. Allergy*, **46**, 107–121 (1970).
10 Knox, R. B., Heslop-Harrison, J., and Reed, C., *Nature*, **225**, 1066–1068 (1970).
11 Knox, R. B., *J. Cell Sci.*, **9**, 209–237 (1971).
12 Wright, W. L. T., and Clifford, H. T., *Med. J. Aust.*, **2**, 74–75 (1965).
13 Augustin, R., *Immunology*, **2**, 148–169 (1959).
14 Marsh, D. G., *Int. Arch. Allergy appl. Immun.*, **41**, 199–215 (1970).
15 Mangelsdorf, P. C., in *Crop Plant Evolution* (edit. by Hutchinson, Sir J.), 23–49 (Cambridge University Press, 1965).
16 Mattsson, O., Knox, R. B., Heslop-Harrison, J., and Heslop-Harrison, Y., *Nature*, **247**, 298 (1974).
17 Oikawa, T., Yanagimachi R., and Nicolson, G. L., *Nature*, **241**, 256–259 (1973).

Absence of adenine nucleotide release from autonomic ganglion

SYNAPTIC vesicles isolated from Torpedo electroplaques contain ATP as well as acetylcholine (ACh)[1,2]. Silinsky and Hubbard[3] showed that ATP is released from the rat hemidiaphragm bathed in a hyperosmotic medium and they suggested that the motor nerve terminal releases ACh and ATP simultaneously. Here we report tests for the release of ATP and ACh from the cat superior cervical ganglion; we demonstrated that ACh, but not ATP, is released during stimulation.

The superior cervical ganglion of the cat was perfused[4,5] either with 'normal' Krebs solution (composition in mM: NaCl, 120; KCl, 4.6; $CaCl_2$, 2.4; $NaHCO_3$, 25; KH_2PO_4, 1.2; $MgSO_4.7H_2O$, 1.2; glucose, 9.9; neostigmine bromide, 0.03) or with a Krebs solution that did not contain $CaCl_2$ or $MgSO_4$ but contained 1 mM EDTA and 3 mM $BaCl_2$. This 'modified' Krebs solution was used to inhibit Ca^{2+}, Mg^{2+} dependent ATPase activity, and it contained Ba^{2+} to provide the divalent cation requirement for ACh release[6]. The release of ACh was evoked either by stimulating the preganglionic sympathetic trunk (20 Hz, 0.3 ms, 5.5 V) or by raising the KCl concentration in the perfusion medium to 50 mM (in which case the NaCl concentration was reduced to 50 mM). The effluent from the ganglion was collected in 5 min aliquots and each sample was assayed for ATP and for ACh. ATP was assayed using a modification of the luciferase method[7] that permitted the determination of as little as 4 pmol ATP; ACh was measured by bioassay on the cat blood pressure[8]. In some experiments adenosine monophosphate (AMP) or adenosine diphosphate (ADP) was measured instead of ATP; they were measured by the luciferase method after being converted to ATP enzymatically[9]. The conversion of AMP or ADP to ATP was checked by thin-layer chromatography[10] using ^{14}C labelled standards.

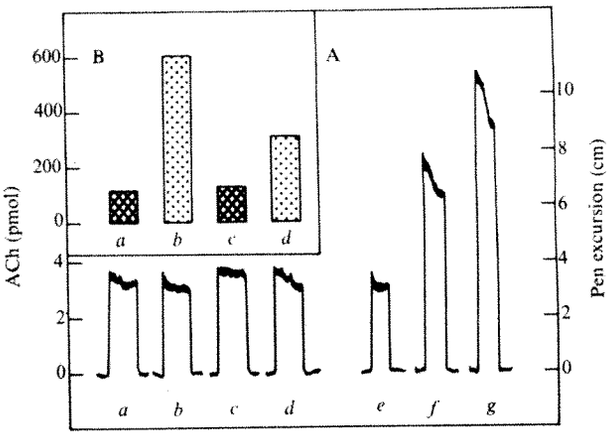


Fig. 1 A, Chart record of light emitted in the ATP assay of perfusate collected from the ganglion during *a*, rest; *b*, stimulation by high K^+; *c*, rest; *d*, preganglionic nerve stimulation. *e*, Control sample of 'modified' Krebs solution with no added ATP; *f*, 7.5 pmol ATP added to 'modified' Krebs medium; *g*, 12.5 pmol ATP added to 'modified' Krebs medium. ATP was assayed as follows. A plastic vial containing 1.5 ml of the sample to be assayed was placed in a light-tight housing system which was connected to a photomultiplier (American Instrument Co., USA) and to a Texas Rectiriter pen recorder. The reaction was initiated by injecting 1.0 ml of firefly lantern extract (Sigma, 5 mg ml^{-1} dissolved in 0.15 M Na_3AsO_4 buffer, *p*H 7.5, containing 0.1 M $MgSO_4$) into the vial through a rubber seal on the top of the housing system. B, The amount (pmol) of ACh contained in the same samples measured on the blood pressure of the eviscerated cat[8]. Before measuring the ACh content of the perfusion medium, barium ions were removed by the addition of 1/10 volume of 0.15 M Na_2SO_4; the precipitated $BaSO_4$ was removed by centrifugation and the supernatant was used for bioassay.

Figure 1 illustrates the result of a typical experiment which measured the release of ACh and ATP by nerve stimulation or by raised K^+ during perfusion with 'modified' Krebs solution. Although ACh was released by perfusion with high K^+ or by nerve stimulation, ATP could not be detected during the stimulation or rest period. Six other experiments confirmed this result. At the end of each experiment, a known concentration of ATP (60 pmol ml^{-1} in 'modified' Krebs solution) was perfused through the ganglion to determine the extent of breakdown. This usually was 10–25%. In contrast, when normal Krebs plus ATP or Krebs medium containing 0.1 mM Ca^{2+} plus ATP (no Mg^{2+}) was perfused through the ganglion, none of the ATP survived degradation. Whittaker *et al.*[1] found that the mean ACh:ATP molar ratio was 11:1 in synaptic vesicles from Torpedo electroplaques. If this ratio were the same

in transmitter stores of cat ganglion, and if all of the ATP were released during stimulation, then in our conditions, in which 150–650 pmol of ACh was released, 14–59 pmol of ATP should have been released; this quantity of ATP would have been readily detected under the conditions of our experiments. It is interesting that ATP release was detected immediately following a change of perfusion medium from a normal Krebs to a 'modified' Krebs solution. At this time a transient depolarisation of the postganglionic fibres occurred, causing a contraction of the nictitating membrane and about 15 pmol of ATP was released; no ACh was released during this period. We did not determine the origin of this ATP.

Other experiments were designed to test the release of ADP or AMP when perfusion was with normal Krebs medium and ACh release was evoked by nerve stimulation. In all experiments, ACh was released during nerve stimulation, but no ADP or AMP release could be detected. At the end of each experiment, ADP or AMP of known concentration was perfused through the ganglion, collected and analysed to determine the extent of breakdown. The ADP and AMP were degraded by 25% and 10% respectively.

In conclusion, we were unable to reproduce the results of Silinsky and Hubbard[3] using a different mammalian preparation. It is possible that the ATP detected in their experiments originated from the muscle rather than from the nerve terminal, as suggested by other workers[11–13]. The perfusion of the superior cervical ganglion avoided any artefacts arising from the presence of muscle, and allowed the collection of more ACh than could be collected from the stimulated rat phrenic nerve diaphragm preparation.

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1 Whittaker, V. P., Dowdall, M. J., and Boyne, A. F., *Biochem. Soc. Symp.* **36**, 49–68 (1972).
2 Bohan, T. P., Boyne, A. F., Guth, P. S., Narayanan, Y., and Williams, T. H., *Nature*, **244**, 32–33 (1973).
3 Silinsky, E. M., and Hubbard, J. I., Nature, **243**, 404–405 (1973).
4 Feldberg, W., and Gaddum, J. H., *J. Physiol., Lond.*, **81**, 305–319 (1934).
5 Collier, B., and Lang, C., *Can. J. Physiol. Pharmac.*, **47**, 119–126 (1969).
6 Douglas, W. W., Lywood, D. W., and Straub, R. W., *J. Physiol., Lond.*, **156**, 515–522 (1961).
7 Strehler, B. L., in *Methods of Enzymatic Analysis* (edit. by Bergmeyer, H.-U.), 559–569 (Academic Press, New York, 1963).
8 MacIntosh, F. C., and Perry, W. C. M., in *Meth. Med. Res.* (edit. by Gerard, R. W.), **3**, 78–92 (Year Book Publishers, Chicago, 1950).
9 Imai, S., Riley, A. L., and Berne, R. M., *Circulation Res.*, **15**, 443–450 (1964).
10 Randerath, K., and Randerath, E., in *Methods in Enzymology* (edit. by Grossman, L., and Moldave, K.), **XIIA**, 323–347 (Academic Press, New York and London, 1967).
11 Forrester, T., and Hassan, M. O., *J. Physiol., Lond.*, **232**, 86P–88P (1973).
12 Boyd, I. A., and Forrester, T., *J. Physiol., Lond.*, **199**, 115–135 (1968).
13 Forrester, T., and Lind, A. R., *J. Physiol., Lond.*, **204**, 347–364 (1969).

Pattern of remyelination in the CNS

It has been suggested that reduction of internodal length may be a feature of remyelinated central nerve fibres as it is of peripheral nerve fibres[1]. As the technique for measuring internodal length of nerve fibres in the central nervous system (CNS) is tedious and requires a high degree of skill and patience from the investigator[2], it is unlikely that this method can be applied to routine neuropathological investigations. Recent ultrastructural studies on remyelination in the mouse, following primary demyelination induced by feeding cuprizone (Bis - cyclohexanone - oxaldihyrozone)[3–5] have shown that measurements of the relationship between myelin sheath thickness and axon diameter provides an easier method of identifying remyelinated axons.

Male weanling albino mice of the ICI strain were fed on a powdered mouse diet containing 0.6% cuprizone. Two mice were killed after 6 weeks to assess the extent of demyelination in the superior cerebellar peduncle (SCP) (ref. 3) (Table 1). As both did not show complete demyelination the remaining nine animals were left on the cuprizone-containing diet for a further 2 weeks. At this time two animals were killed and examined for demyelination. Nearly all fibres in the SCP were demyelinated (Table 1), so the remaining seven animals were placed on a normal diet (previous experiments having shown that under these circumstances remyelination readily takes place[4,5]). Mice were then killed at the times indicated in Table 1 and remyelination assessed. Normal mice of two different ages were also killed and examined in the same way as the experimental animals. All animals were perfused with 4% glutaraldehyde in phosphate buffer and blocks were taken

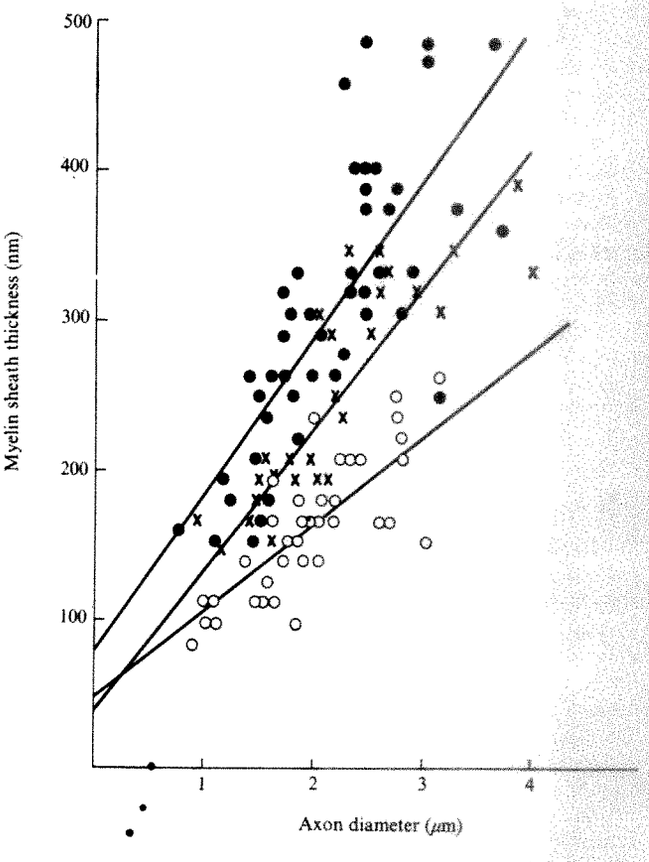


Fig. 1 Relationship between myelin sheath thickness and axon diameter in the superior cerebellar peduncle in a young mouse (×), an old mouse (●), and a mouse 6 months after this tract was demyelinated (○). Animals marked with a †dagger in Table 1.

Table 1 Remyelination in superior cerebellar peduncle

	% myelinated fibres	Mean d/D ratio	SD	Regression equation* $Y = \bar{y} + byx\,(x-\bar{x})$	Correlation coefficient
Old normal	100	0.78	±0.03	$Y = 22.51+1.56\,(x-15.99)$	0.74†
(9 month)	100	0.76	±0.03	$Y = 24.47+1.48\,(x-15.53)$	0.85
Young normal	100	0.81	±0.02	$Y = 15.61+0.95\,(x-14.03)$	0.83
(2 month)	100	0.81	±0.03	$Y = 16.94+0.93\,(x-15.12)$	0.89†
6 weeks cuprizone	98				
	3				
8 weeks cuprizone	15				
	4				
8 weeks cuprizone	90	0.92	±0.03	$Y = 7.33+0.24\,(x-16.65)$	0.50
6 weeks recovery	79	0.91	±0.03	$Y = 7.29+0.09\,(x-16.37)$	0.19
8 weeks cuprizone	56	0.92	±0.03	$Y = 6.61+0.28\,(x-15.26)$	0.46
12 weeks recovery	90	0.90	±0.02	$Y = 8.74+0.55\,(x-15.39)$	0.74
	88	0.90	±0.02	$Y = 7.22+0.32\,(x-14.42)$	0.56
8 weeks cuprizone	88	0.89	±0.03	$Y = 8.87+0.34\,(x-15.30)$	0.48
24 weeks recovery	98	0.86	±0.02	$Y = 12.13+0.58\,(x-14.78)$	0.73†

* In regression equation: $\bar{y}$ = mean myelin sheath thickness; $\bar{x}$ = mean axon diameter; byx = slope of linear regression of myelin sheath thickness on axon diameter; x and y were measured in mm on electron micrographs at 7,200 magnification; y = actual measurement × 10.
† Indicates the three animals depicted graphically in Fig. 1.
d = diameter of axon; D = diameter of fibre.

which contained the SCP cut in transverse section at the point where it passes under the middle cerebellar peduncle[3,4]. Thin sections were prepared for electron microscopy and six electron micrographs were taken at random of the SCP at a magnification of 1,800. These were enlarged photographically a further four times and the following parameters measured: percentage of myelinated fibres, myelin sheath thickness and axon diameter. These were determined for a representative sample of fibres by methods used previously[3,4]. Table 1 gives values for the percentage of myelinated fibres in the SCP, the d/D ratio (diameter of axon/diameter of fibre)—an index of myelin sheath thickness—the slope of the linear regression of myelin sheath thickness on axon diameter, and the correlation coefficient of myelin sheath thickness and axon diameter. An analysis of the significances of differences between the regression lines was carried out[6].

Nearly all axons are remyelinated during the recovery period and with time the myelin sheath increases in thickness (Table 1). But even after six months recovery, myelin sheath thickness has not returned to its normal relationship with axon diameter (Table 1, Fig. 1). There is a significant difference between the index of myelin thickness for the 6 month remyelinated animals and the 2-month-old control animals. Taking into account that the 6-month-recovery animals are 10 months old, it can be seen that the remyelinated axons have much thinner myelin sheaths than would be expected for animals of their age. In addition the slopes of the regression lines for the remyelinated animals are significantly different (at the 0.01 level; sample size 32–47 observations) from either young or old control animals. It seems therefore that although myelin sheath thickness does increase with time in remyelinated animals, it does not return to its normal relationship with axon diameter for a considerable length of time. It is likely, therefore, that the presence of an axon within a myelin sheath which is thinner than would be expected for the diameter of the contained axon, represents a remyelinated fibre. Such an observation will have little significance when applied to a single fibre, but if it can be shown that the axon diameter–myelin sheath thickness relationship for a population of fibres is abnormal, or that two populations of fibres coexist in a single tract then it can be reasonably assumed that remyelination has taken place on the axons possessing thinner than normal myelin sheaths. This experiment supports the many observers who have stated that axons within thinner than normal myelin sheaths represent remyelinated axons[7–12] and suggests a simple method for determining the extent of remyelination in man.

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1 Gledhill, R. F., Harrison, B. M., and McDonald, W. I., *Nature*, **244**, 433 (1973).
2 McDonald, W. I., and Ohlrich, G. D., *J. Anat.*, **110**, 191 (1971).
3 Blakemore, W. F., *J. neurol. Sci.*, **20**, 63 (1973).
4 Blakemore, W. F., *J. neurol. Sci.*, **20**, 73 (1973).
5 Blakemore, W. F., *J. neurol. Sci.* (in the press).
6 Steel, R. G. D., and Torrie, J. H., *Principles and Procedures of Statistics*, 319 (McGraw-Hill, New York, 1960).
7 Bunge, M. B., Bunge, R. P., and Ris, H., *J. biophys. biochem. Cytol.*, **10**, 67 (1961).
8 Lampert, P. W., *J. Neuropath. exp. Neurol.*, **24**, 371 (1965).
9 Hirano, A., Levine, S., and Zimmerman, H. M., *J. Neuropath. exp. Neurol.*, **28**, 234 (1968).
10 Prineas, J., Raine, C. S., and Wisniewski, H., *Lab. Invest.*, **21**, 472 (1969).
11 Wisniewski, H., and Raine, C. S., *Lab. Invest.*, **25**, 73 (1971).
12 Gledhill, R. F., Harrison, B. M., and McDonald, W. I., *Expl neurol.*, **38**, 472 (1973).

Effect of intracellular lithium on snail neurones

It is well established that lithium salts are effective in the treatment of manic depressive illness[1,2], but the way they act is little understood. Some time ago, as part of a study on the electrogenic sodium pump, it was noticed that high levels

of intracellular Li^+ or Na^+ seemed to increase the potassium conductance of the snail nerve cell membrane[3,4]. Because of the greatly increased interest in Li^+ itself, we have now confirmed the original observations, and have shown that detectable effects occur at quite low levels of intracellular Li^+. Such an increased membrane conductance to potassium would tend to decrease the membrane excitability and hence stabilise the cell. If a similar response to intracellular Li^+ occurs in man, it could be the basis for its therapeutic effects.

We performed our experiments on the largest nerve cell at the rear of the right pallial ganglion of the common snail *Helix aspersa*. The brain was dissected from dormant specimens and pinned in a chamber where it was bathed in continuously flowing snail Ringer (KCl, 4 mM; NaCl, 80 mM; $CaCl_2$, 7 mM; $MgCl_2$, 5 mM; Tris-maleate (*p*H 7.5), 20 mM) at room temperature (14–21° C). The bathing solution could be quickly changed to Ringer containing various concentrations of potassium or to normal Ringer with 10^{-4} M ouabain by means of a multiposition tap. The cell was impaled with one double and two single-barrelled microelectrodes. This allowed for the recording of membrane potential, the determination of input resistance by injected current pulses, and the interbarrel injection of LiCl, NaCl or KCl into the cell. In this last technique, current is passed between two intracellular electrodes, ejecting cations from one electrode and anions from the other.

Cell diameter was measured and cell volume calculated assuming a spherical cell. The amplitude and length of the interbarrel injection current was recorded and thus the amount of salt injected could be estimated. (For a more detailed description of these methods, see Thomas[5].)

The effect of a relatively large injection of LiCl is shown in Fig. 1. With the intracellular Li^+ concentration raised to a calculated level of 29 mM, the membrane potential was increased by about 3 mV, and the cell's spontaneous action potentials ceased. (Larger effects were seen with larger injections of LiCl, but in this experiment injection was stopped when the cell ceased firing to ensure reasonably rapid recovery.) There was a simultaneous one-third reduction in input resistance, as is shown by the decrease in the size of the hyperpolarisations resulting from the current pulses. Following the injection, both input resistance and membrane potential gradually returned to their resting values and spontaneous action potentials returned. The lowered resistance after LiCl injection recovered approximately exponentially. The time constant for the recovery shown in Fig. 1 was 40 min. Similar effects were seen in 29 cells, although in several cases the cell did not recover after the injection.

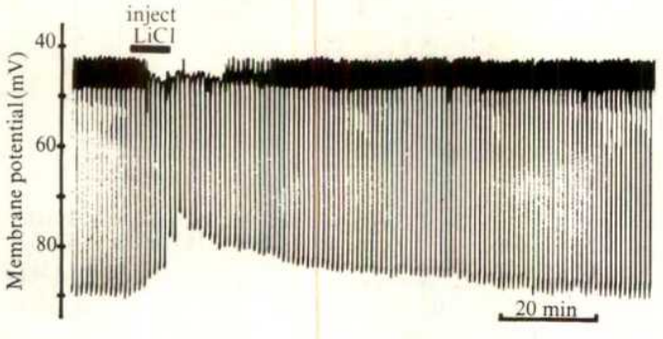


Fig. 1 Pen recording from an experiment to show the effects of injected LiCl on the input resistance of a spontaneously active snail neurone. The slow time response of the recorder has reduced the apparent size of the action potentials to only a few millivolts. Every minute a 5 nA hyperpolarising current was passed across the cell membrane for 10 s to measure the input resistance. The solid bar indicates the 8 min period during which an interbarrel injection current of 25 nA was passed. The cell diameter was 0.2 mm, so that this injection would be expected to raise the intracellular Li concentration by about 29 mM.

We also tested the effects of large injections of KCl and NaCl. An injection of KCl which would be expected to raise the internal K^+ concentration by about 45 mM caused only a slow depolarisation of a few millivolts with no change in input resistance. The injection of NaCl produced a more complex and variable result. Small injections of NaCl consistently caused an immediate ouabain-sensitive hyperpolarisation (as expected from the consequent stimulation of the electrogenic Na pump[4]) with no appreciable change in input resistance. Large injections of NaCl (giving a concentration greater than 20 mM), however, often produced both a considerably greater hyperpolarisation, which was little affected by ouabain, and a noticeable decrease in input resistance.

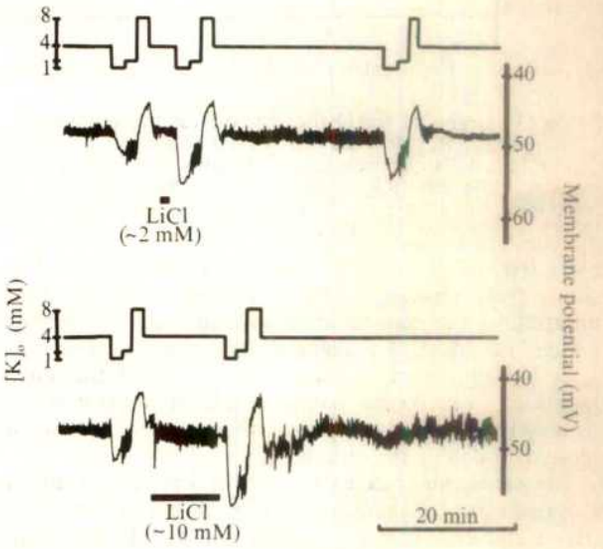


Fig. 2 The effect of injected LiCl on the sensitivity of a snail neurone to changes in external potassium. Two segments from a continuous record showing the effects on the membrane potential of brief exposures to 1, 2 and 8 mM external potassium. The normal external potassium concentration ($[K]_o$) was 4 mM. LiCl was iontophoretically injected where indicated by the solid bars, giving estimated internal concentrations of 2 and 10 mM.

Another way of describing the experiment shown in Fig. 1 is to say that the cell was being held, alternately, at two membrane potentials; one, the resting level at the top of the current pulses (−45 mV) and the other a more hyperpolarised level at the bottom of the pulses (−90 mV). Following a LiCl injection, then, the resting cell is seen to hyperpolarise while a cell held at a hyperpolarised potential depolarises, thus indicating the existence of an equilibrium potential for the process. The large injections of Cl^- which accompany these Li^+ injections would cause the chloride equilibrium potential to be less negative than the resting potential. Potassium is the only ion whose equilibrium potential would be in the range to produce these effects, although some involvement of other ions cannot be ruled out.

Figure 2 shows an experiment where the external K^+ concentration ($[K]_o$) was changed before and after two injections of LiCl. Although quite large injections are necessary to produce the marked changes in input resistance seen in Fig. 1, measurable changes in the relative potassium permeability (P_K) can be produced by much smaller injections. Following both LiCl injections in Fig. 2 the membrane potential becomes more responsive to a change in external K^+. As the injected Li^+ leaves the cell, this increased sensitivity declines gradually to the resting level.

Estimates in the change in P_K before and after LiCl injections, assuming no change in other ion permeabilities, were obtained from linear regression fits to $[K]_o$ against $\exp(FV/RT)$

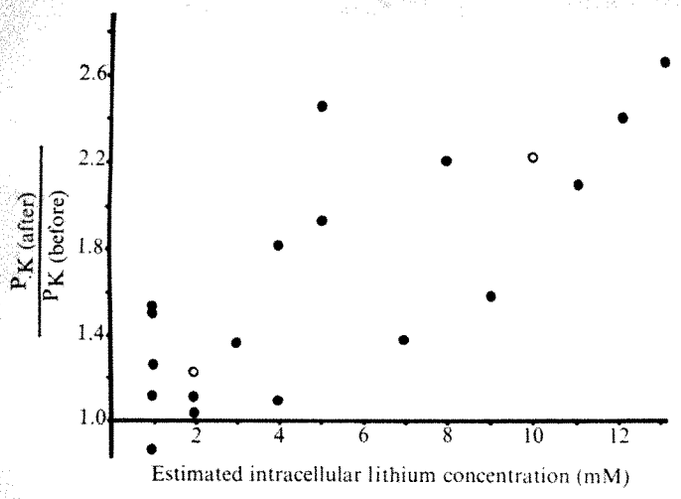


Fig. 3 Graph of the changes in the relative potassium permeabilities (P_K) of snail neurones as a function of estimated intracellular Li concentration. Relative P_Ks are calculated from plots of $[K]_0$ against $\exp(FV/RT)$. Results from 20 injections in seven cells; ○, points calculated from the data shown in Fig. 2.

plots (ref. 6) of 20 LiCl injections in seven cells. Figure 3 shows these changes in P_K as a function of the estimated intracellular Li^+ concentration. Although there is considerable scatter, the trend of a rising P_K following successively larger LiCl injections can be clearly seen. Thus intracellular Li^+ injections giving concentrations of a few mM cause measurable increases in P_K and injections of greater than about 8 mM more than double the resting P_K.

The extracellular concentration of Li^+ that is effective in the treatment of manic depressive illness is around 1 mM (refs 1 and 2). It seems possible that at least some neurones in the human brain will tend to concentrate Li^+ intracellularly, as passive distribution alone would give an internal level of about 10 mM in a cell whose resting potential was 60 mV, and the Na–K pump does not extrude Li[4]. Thus, if intracellular Li^+ has the same effect on some human neurones as on snail neurones, its therapeutic effects could arise from the membrane stabilising effects of the increased potassium permeability.

How does Li^+ cause this rise in potassium permeability? Perhaps the most likely mechanism is by an increase in the intracellular ionised Ca^{2+}. Meech[7] has shown that the injection of $CaCl_2$ into mollusc neurones causes a rise in their potassium permeability, and similar responses have been seen with mammalian motoneurones[8]. There is also evidence that Ca^{2+} is normally extruded from nerve cells by a Na–Ca exchange system[9], so that a rise in intracellular Na^+ will tend to cause a rise in intracellular Ca^{2+}. If Li^+ can substitute for Na^+ in this system then intracellular Li^+ would tend to cause an increase in intracellular Ca^{2+}. We have some preliminary evidence that intracellular calcium is involved, as we have found that the injection of EGTA partially reverses the change in P_K following a large lithium injection.

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[1] Schou, M., *Proc. 6th. Ann. Meet. Am. College Neuropsychopharmacol.* (edit. by Efron, D. H.), 701-718 (1968).
[2] Coppen, A., Noguera, R., Bailey, J., Burns, B. H., Swani, M. S., Hare, E. H., Gardner, R., and Maggs, R., *Lancet*, ii, 275-279 (1971).
[3] Thomas, R. C., *J. Physiol., Lond.*, **195**, 23P (1968).
[4] Thomas, R. C., *J. Physiol., Lond.*, **201**, 495-514 (1969).
[5] Thomas, R. C., *J. Physiol., Lond.*, **220**, 55-71 (1972).
[6] Moreton, R. B., *J. exp. Biol.*, **48**, 611-623 (1968).
[7] Meech, R. W., *Comp. Biochem. Physiol.*, **42A**, 493-499 (1972).
[8] Krnjevic, K., Lisiewicz, A., *J. Physiol., Lond.*, **225**, 363-390 (1972).
[9] Baker, P. F., *Prog. Biophys. molec. Biol.*, **24**, 177-223 (1972).

Periodicity in high sulphur proteins from wool

In a recent publication[1], Elleman, Lindley and Rowlands claimed that there is no evidence to support the view that high-sulphur proteins from wool have a common ancestral protein partly composed of a repeating unit of five amino acids[2,3]. Their evidence for a repeating decapeptide unit is restricted almost entirely to the region between residues 22 and 64 of protein SCMKB2B. Although the C terminal region of this sequence shows some correlation to the repeating decapeptide unit, it correlates better with a pentapeptide repeating unit as shown previously (ref. 3).

It is informative to compare the 'decapeptide rich' regions of the sequences of the three homologous SCMKB2 proteins[4] as shown in Fig. 1. Protein B2C contains two decapeptide repeating units while B2B and B2A contain three and four units, respectively. These differences probably resulted from gene elongation due to unequal crossing over of DNA during meiosis. The original decapeptide unit would then have been two adjacent pentapeptide units differing as a result of mutations. This would explain the 'partial symmetry' referred to by Elleman *et al.*[1].

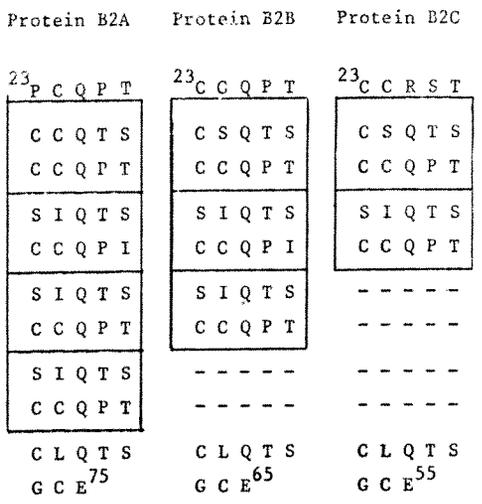


Fig. 1 The partial amino acid sequences[4] of protein SCMK-B2A (residues 23–75), protein SCMK-B2B (residues 23–65) and protein SCMK-B2C (residues 23–55) from the high-sulphur fraction of Lincoln wool, arranged to show maximum homology based on a pentapeptide repeat. The single letter notation for amino acids[5] has been used.

Lindley and Elleman[6] showed homology between 14 residues at the C terminal regions of the IIIB and B2 proteins. This homology was further extended by aligning a part of the sequence of protein IIIA3 with these regions[3], which proves a common origin for all these high-sulphur proteins. Yet Elleman *et al.*[1] derived different homologous decapeptide units for proteins IIIA3 and B2B, and none for the IIIB proteins, which they claimed to be random. We found[3] a single primordial pentapeptide unit, Cys-Cys-Gln-

Pro-X, which fits the IIIA3 and B2 groups of proteins equally well and, in addition, nearest neighbour statistical analysis shows that the sequences of the IIIB proteins are not random and are derived from the same unit with a probability of the order of 8×10^6 to one.

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1 Elleman, T. C., Lindley, H., and Rowlands, R. J., *Nature*, **246**, 530 (1973).
2 Ycas, M., *J. molec. Evol.*, **2**, 17 (1972).
3 Swart, L. S., *Nature new Biol.*, **243**, 27 (1973).
4 Elleman, T. C., *Biochem. J.*, **130**, 833 (1972).
5 Dayhoff, M. O., *Atlas of Protein Sequence and Structure (National Biomedical Research Foundation*, Silver Spring, Maryland, 1972).
6 Lindley, H., and Elleman, T. C., *Biochem. J.*, **128**, 859 (1972).

Platelet-activating factor, a new mediator of anaphylaxis and immune complex deposition from rabbit and human basophils

AN increase in vascular permeability as a result of the release of vasoactive amines has been implicated as a 'trigger mechanism' of immune complex deposition[1,2]. Several immunological mechanisms of vasoactive amine release from rabbit platelets have been described[3]. Experiments have correlated the presence of one of these mechanisms, a complement-independent, leukocyte-dependent reaction, with the vascular deposition of complexes in acute[4] and chronic[5] serum sickness of rabbits. We have obtained direct evidence that this mechanism is due to basophils sensitised with IgE (ref. 6). Antigen-induced *in vitro* degranulation of basophils from rabbits immunised with peroxidase yielded a soluble factor (platelet-activating factor (PAF)) which aggregated rabbit platelets in leukocyte-free solutions[6]. Here we describe several characteristics of rabbit PAF and a PAF-like factor obtained from human leukocytes.

Procedures used to obtain PAF from rabbit leukocytes have already been described in detail[6]. Briefly, buffy coat from rabbits immunised with peroxidase was cleared of erythrocytes by sedimentation in phosphate buffered saline (PBS) with 2.5% gelatin followed by osmotic shock, and cleared of platelets by repeated low speed centrifugation. Leukocytes were incubated in Tris-buffered Tyrode's solution (*p*H 7.4), with 0.25% bovine serum albumin (TTBSA), in the presence of antigen for 15 min at 37° C. Absence of platelets in buffy coat and addition of BSA to the medium as a PAF carrier are prerequisites for recovery of PAF. Cell-free supernatant was obtained by centrifugation, dialysed with 0.01 M phosphate buffer (*p*H 8.0), chromatographed on a DEAE-Sephadex A 50 anion exchange column, with a gradient of NaCl. PAF activity, measured in an aggregameter by aggregation of rabbit platelets in Tyrode's added with 0.25% gelatin (TG), was eluted with BSA. After dialysis with 0.01 M Tris 0.15 NaCl buffer (*p*H 7.4), PAF was separated from BSA by precipitation in 80% ethanol. After centrifugation at 60,000*g*, the supernatant was evaporated to dryness and PAF was resuspended in 80% ethanol. Throughout purification and characterisation of PAF, controls were TTBSA with leukocytes and peroxidase but maintained at 4° C for 15 min, instead of 37° C, or TTBSA without Ca^{2+} and Mg^{2+} used instead of TTBSA in the usual degranulation method. In a second step, leukocytes used for preparation of control buffers were resuspended in TTBSA at 37° C to release PAF in the presence of peroxidase.

PAF in 80% ethanol was filtered through a Sephadex LH 20 column equilibrated with 80% ethanol at *p*H 9.5. Markers were ^{125}I-labelled glucagon, bacitracin, vitamin B_{12}, ^{3}H-angiotensin and PSP. PAF from rabbit leukocytes (R-PAF) was eluted between vitamin B_{12} and angiotensin, exhibiting an apparent molecular weight of 1,100.

PAF filtered through LH20 in 80% ethanol was evaporated to dryness and resuspended in distilled water or 80% ethanol. PAF activity disappeared from distilled water-containing tubes in about 24 h, whereas PAF activity remained unchanged in 80% ehtanol for several weeks at 4° C. Varying the *p*H of distilled water between 5 and 10 did not influence the decrease of PAF activity. To explore this phenomenon further, the same experiment was carried out in distilled water, using tubes used for platelet aggregation. Tubes from which PAF activity had disappeared were emptied, rinsed once and filled with TG. Platelet aggregation occurred in these tubes, showing that PAF in distilled water had been adsorbed on the plastic walls of the tubes. No PAF activity was found on the walls of the tubes which had contained PAF in 80% ethanol. This result indicated that further studies of PAF had to be carried out in 80% ethanol.

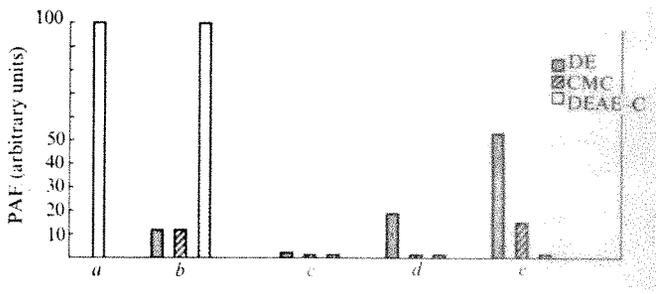


Fig. 1 Adsorption of PAF on charged particles in 80% ethanol. PAF activity was measured by aggregation of 3×10^7 rabbit platelets in 0.3 ml Tyrode's in an aggregameter (Icare, Marseille, France). *a*, Supernatants before adsorption at *p*H 6.50; *b*, Supernatants after adsorption at *p*H 6.50; *c*, elution from washed particles at *p*H 7.00; *d*, at *p*H 8.00; *e*, at *p*H 9.50.

We attempted to determine the electrostatic charge of PAF by adsorption and elution experiments with negatively charged particles, such as diatomaceous earth (DE) and carboxymethylcellulose (CMC) (Fig. 1). PAF in 80% ethanol at *p*H 6.5 was incubated with DE or CMC. PAF activity disappeared from the solutions. DE and CMC were washed once with 80% ethanol at *p*H 6.5, then the *p*H was gradually raised to *p*H 11. Elution of PAF was obtained at a *p*H between 9.2 and 9.7 with a maximum most frequently obtained at *p*H 9.5. The same experiment was performed in distilled water at *p*H 6.5 with similar results but very low yield of eluted PAF. These results are compatible with a PAF isoelectric point between *p*H 9 and 10. Controls for this experiment were: first, binding of arginine by DE or CMC in 80% ethanol, showing that the particles were charged in this medium; second, a lack of binding of PAF and arginine at neutral *p*H by DEAE-cellulose, a positively charged particle; and third, binding of aspartic acid in 80% ethanol by DEAE-cellulose but not by DE or CMC. Experiments were undertaken to obtain release of PAF from rabbit basophils without antigen–IgE interaction. Overnight slow agitation of leukocyte preparation from immunised or normal rabbits in TTBSA (*p*H 7.4) at 22° C released small amounts of a PAF-like material (spontaneous release (SR)). In keeping with the basic nature of the molecule, it was postulated that a better release would be obtained at a *p*H where the molecule has a neutral electrostatic charge. Indeed, SR was

obtained readily at a starting *p*H of 10.5 (final *p*H 9.5) within 90 min at 22° C. Through the whole procedure of purification, the molecule obtained by spontaneous release at *p*H 9.5 showed exactly the same characteristics as PAF actively released at *p*H 7.4. At *p*H 9.5, no metabolic activity of the cells was necessary for PAF release—in contrast with the metabolic activity required for release at *p*H 7.4 (J. Benveniste, unpublished). Human leukocytes were prepared by sedimentation of blood in 2.5% gelatin in PBS, followed by osmotic shock and several washings with TTBSA. Final leukocyte preparations contained 0.2–1% basophils with other leukocytes in normal proportion. Incubation of leukocytes in TTBSA at 22° C gave exactly the same results as in rabbits, that is, small amounts of a PAF-like factor at *p*H 7.4 and much higher amounts, comparable to those obtained from rabbit leukocytes, at *p*H 10.6. This human factor bound to BSA, was soluble in 80% ethanol, showed neutral electrostatic charge at *p*H 9.5 and an apparent molecular weight of 1,100. Further purification and identification of the molecule is under way in our laboratory.

These results indicate that PAF is a basic molecule with a molecular weight of 1,100. Its highly positive charge at physiological *p*H could explain its tendency to bind to negatively charged surfaces. It is likely that the molecule also possesses hydrophobic sites responsible for its binding to BSA and plastic walls in aqueous but not in 80% ethanol solution. PAF represents a new mediator of anaphylaxis, different from those already described: histamine, SRS-A (ref. 7) and ECF-A (refs 8 and 9). In rabbits, PAF has been clearly identified as of basophil origin: we have obtained the release of PAF by anti-IgE antiserum but not anti-IgG (ref. 6). It has been implicated in immune complex deposition through its aggregating and releasing action on platelets. The identity of the factors obtained from human leukocytes and from rabbit basophils makes the basophil a likely source in the human. Preliminary results in our laboratory show that leukocytes from patients having undergone basophil degranulation during anaphylactic shock do not release PAF, a situation very close to immunised rabbits injected daily with small doses of antigen. The direct evidence of the basophil origin of human PAF remains to be demonstrated, however. If the existence of PAF is firmly established in the human, it could represent, according to the experimental model, the mediator of a mechanism involving IgE-sensitised basophils in the pathogenesis of immune complex deposition. This mechanism would be a link between immediate hypersensitivity and immunological diseases associated with severe structural injury.

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1 Benacerraf, B., McCluskey, R. T., and Patras, D., *Am. J. Path.*, **35**, 75 (1959).
2 Cochrane, C. G., *J. exp. Med.*, **113**, 489 (1963).
3 Henson, P. M., *Fedn Proc.*, **28**, 1721 (1969).
4 Henson, P. M., and Cochrane, C. G., *J. exp. Med.*, **133**, 554 (1971).
5 Wilson, C. B., and Henson, P. M., cited by Benveniste, J., Henson, P. M., and Cochrane, C. G., in *Inflammation*, (edit. by Lepow, I. H., and Ward, P. A.), (Academic Press, New York and London, 1972).
6 Benveniste, J., Henson, P. M., and Cochrane, C. G., *J. exp. Med.*, **136**, 1356 (1972).
7 Orange, R. P., and Austen, K. F., *Adv. Immun.*, **10**, 105 (1969).
8 Kay, A. B., Stechshulte, D. J., and Austen, K. F., *J. exp. Med.*, **133**, 602 (1971).
9 Parish, W. E., *Clin. Allergy*, **2**, 381 (1972).

Organochlorine residues, eggshell thinning and hatching success in British sparrowhawks

SPARROWHAWKS (*Accipiter nisus*) in Britain have laid thin-shelled eggs throughout the period of DDT usage[1], and in several predatory species in North America an inverse correlation has been shown between shell thickness and DDE levels in eggs, both in natural and in experimental situations[2-4]. It has been claimed that eggshell thinning is associated with reduced hatching success, and that both effects could be implicated in a sequence leading to population decline. We have examined these relationships up to hatching stage in the sparrowhawk in Dumfriesshire, southern Scotland, where it still breeds in good numbers. During 1971–73 full details were obtained from 325 nests, and eggs from 130 clutches were collected for analysis.

Egg contents were homogenised; a 5 g aliquot was mixed with anhydrous sodium sulphate and sand, and extracted overnight in a soxhlet with hexane:acetone 2:1. The extract was evaporated to dryness in a tared beaker and total lipid estimated. The extract was then reconstituted in 25 ml hexane, and 5 ml was cleaned up by florisil column chromatography. (Liquid-liquid partitioning of the extract to remove fat was found to be unnecessary.) A further 5 ml aliquot was used for a duplicate estimate of lipid content. Hexane and hexane/ether chromatography fractions were used directly for analysis, using standard gas chromatography methods on columns packed with SE 30, SE 30-OV 210, and GE-XE 60 (after Richardson[5]). Results are given as parts per million (p.p.m.) in lipid, as the most valid basis for expressing organochlorine residues in eggs. On average, fresh sparrowhawk eggs contain approximately 6% by weight of lipid, so results can be converted to a wet weight basis by dividing them by 17.

The main residues found were DDE (the principal metabolite of DDT), polychlorinated biphenyls (PCB) and dieldrin (Table 1). Only results from eggs with embryos less than half grown were included. By this stage only a small reduction in lipid content (< 10%) and negligible removal of calcium from the shell has occurred[6]. When eggs from the same clutch, but at different stages of development, were analysed, no evidence was found that residues were metabolised, as proposed by Hazeltine[7]. Residues remained in the same relative proportions, and the increase in concentration in lipid during development was of the extent predicted from the amount of lipid metabolised.

Table 1 Organochlorine content and shell indices of sparrowhawk eggs from southern Scotland 1971-73

	Mean + s.e. (p.p.m. in lipid)	
DDE	148 ± 8	(n = 131)
PCB	66 ± 4	(n = 131)
Dieldrin	26 ± 1	(n = 131)
Total organochlorine	240 ± 11	(n = 131)
Shell index	1.17 ± 0.01	(n = 122)

Numbers refer to clutches. Each clutch represented once only by mean values and shell indices calculated by Ratcliffe's method[1].

Compared with 71 clutches collected in the same general area in 1900-45, which had a mean shell index of 1.43 ± 0.01, those collected in 1971-73 showed an 18% reduction in shell index ($P < 0.001$). This is of the same order as that found for a wider part of Britain by Ratcliffe[1]. Using all recent eggs, with each clutch represented only once by mean values, eggshell index was inversely correlated with the logarithm of DDE concentration (index = 1.568 − 0.186 ($\log_{10}$ DDE), $r = -0.359$, $P < 0.001$). It was also inversely correlated with $\log_{10}$ PCB concentration, though less well ($r = -0.231$, $P < 0.01$), and only poorly correlated with dieldrin concentration ($r = -0.101$, not significant). The correlation of PCB with shell thinning

in our eggs was probably because PCB was itself correlated with DDE ($r=0.480$, $P<0.001$). Lincer[8], however, has shown that, while PCB had no direct effect on shell thinning in captive American kestrels, it potentiated the effect of DDE.

Among 325 study nests, 110 (34%) showed 'normal' hatching success, 101 (31%) showed partial success, and 114 (35%) failed completely. Success was classed as 'normal' if all eggs hatched, or if no more than one egg was addled (watery), and there were no egg breakages and no 'dead-in-shell' embryos. This criterion was established from extensive hatching data collected in Britain before 1946 (ref. 9). The hatching success of these various recent clutches was inversely related to their organochlorine content (Table 2).

Table 2 Hatching success and shell indices in relation to organochlorine content

Success	*n*	Total organochlorine content (mean ± s.e.)	*n*	Shell index
1. Normal*	17	157 ± 13	15	1.27 ± 0.03
2. Partial†	74	226 ± 11	70	1.20 ± 0.01
3. Nil	40	293 ± 25	37	1.11 ± 0.02

Each clutch represented once only by mean values; shell indices calculated by Ratcliffe's method[1].

* No more than one egg addled, no breakage and no 'dead-in-shell' embryos.

† One or more eggs broken or 'dead-in-shell' embryos but at least one young hatched.

The main cause of unsuccessful breeding was failure to lay (having built a nest). This occurred at 49 (15%) of 325 nests, and accounted for 43% of all complete failures. It was apparently not recorded in British sparrowhawks before 1946 (ref. 9). Egg breakage was the second commonest cause of failure, the 35 instances accounting for 11% of all clutches and 31% of all failures. Breakage was in general related to the degree of shell thinning (Table 3), but the facts that eggs in 22% of clutches with normal shell indices were broken, whereas eggs in 66% of those with low indices survived, imply that some other factor, such as parental care, was also involved. Usually the eggs in a clutch were broken individually over several days, until only one remained, which was then deserted. Analyses

Table 3 Incidence of egg breakage* in clutches with different shell indices

Shell index	0.80–1.00	1.01–1.20	1.21–1.40	1.41–1.60
No. of clutches	9	52	57	3
No. with breakage	7	20	12	1

* One or more eggs broken; clutches deserted fresh excluded.

of those remaining eggs from clutches gave organochlorine levels of 350 ± 47 p.p.m. The third commonest cause of failure was desertion, which occurred at 5% of all nests and accounted for 15% of all failures. The 16 clutches involved showed a wide range of organochlorine contents, with a mean of 200 ± 23 p.p.m., lower than that in other types of complete failure. The remaining 13 clutches were unproductive because the eggs, though incubated and undamaged, failed to hatch. This involved 4% of all nests, and 11% of all failures. Such failures might have been due to poor parental care, impaired gas and water exchange through a thin shell or a direct toxic effect of high residue levels. Embryo deaths from thin shells alone seems unlikely, as the 10 clutches involved had by no means the thinnest shells (mean 1.15 ± 0.01), but by far the highest organochlorine levels (mean 363 ± 19 p.p.m.). Among nests which produced at least one young, unhatched eggs were found in 51 (19% of all nests), broken eggs in 27 (8% of all nests), and both unhatched and broken eggs in another 23 (7% or all nests). Once the eggs hatched, survival of young in all groups of nests was greater than 90%.

In conclusion, in spite of widespread shell thinning, egg breakage was a less important cause of unsuccessful breeding than failure to lay (having built a nest), while desertions and embryo deaths also accounted for a large proportion of failures in clutches. Clutches failing in all these ways, except desertions, showed organochlorine levels significantly higher than those showing normal success.

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[1] Ratcliffe, D. A., *J. appl. Ecol.*, 7, 67 (1970).
[2] Cade, T. J., Lincer, J. L., White, C. M., Roseneau, D. G., and Swartz, L. G., *Science*, **172**, 955 (1971).
[3] Porter, R. D., and Weimeyer, S. N., *Science*, **165**, 199 (1969).
[4] Enderson, J. H., and Berger, D. D., *Bioscience*, **20**, 355 (1970).
[5] Richardson, A., *6th Int. Pl. Pro. Cong.*, (Vienna, 1967).
[6] Romanoff, A. L., *Pathogenesis of the Avian Embryo* (Wiley Interscience, New York, 1972).
[7] Hazeltine, W., *Nature*, **239**, 410 (1972).
[8] Lincer, J. L., thesis, Cornell University, (1972).
[9] Newton, I., *J. appl. Ecol.*, **11**, (in the press).

Species diversity in temperate and tropical Ichneumonidae

One of the axioms of ecology is that the diversity of species is greater at low than at high latitudes. This was appreciated by Alfred Russell Wallace more than a century ago and has been repeatedly demonstrated in the more conspicuous groups of organisms such as trees, birds, and butterflies. Ecologists have produced a variety of theories, none entirely satisfactory, to explain latitudinal gradients in diversity, but an understanding of the phenomenon remains as elusive today as in Wallace's time.

Here we report an apparent exception to the rule in a family of parasitic Hymenoptera, the Ichneumonidae, which are mostly small, wasp-like insects. There are, however, more species of Ichneumonidae than of vertebrates, and in few groups of animals can there remain so many undescribed species. Virtually all ichneumonids are parasitic on the larvae and pupae of insects with a complete metamorphosis, especially Lepidoptera, Diptera, Neuroptera, Coleoptera, and Symphyta. The dependence of ichneumonids on herbivorous insects indicates that they have an enormous potential, as yet hardly explored, for programmes of biological control of pests.

Ichneumonids are abundant wherever there is vegetation and where it is not too dry, but few species are common. We have obtained large samples of them from three gardens: at Kampala in Uganda, Freetown in Sierra Leone, and Leicester in England. We have available another sample collected in Skåne, southern Sweden, not from a garden, but from disturbed land along a small stream. All four samples were collected in Malaise traps[1], tent-like constructions into which flying insects wander to fall eventually into a bottle of alcohol attached to the top of the trap. Malaise traps employ neither bait nor light and do not therefore attract insects, although undoubtedly some insects are more likely to be caught than others because of their behaviour. The only assumption we have made in our calculations of species diversity is that the traps do not select for either common or rare species.

The three gardens are not of course ecologically identical—Kampala and Freetown are on opposite sides of tropical Africa; the garden at Leicester is in the suburbs of a large city—nevertheless they share features common to all gardens: an abundance of flowers, and a wide variety of plants and trees introduced from other parts of the world. The Skåne sample should perhaps be considered separately and is added only to provide further evidence of the pattern of species diversity discovered.

Results of an analysis of the four samples are given in Table 1. The traps were operated day and night in all weather, and the sampling period in each locality included all months in which insects are flying. The differences in sample size shown in Table 1 do not necessarily reflect differences in abundance; much depends on the habitat and the efficacy of the traps in different situations, but we have the impression that, as a group, ichneumonids are rather more abundant in the temperate than in the tropical localities. The number of species recorded in each of the four samples is astonishing; the figure of 326 species in the Leicester garden represents about 16% of the species on the British list[2].

The information theory index of diversity[3], H, enables an overall comparison of the four samples. The values of H for Freetown and Leicester are not significantly different but all other paired samples are different from each other at the 1% level. The value of H is significantly smaller for the Kampala than for the Leicester and Skåne samples and is smaller for the Freetown than for the Skåne sample.

The massive species diversity in the Ichneumonidae is further reflected in Table 1 by the calculation of the number of species taken once only and the percentage of the commonest species in each of the four samples. A quite extraordinary number of species was found once only and no species is really common.

Table 1 Two tropical and two temperate samples of Ichneumonidae compared

	Sample size	Species	Diversity index, H, and s.e.	Species taken once	% commonest species
Kampala	2,268	293	4.524±0.032	116	10.1
Freetown	1,979	319	4.934±0.029	117	4.9
Leicester	2,495	326	4.937±0.024	122	3.2
Skåne	10,994	758	5.481±0.014	203	5.5

There is no evidence of the expected greater diversity in the two tropical localities. A large (but for the tropical species, unknown) proportion of the species of ichneumonid parasitise Lepidoptera caterpillars. There is no doubt about the greater diversity of Lepidoptera in the tropics. We estimate that there are 300 species of butterflies in the Freetown garden[4] but have found only 15 species in the Leicester garden. Butterflies comprise only a small fraction of the total species of Lepidoptera but probably reflect the situation in the remainder. The explanation of the high diversities in both the tropical and the temperate samples is probably associated with the fact that each species of ichneumonid tends to be niche specific rather than host specific[5]. In the parasite-host relationships considered here there may be no more niches in the tropics than in the temperate region, in contrast to the situation that probably prevails for most other feeding relationships.

We suggest on the basis of these samples that the Ichneumonidae are an exception to the rule that species diversity decreases from the equator to the poles, and that this single, little known family of insects can produce, even within a small area, a variety of species unparalleled in any other family of organisms. The Leicester sample is from an ordinary suburban garden. The trap sampled insects flying below 1.1 m over an area of only 2.6 m^2 and yet in one season produced 326 species, seven times as many as have been recorded in Monks Wood National Nature Reserve[6]. Both tropical samples contain many species and some genera new to science[7] and the entire collection has been given to the American Entomological Institute.

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1 Townes, H., *Ent. News*, **83**, 239 (1972).
2 Kloet, G. S., and Hincks, W. D., *A check list of British insects* (Stockport, 1945).
3 MacArthur, R. H., *Biol. Rev.*, **40**, 510 (1965).
4 Owen, D. F., *Tropical butterflies: the ecology and behaviour of butterflies in the tropics with special reference to African species* (Oxford, 1971).
5 Townes, H., *Proc. Tall Timbers Conf. Ecol. Animal Control Habitat Management*, **3**, 235 (1972).
6 Moller, G. J., *in Monks Wood: a nature reserve record* (edit. by Steele, R. C., and Welch, R. C.), (Cambridge 1973).
7 Townes, H., and Townes, M., *Mem. Am. ent. Inst.*, **19**, 1 (1973).

Role of antennae of the dragonfly *Orthetrum cancellatum* in flight control

The function of the antennae of insects as air current sense organs and their relationship to flight control has already been investigated by behavioural studies in flies, bees and locusts[1–6], all of which are excellent fliers. The dragonfly is also an excellent flier although it possesses a very different flight apparatus[7–9]. Even though flight is apparently influenced by the eyes and the neck sense organs of the dragonfly[10,11], it seems unlikely that these organs have anything to do with the control of movements which are dependent on the surrounding air for their stimulation. For the measurement of flying speed relative to the surrounding air, for example, one might expect that, just as in the other insects, the antennae play an important and specific role. It is this hypothesis which has been tested in the present work, the results of which are reported below.

Experiments were carried out on the dragonfly *Orthetrum cancellatum L.* (Anisoptera, Odonata) 2–6 d after the imaginal moulting of larvae, which had been collected from various ponds in the area. For the flight experiments the foremost dorsal part of the mesepisterna of the animal was firmly fixed to a rod by the application of rosin wax. The wing-stroke angles, φ (Fig. 2*a*) of the fore and hindwings of the animal flying tethered in front of a wind tunnel were measured from photos (red light; exposure time, 1 s) which were taken just after the start of flight. The optical axis of the camera was perpendicular to the wing-stroke plane which formed an angle of about 45° with the vertical. The room temperature was 29° C. Control measurements at the end of each experiment showed that the animals were not noticeably exhausted.

The two antennae of the dragonfly are relatively small and are located in the antennal sockets between the frons, the vertex and the compound eyes (Fig. 1). Each antenna is composed of the basal small conical scapus, the somewhat elongated cylindrical pedicellus and the distal bristle-like flagellum pointing frontally, upwards and laterally. It is the scapus–pedicellus joint which is primarily responsible for active movements of the antenna even though passive

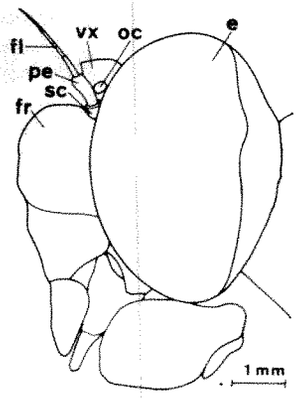


Fig. 1 Lateral view of the head of *Orthetrum cancellatum* (♀). The antenna consists of the scapus (sc), pedicellus (pe) and flagellum (fl). e, Eye; fr, frons; oc, ocellus; vx, vertex.

movements can be effected by external forces in the joints between the four flagellar segments as well as in the pedicellus–flagellum joint. For example, during flight, the flagellum is bent backwards at these joints by the impact pressure from the frontal air current. It is possible that it is this movement which stimulates mechanoreceptors situated at these joints (for example, Johnston's organ located in the pedicellus) and which consequently may allow the dragonfly to register its flight speed relative to the surrounding air, as already shown in other insects[1–6]. To test this hypothesis, the flight behaviour of normal insects and insects without flagellae was compared, using the total wing-stroke angles (φ_t) of the fore and hindwings as behavioural flight parameters. The dorsal (φ_d) and ventral components (φ_v) of the wing-stroke angles were also evaluated as shown in the insert of Fig. 2*a* ($\varphi_t = \varphi_d + \varphi_v$).

In normal animals, the total wing-stroke angles (φ_t) of the fore and hindwings in the absence of air currents are approximately of the same value (Fig. 2*a* and *d*). These wing-stroke angles (φ_t) are reduced equally in both pairs of wings as air speed increases (Fig. 2*a*: *n*; $\triangle\varphi_{t0-4}=48°$. Fig. 2*d*: *n*; $\triangle\varphi_{t0-4}=40°$. $P=0.18$). This decrease depends only on the reduction of the dorsal component (φ_d; Fig. 2*b*, *e*: *n*) since the ventral component (φ_v) is nearly independent of the air speed (forewing: Fig. 2*c*: *n*, $\triangle\varphi_{v0-4}=8°$; $P=0.08$. Hindwing: Fig. 2*f*: *n*; $\triangle\varphi_{v0-4}=7°$; $P=0.045$).

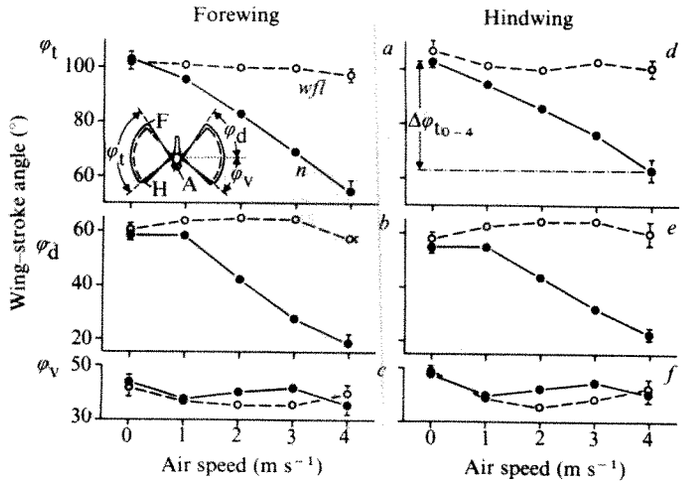


Fig. 2 *a–f*, Dependence of the wing-stroke angle (φ) on the air speed of a wind tunnel. Each point represents the mean of 70–110 measurements from 11 females and 8 males. Vertical bars indicate the standard error (± s.e.). A, antenna; F, forewing; H, Hindwing; *n*, normal animals; *wfl*, animals without flagellae: φ_t, total wing-stroke angle; φ_d (φ_v), dorsal (ventral) component of the wing-stroke angle; $\triangle\varphi_{t0-4}$, difference between the wing-stroke angle without air current (0) and with frontal air current with a speed of 4 m s^{-1} (ref. 4).

On the contrary, in insects whose flagellae had been cut off at their base 30 min or more before the experiments, the decrease of the wing-stroke angles of the forewings with increasing air speed ($\triangle\varphi_{t0-4}=7°$; Fig. 2*a*: *wfl*) is much smaller than in normal insects ($\triangle\varphi_{t0-4}=48°$; Fig. 2*a*: *n*; $P<0.0002$). This is also true for the wing-stroke angles of the hindwings (Fig. 2*d*: *wfl*, $\triangle\varphi_{t0-4}=6°$; *n*, $\triangle\varphi_{t0-4}=40°$, $P<0.0002$).

It seems, then, that the different flight behaviour of normal insects and those without flagellae is reflected by the distinct change in the dorsal component of the wing-stroke angle (φ_d). More specifically, the decrease in dorsal component (φ_d) as a function of increasing air speed is much reduced in both the forewing (Fig. 2*b*; $P<0.0002$) and the hindwing (Fig. 2*e*; $P<0.0002$) of dragonflies without flagellae. The ventral component (φ_v) of both wing pairs, however, is independent of the air speed both in animals without flagellae and normal animals (Fig. 2*c*, *f*).

The results indicate that some mechanoreceptors in the antennae, stimulated during flight by the action of air currents on the flagellae, are responsible for relaying information about the air speed necessary for the control of wing movements. These receptors, however, affect only the dorsal wing-stroke angle, the ventral components of both wings functioning independently of the surrounding air. That means that the antennae of dragonflies may be air-current sense organs which control flight performance, probably the flying speed, a finding which is consistent with those previously reported for other insects[1–6].

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1 Burkhardt, D., Schneider, G., *Z. Naturf.*, **12b**, 139–143 (1957).
2 Burkhardt, D., Gewecke, M., *Cold Spring Harbor Symp. quant. Biol.*, **30**, 601–614 (1965).
3 Heran, H., *Z. vergl. Physiol.*, **42**, 103–163 (1959).
4 Gewecke, M., *Nature*, **225**, 1263–1264 (1970).
5 Gewecke, M., *J. comp. Physiol.*, **80**, 57–94 (1972).
6 Gewecke, M., in *Experimental Analysis of Insect Behaviour*, edit. by Barton Browne, L., 100–113 (Springer-Verlag, Berlin, 1974).
7 Chadwick, L. E., *Bull. Brooklyn ent. Soc.*, **35**, 109–112 (1940).
8 Russenberger, H., Russenberger, M., *Mitt. naturf. Ges. Schaffhausen*, **27**, 1–88 (1959).
9 Neville, A. C., *J. exp. Biol.*, **37**, 631–656 (1960).
10 Mittelstaedt, H., *Z. vergl. Physiol.*, **32**, 422–463 (1950).
11 Hisada, M., Tamasige, M., Suzuki, N., *J. Fac. Sci. Hokkaido Univ.*, (Ser. 6, Zool.), **15**, 568–577 (1965).

Evidence for a gene-specific RNA determining resistance in wheat to stem rust

Although the inheritance of resistance[1] in wheat (*Triticum aestivum* L.) to stem rust (*Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn.) is fairly well understood, most efforts to elucidate the biochemical mechanism involved have been unsuccessful. Biochemical studies of plant diseases involving rusts and other obligate parasites[2] have contributed to knowledge of biochemical symptomatology, but no chemical changes have been correlated with the action of specific genes for resistance. Future advances it seemed,

would depend on a reliable test to facilitate such correlations, and so we have developed a bioassay with the necessary specificity, which detects the presence of metabolites involved in the expression of the resistance. We have thus been able to demonstrate a gene-specific RNA that seems to be directly responsible for initiating the resistant reaction in wheat against stem rust. A preliminary report of some of these findings has been presented[3].

In our work with the wheat/rust system we were interested in the highly specific interactions that are consistent with the gene-for-gene hypothesis[4]. Briefly, this hypothesis states that for every gene for resistance in the host there is a specific and related gene for virulence in the parasite. Interaction between these genes may involve transfer of information across the host/parasite interface. The substances involved in this transfer must be able to carry a high informational content because many genes for resistance have already been identified. Nucleic acids, proteins and complex carbohydrates fulfil this requirement. We believed that compounds involved in the expression of resistance could best be obtained from resistant reacting tissues containing a large number of haustoria, for histological observations[5] of the resistant interaction have shown that cell necrosis, which is characteristic of resistance, is always associated with the establishment of haustoria. To obtain such tissue we exploited the temperature lability[6] of the *Sr6* gene at temperatures above 25° C. Wheat leaves containing this gene were inoculated with spores of the avirulent race C17 (56), and maintained at 26° C to suppress host cell necrosis that would normally occur in this interaction at lower temperatures. Seventy-two hours after inoculation, when the leaves were extensively colonised, plants were transferred to 20° C for a further 30 h to initiate the necrotic reaction, then collected for the preparation of extracts.

In developing a useful bioassay it was desirable to simulate natural conditions as closely as possible. Normally, in interactions involving the *Sr6* gene for resistance and the *P6* gene for avirulence, necrotic cells develop at most infection sites shortly after haustorial formation, whereas few necrotic cells are produced in susceptible interactions[5]. Our objective was to test extracts for their ability to induce the formation of similar necrotic cells in wheat leaves inoculated with a virulent race of stem rust. We defined an active extract as one that induces significantly more necrotic cells in the genotypically resistant leaves (R line) than in the genotypically susceptible leaves (S line) of wheat.

For statistical purposes, necrotic sites consisting of one or several contiguous necrotic cells were counted. If the average incidence of necrotic sites is the same in leaves of both lines, then we would expect, if we had equal numbers of observations in both cases, that half the necrotic sites would appear in the sample of the R line. This property can be used as the basis for a statistical test[7] which has certain optimal properties if the necrotic site counts follow a Poisson law. If N_R and N_S necrotic sites occur in n_R and n_S observations in leaves of the R and S lines, respectively, then we can use the properties of the binomial distribution to test if N_R necrotic sites occur in a total random sample of (N_R+N_S) necrotic sites with probability $n_R/(n_R+n_S)$ expected if the average incidence of necrotic sites is the same in leaves of both R line and S line. For $N_R+N_S > 20$, a normal approximation was used.

Preliminary experiments with crude nucleic acid preparations showed that significantly more ($P < 0.005$) necrotic sites were produced when the extract obtained from the *Sr6/P6* interaction was injected into bioassay plants of the R line (containing the *Sr6* gene), than when it was injected into plants of the S line (Fig. 1). Non-injected leaves of either line, and leaves that were injected but not infected, contained virtually no necrotic sites. The presence of necrotic sites in infected leaves of the S line suggested that the crude nucleic acid extract also contained substances which caused nonspecific necrosis in infected leaves of both lines, in addition to the component that showed specificity towards the *Sr6/p6* interaction.

Typically, crude nucleic acid extracts contained RNA, DNA and protein in a ratio of 5:4:1, by weight. Because of their high DNA content these crude extracts were viscous and difficult to inject. Treatment with cetyltrimethylammonium bromide (CTA), DNase and protease yielded preparations with higher specificity towards bioassay plants

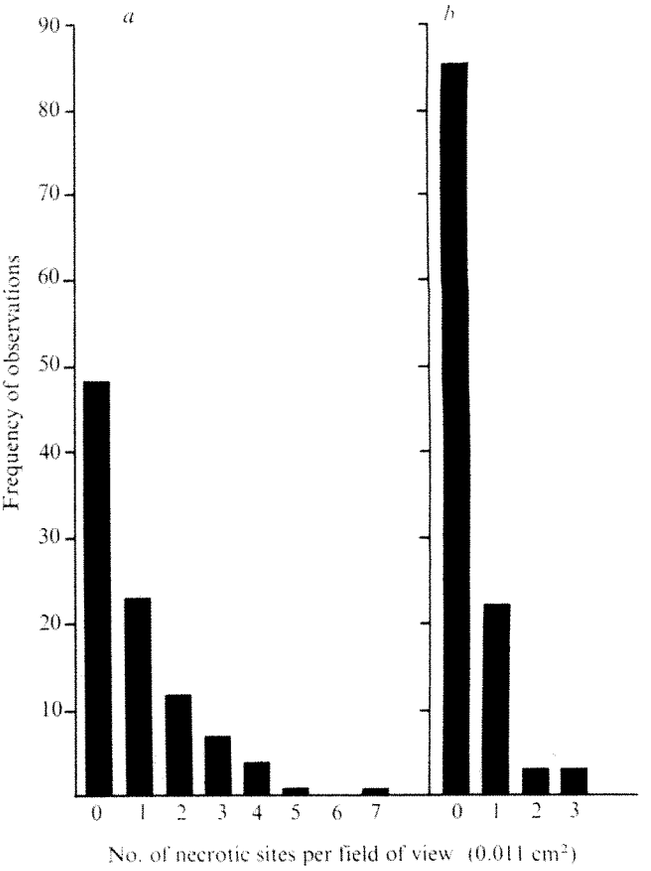


Fig. 1 Bioassay of a crude nucleic acid extract prepared from Chinese Spring wheat (containing the *Sr6* gene for resistance) infected with race C17(56) of wheat stem rust (containing the *P6* gene for avirulence). The extract was prepared[8] by homogenising plant material with *n*-butanol/chloroform/1M K_2HPO_4 (1:1:2 v/v/v), followed by deproteinisation of the aqueous phase with phenol. Nucleic acids were recovered by precipitation with ethanol and redissolved in 15 mM NaCl, 1.5 mM sodium citrate, *p*H 7.0 (1/10 SSC). Primary leaves of bioassay plants (7-d-old) were heavily infected with a virulent race (C45(56A)) to give 800–1,600 appressoria cm^{-2}. Uredospore suspensions (6 mg ml^{-1}) in 0.1% gelatin and 0.001% Tween 20 were applied with cotton swabs along 2 cm of the adaxial (upper) leaf surface, 2–3 cm from the leaf tip. Inoculated plants were incubated in the dark (16 h, 20°C) then transferred to a growth chamber (16 h 20,000 lux; 8 h darkness; 20°C). This resulted in near-synchronisation of fungal development[5]. Twenty-four hours after the beginning of the first photoperiod, extracts in 1/10 SSC were injected[9] into leaves between the tip and the inoculated area, so that the intercellular spaces in the inoculated region were flooded with extract. Six hours later the infected regions were excised, cleared and stained with Trypan blue (C.I. No. 23850) for microscopic examination[5]. Necrotic cells appeared to have collapsed and stained dark blue. They were also identified in unstained tissue by their characteristic fluorescence[10]. Resistant and susceptible near-isogenic lines of wheat produced by W. Q. Loegering[11] from chromosome substitution lines of Chinese Spring wheat were used in these studies. *a*, R line (*Sr6/p6* interaction); mean number of necrotic sites per field of view was 1.0 *b*, S line (*sr6/p6*) interaction; mean number of necrotic sites per field of view was 0.246.

Table 1 Characterisation of the active component in a crude extract from resistant reacting wheat leaves (*Sr6/P6* interaction)

Treatment of crude extract	Chemical analysis (μg ml⁻¹)				Necrotic sites per cm⁻² leaf of bioassay plants		
	A_{260}	RNA	DNA	Protein	R line (*R*)	S line (*S*)	*R/S*
None	40	1074	888	188	97.4	62.6	1.6**
Cetyltrimethyl-ammonium bromide (CTA) precipitation[12]	40	1064	968	53	20.2	7.6	2.7**
CTA precipitation followed by DNase*	86	1780	220§	—	56.0	18.6	3.0**
	66	1369	169§	—	44.4	12.2	3.6**
CTA precipitation followed by DNase and protease†	70	—	—	384¶	48.5	8.1	6.0**
CTA precipitation followed by RNase‡	41	0	2440	—	8.5	20.2	0.4**

Details of the preparation of the crude extract are given in the legend to Fig. 1.

RNA and DNA were determined with ethidium bromide[13] using highly polymerised calf thymus DNA (Sigma) and wheat germ RNA (Calbiochem) as standards. Protein was determined with Folin reagent[14] using bovine serum albumin as standard. Plants used for the bioassay were: for R line, Chinese Spring wheat containing the *Sr6* gene; and for S line, Chinese Spring wheat containing the *sr6* gene. Both were infected with race C45(56A) of wheat stem rust (containing the *p6* gene).

* We used 100 μg of RNase-free DNase (Worthington) and 30 A_{260} units nucleic acid ml⁻¹, 500 mM NaCl, 50 mM $MgCl_2$, 10 mM Tris-HCl (*p*H 7.4) 1 h, 20° C. After incubation the mixture was deproteinised with phenol, extracted five times with ether, and then nucleic acid precipitated with ethanol and dissolved in 1/10 SSC.

† We used 10 mg of predigested insolubilised protease (Miles) and 30 A_{260} units nucleic acid ml⁻¹, in 1/10 SSC, 1 h, 37° C. Protease was removed by centrifugation, nucleic acid precipitated with ethanol and dissolved in 1/10 SSC.

‡ We used 100 μg pancreatic RNase (Sigma), 25 units T_1 RNase (Miles) and 30 A_{260} units nucleic acid ml⁻¹, 1 h, 37° C. Enzyme was removed with phenol as described above.

§ Oligonucleotides, as determined by acrylamide gel electrophoresis.

¶ Apparent high protein content due to products from self-digestion of protease.

** *R* significantly greater than *S* at 0.05 level of probability.

of the R line (Table 1). Digestion with RNase, however, destroyed this specificity, indicating that the active component was a ribonucleic acid. CTA precipitation of the crude nucleic acid, followed by digestion with DNase, resulted in preparations containing RNA, oligonucleotides of DNA and protein in a ratio of 94 : 5 : 1, by weight. Such preparations had a low viscosity at high RNA concentrations, and could be readily injected into wheat leaves. Eight separate RNA extracts prepared in this way were tested in this bioassay system and they all caused more necrosis in plants of the R line than of the S line.

Having demonstrated that an active RNA can be obtained from the *Sr6/P6* interaction, we wished to know if active extracts could be obtained from other gene interactions, and whether active RNA isolated from one system was active in systems containing other genes. An active RNA extract obtained from the *Sr6/P6* interaction was not active in the *Sr5* bioassay system (Table 2). When a separate extract prepared from leaves of the *Sr6* R line, infected with a different race (avirulent to *Sr6*) was used, it was active in the *Sr6* bioassay system but inactive in the *Sr11* bioassay system. Conversely, an extract from leaves of the *Sr11* R line, maintained at 20° C and infected with a race avirulent to *Sr11* (*Sr11/P11* interaction), was active in the *Sr11* bioassay system but inactive in the *Sr6* bioassay system. It was not possible to obtain an active extract from the *Sr5/P5* interaction. We attributed this lack of activity to the fact that the host/parasite interaction conditioned by the *Sr5* gene is usually restricted to very few cells and permits little potential for the production of detectable amounts of an active component. Since the *Sr5* gene is not temperature sensitive, it is not possible to obtain more extensive colonisation of the leaves by growing the inoculated plants at higher temperatures.

In summary, our experiments have demonstrated that RNA is directly involved in the resistant reaction of wheat to stem rust and that the active RNA shows specificity comparable to the specificity of the host/parasite system. Our bioassay should make it possible to obtain further information on the chemical nature of the active RNA and its production, and on the mechanism of action leading to cell necrosis. We believe that this approach to the study of host/parasite interactions may be applicable to other host/parasite systems for which a gene-for-gene relationship exists.

Table 2 Specificity of RNA from rust-infected resistant reacting wheat leaves towards host genes for resistance

Host-parasite complex from which RNA extract was obtained		Bioassay system Gene interaction			Necrotic sites per cm⁻² leaf of bioassay plants		
Gene interaction	Avirulent race* used	R line	S line	Virulent race* used	R line (*R*)	S line (*S*)	*R/S*
Sr6/P6	C17(56)	*Sr6/p6*	*sr6/p6*	C45(56A)	15.9	9.1	1.8†
		Sr5/p5	*sr5/p5*	C45(56A)	3.9	5.8	0.7
Sr5/P5	C5(29–1)	*Sr5/p5*	*sr5/p5*	C11(15B-4)	3.6	3.6	1.0
		Sr6/p6	*sr6/p6*	C45(56A)	1.0	5.6	0.2
Sr6/P6	C18(15B-1L)	*Sr6/p6*	*sr6/p6*	C22(32)	9.5	3.2	3.0†
		Sr11/p11	*sr11/p11*	C22(32)	1.8	3.0	0.6
Sr11/P11	C21(32)	*Sr11/p11*	*sr11/p11*	C22(32)	4.9	2.1	2.3†
		Sr6/p6	*sr6/p6*	C22(32)	2.9	3.7	0.8

RNA was purified from crude extracts using CTA precipitation and digestion with DNase (see Table 1).

* C numbers refer to Canadian designations of stem rust races[15]; numbers in brackets refer to standard race designations[16].

† *R* significantly greater than *S* at 0.05 level of probability.

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1 McIntosh, R. A., *Proc. fourth int. Wheat Genet. Symp.*, Columbia, Missouri (Agricultural Experiment Station, University of Missouri, in the press).
2 Scott, K. J., *Biol. Rev.*, **47**, 537–572 (1972).
3 Howes, N. K., Kim, W. K., Rohringer, R., and Samborski, D. J., *Proc. second int. Congr. Pl. Path.*, Abstr. 1004 (1973).
4 Flor, H. H., *A. Rev. Phytopath.*, **9**, 275–296 (1971).
5 Skipp, R. A., and Samborski, D. J., *Can. J. Bot.* (in the press).
6 Forsyth, F. R., *Can. J. Bot.*, **34**, 745–749 (1956).
7 Lehmann, E. L., *Testing statistical hypotheses*, 140 (Wiley, New York, 1959).
8 Diener, T. O., *Virology*, **45**, 411–428 (1971).
9 Hagborg, W. A. F., *Can. J. Bot.*, **48**, 1135–1136 (1970).

[10] Marte, M., and Montalbini, P., *Phytopath. Z.*, **75**, 59–73 (1972).
[11] Loegering, W. Q., and Harmon, D. L., *Phytopathology*, **59**, 456–459 (1969).
[12] Bellamy, A. R., and Ralph, R. K., *Methods in Enzymology*, **12B**, 156–160 (1968).
[13] Le Pecq, J.-B., and Paoletti, C., *Analyt. Biochem.*, **17**, 100–107 (1966).
[14] Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265–275 (1951).
[15] Green, G. J., *Can. J. Bot.*, **49**, 1575–1588 (1971).
[16] Stakman, E. C., Stewart, D. M., and Loegering, W. Q., *U.S. Dep. Agric. A.R.S., Bull.*, E 617 (1962).

Virus-like particles in *Leishmania* parasites

VIRUSES or virus-like particles (VLPs) have been found in *Entamoeba histolytica*[1], in the amoeboflagellate *Naegleria gruberi*[2] and in malaria parasites[3–5], but there have been no reports as yet of such organisms being found in the Trypanosomatidae. During investigations on the ultrastructure of various members of the genus *Leishmania*, however, VLPs have been found in the promastigote forms of an isolate of *Leishmania hertigi*. *Leishmania hertigi*[6] was obtained from Panama and kept in our laboratory on blood agar slopes or as cryopreserved material at the temperature of liquid nitrogen. Examination of the promastigotes of *L. hertigi* immediately revealed cytoplasmic inclusions of a type which had not been observed in any of our previous studies on the promastigotes of *Leishmania* species in culture.

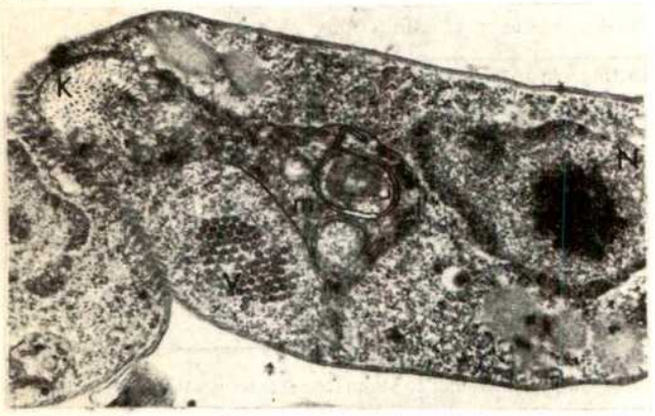


Fig. 1 Electron micrograph of section of promastigote of *L. hertigi* showing group of VLPs (V), and hollow core of some of the particles can be seen. Material from 6 d culture fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer, post-fixed in 1% osmium tetroxide and embedded in Araldite. K, Kinetoplast; m, mitochondrion; N, nucleus (× 22,000).

The particles are distributed throughout the cytoplasm of the promastigotes (Figs 1 and 2), and are seen in a high proportion of sections examined under the electron microscope. It is likely therefore that all parasites of this isolate are infected. The particles have no effect on the growth pattern or division of the *Leishmania* promastigotes in culture and no apparent detrimental effect on cell structure. The promastigotes divide by longitudinal binary fission in the cultures and the particles are present in the two halves of the dividing parasite and are maintained in this way. The individual particles are usually spherical in section 55–60 nm in diameter and packed in a manner typical of virus organisation, (Figs 1, 2, and 3). There is an electron-dense outer ring and an electron-lucid core, 17–20 nm in diameter (Fig. 3). The number of particles in each group is variable.

Within the cytoplasm of parasites which are known to contain VLPs, unusual rod-like structures are found which are not usually seen in trypanosomatid cells. The presence of VLPs may induce the production of these inclusions. It is not known if *L. hertigi* acquired the VLPs in natural conditions from a sandfly vector or from the mammalian

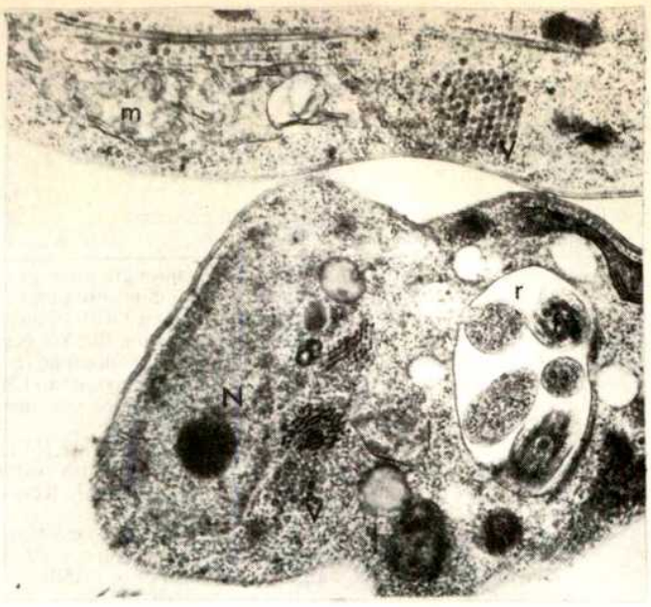


Fig. 2 Section through two promastigotes of *L. hertigi*; both parasites show VLPs (V) in their cytoplasm. Unusual cytoplasmic inclusions are present in both these parasites and may be related to the presence of VLPs. Fixation as in Fig. 1. r, Reservoir; f, flagellum (× 22,000).

host, the tropical porcupine *Coendou rothschildi*. It is unlikely the VLPs were acquired in laboratory conditions as no other *Leishmania* isolates examined have been found infected, but even if the parasites were infected in the laboratory such an observation in itself would be of great interest.

Further studies on these inclusions are in progress to characterise their nature and to determine if they are infective to other species of *Leishmania* and *Trypanosoma* and to mammalian cell cultures. The effects of any virus on the parasite genome have obvious evolutionary implications. It is also possible that intercurrent virus infections may play a hitherto unrecognised role in the clinical manifestations of *Leishmania* and *Trypanosoma* infections. Relatively few isolates have been examined with the electron microscope and such associations may be common with resulting important clinical and chemotherapeutic implications, both in treatment and possibly in transfer of drug resistance between parasites.

I thank Dr A. Herrer for supplying the isolate of *L. hertigi* and to my colleagues in the Department for support and encouragement, particularly Professor W. Peters and for the advice of Professor P. C. C. Garnham and Dr

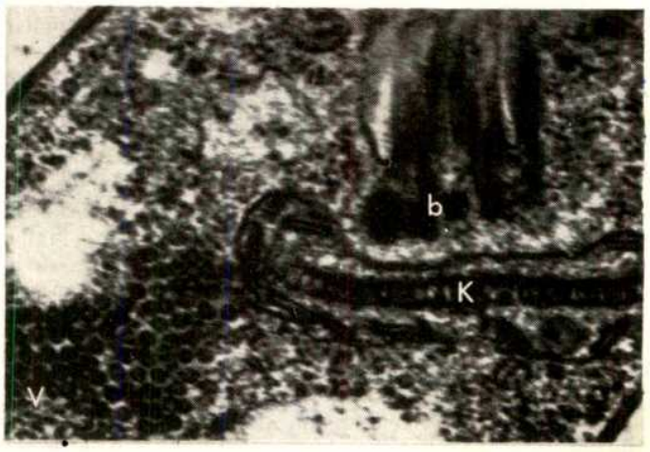


Fig. 3 Showing aggregation of VLPs adjacent to the kinetoplast (K) of *L. hertigi*. The dense outer layer and electron lucid core can be seen in some particles. Fixation as in Fig. 1. b, Basal body (× 120,000).

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1 Mattern, C. F. J., Diamond, L. S., and Daniel, W. A., *J. Virol*, **9**, 342–358 (1972).
2 Schuster, F. L., and Dunnebacke, T. H., *J. ultrastruct. Res.*, **36**, 659–668 (1971).
3 Terzakis, J. A., *Milit. Med.*, **10** (Supplement), **134**, 916–921 (1969).
4 Davies, E. E., Howells, R. E., and Venters, D., *Ann. trop. Med. Parasit.*, **65**, 403–408 (1971).
5 Bird, R. G., Draper, C. C., and Ellis, D. S., *Bull. Wld Hlth Org.*, **46**, 337–343 (1972).
6 Herrer, A., *J. Parasit.*, **57**, 626–629 (1971).

Devastation of a fringing coral reef by *Acanthaster*

A RECENT synopsis of reports on the status of populations of the Crown of Thorns Starfish, *Acanthaster planci* (L.), in the Indo-West Pacific[1], indicates that no extensive destruction of living corals by this starfish has yet been recorded in the western Indian Ocean. Here I present a preliminary account of an aggregated starfish population and extensive mortality of hard corals, observed in May 1973 off the north coast of Grande Comore, Comoro Islands.

Examination of a portion (about 150×300 m) of the fringing reef off Lac Salé, north-east Grande Comore (at 11° 21′S, 43° 22′E), showed an approximate 90% mortality of hard corals; this was estimated by eye at four points on the reef slope as the percentage of recently dead coral (skeletons both white and various shades of green, being in different stages of colonisation by green algae) over total coral (recently dead plus still living coral).

That the mortality was due to *Acanthaster* was inferred from the presence on the Lac Salé reef of large numbers of these starfish and from the discovery of many more at Mitsamiouli on the north-west corner of Grande Comore, where an estimated 40% of the hard corals had recently been killed. Coral predation became less frequent as I moved to the south off Mitsamiouli; my visit to Grande Comore coincided, therefore, with the recent devastation of the Lac Salé reef and the incipient devastation of the Mitsamiouli reef. The indication was that a large number of *Acanthaster* had moved as a front in an anti-clockwise direction around the northern coast of Grande Comore. Thus, the starfish that I found at Lac Salé would only have been the remnants of this mobile population. The survival of many corals in shallow water and of a small percentage of them on the reef slope, which was dominated by 'table' forms of the genus *Acropora*, rules out freshwater run off or upsurge of cold water as reasons for mortality, and further, nearly all the *Acropora* tables showed no sign of mechanical damage, although denuded of their tissue; thus changes in purely physical parameters cannot explain this mortality. A similar level of hard coral mortality due to *Acanthaster* has been observed on other badly affected coral reefs[2,3].

Hard coral colonies on the reef slope survived especially in the wider, outer parts of the surge channels which dissect the reef slope; here the coral colonies were separated from the main reef by a sandy floor, which may give partial protection from *Acanthaster*[4]. Many colonies also had been only partly denuded of their living tissue. Surviving colonies and surviving patches of partially denuded colonies are probably important in reef recovery[5].

The number of fish species recorded by sight on this reef was consistent with values for other western Indian Ocean island reefs where little or no evidence of *Acanthaster* was found (Table 1). Out of a total of 20 species of chaetodontids, which are typical 'coral fishes'[6], recorded by sight at the 3 localities, there were 13 at Lac Salé (90% hard coral mortality), 12 at Iconi (hard corals uncommon), and 12 at Mitsamiouli (40% hard coral mortality in places). Thus while *Acanthaster* and other factors can have a destructive effect on the hard coral cover, they may not affect the number of fish species. Porter[7] found that *Acanthaster* predation can have a beneficial effect on coral species diversity. Heavy stressing of Grande Comore reefs by bottom fishing and spear fishing inhibits more detailed analysis of fish faunal differences between the affected and unaffected reefs; certain species, however, such as the herbivorous acanthurids[8] *Ctenochaetus striatus* and *Acanthurus leucosternon*, may have increased on the Lac Salé reef.

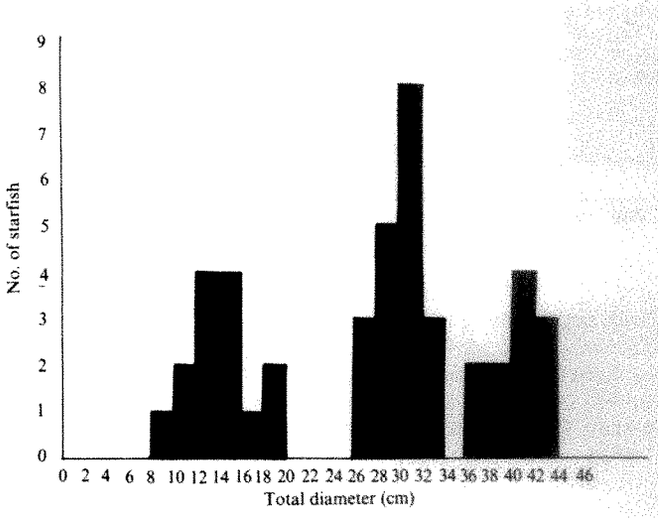


Fig. 1 Size-frequency histogram (total diameter) of 44 *Acanthaster planci* collected between 1300 and 1500 h on May 24, 1973 in the same reef flat habitat of the Lac Salé, Grande Comore, fringing reef.

Few *Acanthaster* were found in the zone of high hard coral mortality. Nearly all the individuals found were confined to the reef flat, where the coral cover was poor, but a higher proportion of the corals still survived. Conditions of swell were more marked on the reef flat than on the reef slope; starfish detached from the substrate on this reef flat had great difficulty in regaining their hold. No starfish were found in the act of feeding during the daytime, and very few individuals were visible from the sea surface. Small individuals were found only by breaking open clumps of branching corals. The size-frequency histogram (total diameter) for 44 starfish collected in the same reef flat habitat indicates the presence in the population of at least two size-classes (Fig. 1). The population may still be a mixed one, however, with animals contributed from the once-rich reef slope and from the impoverished reef flat; Dana *et al.*[9] suggested that the bimodal size distribution reported for some Sudanese Red Sea *Acanthaster*[10] might not be due to the presence of two age classes, and could have resulted from the sampling of different habitats, where coral food was more or less abundant.

H. R. Lubbock (personal communication, 1971) found large numbers of *Acanthaster* in September 1971 in Moroni harbour, Grande Comore, and from discussions with a local diver (J. Nicholas, personal communication) it seems probable that this starfish has been common on Grande Comore for even longer.

In addition to the 18 localities recorded in Table 1, I have examined nine others in the granitic Seychelles; only off Lac

Table 1 Eighteen western Indian Ocean island localities visited between April 19 and July 10, 1973

Locality	Observation time (h)	Maximum depth (m)	Fish species seen (No.)	*Acanthaster*: type and state of reef
Lac Salé, Grande Comore	3	9	104	Many starfish present; ~ 90% dead coral on reef slope. Fringing. Reef once rich.
Mitsamiouli, Grande Comore	1	13	73	Many starfish present; ~ 60% corals still living. Fringing. Reef still rich.
Iconi, Grande Comore	1	26	82	Three seen; almost no live hard corals. Fringing. Reef long 'dead'.*
Récif Vailheu, Grande Comore	1	37	85	None seen; no evidence of predation. Rich table reef.
Canzoni I., Moheli	1	26	87	None seen; no evidence of predation. Fringing. Rich coral in shallows.
Ouénéfou I., Moheli	1	19	106	None seen; no evidence of predation. Fringing. Rich coral in shallows.
Andrema I., Mayotte	4	34	154	One individual seen; no evidence of predation. Fringing. Very rich coral in shallows.
Banc du Geyser	1	28	80	None seen; no evidence of predation. Atoll. Rich coral on outer reef flat.
Banc Suzy, Nossi Bé	1	16	57	None seen; no evidence of predation. Patches.
Tsara Bajina, Mitsio Is.	2	6	97	None seen; no evidence of predation. Patches.
Behangovo, Mitsio Is.	0.5	28	43	Four seen; no evidence of predation. Rare patches on desolate slope.
Ankarea, Mitsio Is.	3	9	113	None seen; no evidence of predation. Patches.
Radam Is.	1	9	76	None seen; no evidence of predation. Patches.
Poivre, Amirante Is.	2.5	19	93	None seen; no evidence of predation. Atoll. Rich coral in places.
Alphonse, Admirante Is.	4	43	152	None seen; no evidence of predation. Atoll. Rich coral in shallows.
West North I., Cosmoledo	4	37	152	None seen; no evidence of predation. Atoll (elevated). Rich coral on outer reef flat.
Assumption	4	56	160	None seen; no evidence of predation. Elevated reef. Rich coral on outer reef flat in places.
Johnny Channel, Aldabra	1.5	9	84	None seen; no evidence of predation. Atoll (elevated). Some rich coral on channel edges.

Notes were taken underwater.
*No evidence for implication of *Acanthaster*.
Observation time is given to the nearest half hour. Also recorded are maximum depth reached, number of fish species recorded by sight and sightings of *Acanthaster*, with notes on the type and state of the reef.

Salé and Mitsamiouli, Grande Comore, have I found large numbers of *Acanthaster* and an extensive, recent mortality of hard corals. There is evidence, however, of recent population increases of *Acanthaster* elsewhere in the western Indian Ocean: on a reef off Mauritius[1], on Kenya reefs (ref. 1 and R. Tauber, personal communication) and on the 'Grand Récif' off Tuléar, Madagascar (L. A. Maugé, personal communication). It is interesting to note that de Loriol[11] reported in 1885 that specimens of *Acanthaster* were *assez fréquents* (quite common) in Mauritius.

There is no doubt that aggregations of *Acanthaster* can be responsible for extensive mortality of reef corals. Recent studies have shown that high levels of reef coral predation may be concomitant with active reef growth[12], and that in the case of patch reefs in Bermuda[13], a phase in their development is burial by sufficient particulate sediment to kill the sessile reef organisms. Aggregations of *Acanthaster* are destructive in the short term, but they may be an important factor in coral reef development in the long term[5,9,12,14,15].

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1 Endean, R., and Chesher, R. H., *Biol. Conserv.*, **5**, 87 (1973).
2 *Aust. Acad. Sci. Publ.*, **11**, 20PP (1970).
3 Chesher, R. H., *Science*, **165**, 280 (1969).
4 *New Studies on the Coral Predating Starfish Acanthaster planci* (edit. by Roads, C. H., and Ormond, R. F. G.), Report of the 1970 Cambridge Red Sea Expedition (1971).
5 Glynn, P. W., *Science*, **180**, 504 (1973).
6 Gerlach, S. A., *Atoll Res. Bull.*, **80**, 6 pp. (1961).
7 Porter, J. W., *Am. Nat.*, **106**, 487 (1972).
8 Jones, R. S., *Micronesica*, **4**, 309 (1968).
9 Dana, T. F., Newman, W. A., and Fager, E. W., *Pacif. Sci.*, **26**, 355 (1972).
10 Ormond, R. F. G., and Campbell, A. C., *Symp. zool. Soc., Lond.*, **28**, 433 (1971).
11 Loriol, P. de, *Mém. Soc. Phys. Hist. nat. Genève*, **29**, No. 4 (1885).
12 Glynn, P. W., Stewart, R. H., and McCosker, J. E., *Geol. Rdsch.*, **61**, 483 (1972).
13 Scoffin, T. P., *Science*, **178**, 1280 (1972).
14 Newman, W. A., *Science*, **167**, 1274 (1970).
15 Dana, T. F., *Science*, **169**, 894 (1970).

Rats learning to work for alcohol

If animals are to be used in the study of alcoholism, it is important that they show characteristics similar to human alcoholics. It has been demonstrated that animals can show tolerance and withdrawal symptoms to alcohol[1]: therefore, animals can legitimately be used to study these aspects. There has, however, been little evidence that animals develop the most important factor in alcoholism, a strong motivation to obtain alcohol for drinking. As there have been no demonstrations of animals learning a new response, or even continuing to make a previously learned response, to obtain drinking alcohol, in the presence of food and water *ad libitum*, it has been possible to claim that their alcohol consumption is 'accidental and inadvertent'[2]. If this were true, experiments on voluntary alcohol drinking by animals would be of little value for generalising to humans.

It therefore seemed critical to determine if animals could learn to work for drinking alcohol and to assess their motivation, if any, for alcohol. Animals which had developed a strong preference for alcohol in a free-choice situation were thought to be most likely to be motivated to obtain alcohol. Therefore, 60 male albino rats of three different strains were given at least 55 d free access to a 10% (v/v) ethanol solution, tap water and the standard Alko rat food. From these, six were selected for testing in the Skinner box because of their consistent high preferences for alcohol. Three were of the AA strain developed for high alcohol consumption[3,4], two were Sprague-Dawleys, and one was of the ANA strain[3,4]. During the week before testing, their ratios of ethanol solution to total fluid consumption ranged from 0.60 to 0.98.

These rats were then each housed continually in a standard two-bar Lafayette operant conditioning cage, modified

so that pressing one bar yielded a drop of about 0.1 ml of 10% alcohol solution, and pressing the other bar produced a similar drop of water. An open jar of food and a bottle of water were also available *ad libitum* at all times in the Skinner box. A 10 h dark, 14 h light illumination schedule was used.

On the first day of testing, before each rat was placed in the Skinner box, drops of water were placed on the water bar, and alcohol solution on the alcohol bar. No other shaping of any kind was used. A rat was arbitrarily considered to have learned to work for alcohol if it responded more than 100 times d^{-1} for alcohol and at the same time pressed at least twice as often for alcohol as for water.

All six rats met this criterion, four during the first night, one on the second night and one on the third night in the Skinner box. Once a rat had learned to work for alcohol, it continued to meet the criterion every day thereafter (Fig. 1.)

Subsequently, a reversal test was made by interchanging the positions of the alcohol and water bars. All but one of the animals learned the reversal: two during the first night, two on the second night, and one on the fourth night.

Two ANA rats which had not developed alcohol preferences[3,4] were also selected from the original 60 animals for testing as controls in the Skinner box, using the same procedure as before. Neither rat met the criterion during 10 d of testing. One ignored both bars and obtained its fluid from the free water bottle. The other pressed both bars about 50 times per night, but still got most of its liquid from the free water bottle.

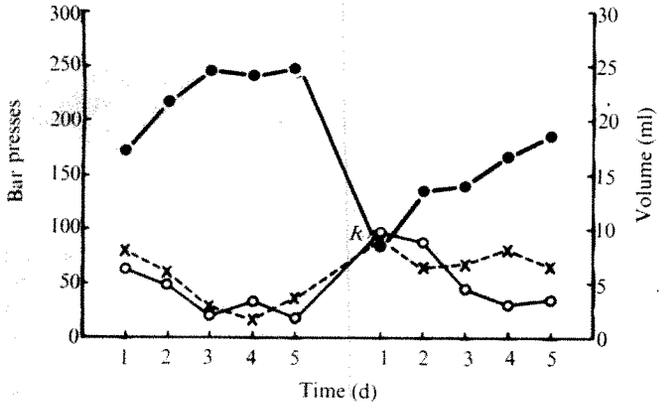


Fig. 1 Mean daily responding for 10% (v/v) ethanol solution (●) and for earned water (○) by six rats. As each bar press produced about 0.1 ml, the approximate volumes earned can be read from the ordinate on the right. Consumption of 'free water' (X) from a bottle available *ad libitum*, is also shown. After the 'reversal' (R) the alcohol and water bars were interchanged. The lower alcohol responding and higher free water intake at this time is caused by one rat that failed to learn the reversal.

The motivation for alcohol was tested in two of the rats which had previously learned to work for alcohol: one was an AA, weighing 421 g, the other a Sprague-Dawley, weighing 440 g. Weights were added progressively each day to the back of the alcohol bar, thus increasing the effort required to press it. On a continual reinforcement schedule, such as used here, this procedure should successfully measure motivation, since it has been found that rats otherwise prefer a bar requiring less effort to press[5]. This method was chosen rather than, for instance, electrifying the alcohol bar, because it should be less likely to produce confounding stressful effects.

Both rats continued pressing the alcohol bar until over 140 g were attached to it. The AA rat responded at a relatively constant rate until 137 g were attached, but showed a sharp decrease when additional effort was required. Responding by the Sprague-Dawley decreased continually throughout the experiment as a nearly linear function of the weight attached to the alcohol bar (Fig. 2).

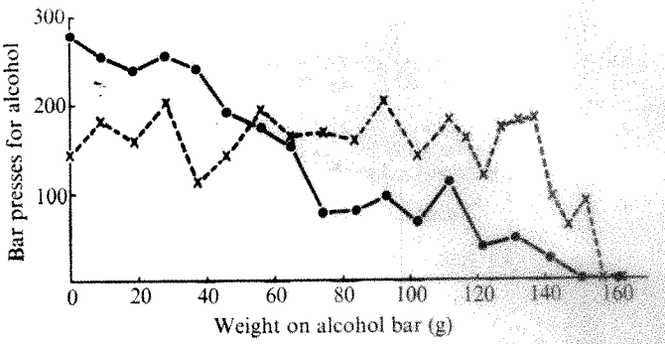


Fig. 2 Daily responses for 10% alcohol solution by rats SD15 (●) and AA141 (X), as a function of the effort required to press the alcohol bar. Each day progressively larger amounts of weight were attached to the alcohol bar, at a distance from the fulcrum equal to that of the portion upon which the rats pressed. Free food and water, and a bar, without weights, producing water, were continually available.

Unlike previous studies, in which rats have bar pressed for alcohol[6–10], the present findings clearly cannot be attributed to motivation from hunger or thirst, as food and water were concomitantly available *ad libitum*. The inclusion of a bar producing water controls for spontaneous bar pressing or responding motivated by stimulus change or mastery. The reversal task controls for position preference. Since no shaping was used, the results are not a function of the skill of the experimenter in shaping procedures. Thus it seems that the learning was reinforced by obtaining alcohol *per se* to drink.

Some animals have previously been found to work for intragastric or intravenous injections of alcohol, and in a few cases to learn new responses for the alcohol injections[11–14]. These results showed that alcohol could be a reinforcer, but they could not eliminate the possibility that the animal's motivation for alcohol injections was somehow different from the alcoholic's motivation for a drink of alcohol. As the present study shows that animals will also learn to work for drinking alcohol, it is less likely that a fundamental difference exists between the motivations of humans and these animals for alcohol.

From a comparison with two unsuccessful (and unpublished) previous attempts to train rats to work for alcohol, it seems likely that the present procedure was successful because, first, rats were used that had demonstrated long-standing, consistent, strong preference for the alcohol solution; second, the animals remained in the Skinner box during the night, which is when rats normally drink alcohol[15], rather than being tested only in daytime sessions; and third, they had not previously been taught to bar press for water, a procedure which in one of the unsuccessful previous attempts seemed to produce a subsequent decrease in voluntary alcohol drinking.

In conclusion, humans are not the only creature sometimes motivated to obtain alcohol for drinking: some rats seem to develop a similar motivation which can lead them to learn new responses for alcohol reinforcement and to expend rather large amounts of effort to obtain it. These findings also suggest the possibility of opening many questions concerning alcohol drinking by animals to sophisticated analysis with the use of operant techniques.

I thank K. Eriksson, O. Forsander and K. Lindros for their help and advice, and P. Neuenschwander for modification of the Skinner box.

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1 Mello, N. K., *Pharmac. Biochem. Behav.*, **1**, 89 (1973).
2 Lester, D., and Freed, E. X., *Pharmac. Biochem. Behav.*, **1**, 103 (1973).
3 Eriksson, K., *Science*, **159**, 739 (1968).
4 Eriksson, K., *Ann. Med. exp. Biol. Fenn.*, **49**, 67 (1971).
5 Kanarek, R. B., and Collier, G., *J. comp. physiol. Psychol.*, **84**, 332 (1973).
6 Mello, N. K., and Mendelson, J. H., *Q. Jl Stud. Alcohol.*, **25**, 226 (1964).
7 Mello, N. K., and Mendelson, J. H., *J. Psychiat. Res.*, **3**, 145 (1965).
8 Meisch, R. A., and Thompson, T., in *Biological Aspects of Alcohol Consumption* (edit. by Forsander, O., and Eriksson, K.) (Finnisa Foundation for Alcohol Studies, Helsinki, 1971).
9 Meisch, R. A., and Thompson, T., *Psychopharmacologia Laboratories*, No. PR-71-2 (Department of Psychiatry, University of Minnesota, 1971).
10 Meisch, R. A., and Thompson, T., *Psychopharmacologia (Berl.)*, **28**, 171 (1973).
11 Deneau, G., Yanagita, T., and Seevers, M. H., *Psychopharmacologia (Berl.)*, **16**, 30 (1969).
12 Winger, G. D., and Woods, J. H., in *Alcoholism and the Central Nervous System* (edit. by Seixas, F. A., and Eggleston, S.), *Ann. N.Y. Acad. Sci.*, **215**, 162 (193).
13 Woods, J. H., Ikomi, F., and Winger, G., in *Biological Aspects of Alcoholism* (edit. by Roach, M. R., McIsaac, W. M., and Creaven, P. J.) (University of Texas Press, Austin, 1971).
14 Yanagita, T., Deneau, G. A. and Seevers, M. H., *23rd Int. Congr. Physiol. Sci.* (Abstr. No. 66) (Tokyo, 1965).
15 Eriksson, K., in *Biological Aspects of Alcohol Consumption* (edit. by Forsander, O., and Eriksson, K.) (Finnish Foundation for Alcohol Studies, Helsinki, 1971).

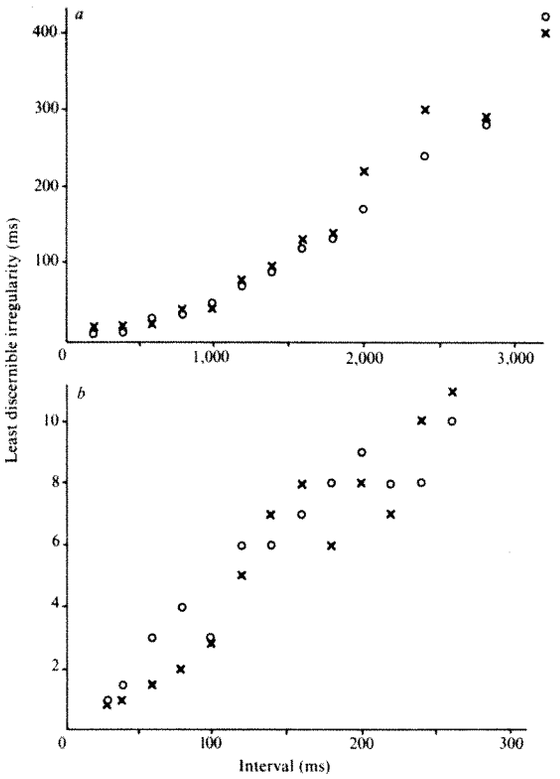


Fig. 1 The accuracy with which rhythm can be heard, or brief times assessed, plotted as least discernible deviation from regularity of a metronome, of which every fourth beat interval between beats. *a*, Intervals 260–3,200 ms; *b*, intervals 30–260 ms. Two runs are shown by the same operator on is controllably irregular, against overall tempo shown as different days. Considerable practice is needed to obtain results as consistent as these. ○, first run; ×, second run.

GENERAL

Time as heard in speech and music

THE time intervals of consequence in music range from about 50 ms, the fastest that notes may be separately fingered on the piano, to about 1 s, the tempo of slow largo. Poetry is customarily recited with beats every 600–800 ms, and the semi-isochronous stresses of speech rhythm are, according to Classe, of the order of 600 ms apart[1]. We take pride in the accuracy with which we can produce and appreciate these intervals or deviate from them at will. That we require an auditory information storage of brief duration, briefer than that customarily named short-term memory, is known, Neisser calling it echoic memory[2]; but, as distinct from our other kinds of memory which memorise events in rank order of occurrence only, what our talent for music and speech shows is that in this brief storage we possess, with a working range of a second or so, a comprehension of those things we have just heard, arranged quite accurately in true time relationship.

This was explored by devising an electronic metronome in which every fourth beat was irregular by a controllable amount, the overall tempo or interval being also under control. It was set up connected to a loudspeaker in a studio with good listening conditions, and to a timer. The interval is first set to the required figure and the irregularity to zero, using the timer. The timer dial is then obscured, and the operator's task is to increase the irregularity himself until it becomes just evident to his ear. He may repeat until satisfied. Subdividing the intervals either overtly (by foot-tapping) or covertly (for which the operator's integrity must be relied on) is barred. This least discernible irregularity is then read from the timer.

A plot of two sequences of readings for various intervals obtained by the author after several weeks' practice is shown in Fig. 1. Similar plots obtained by other operators indicate that the limit of discrimination is biologically imposed. The experiment is difficult to perform but the effect of practice is to improve the consistency of results, not the discrimination under test.

The form of the plot is striking. There are no periodicities as might be expected from cyclic brain timing mechanisms, suggested by Lenneberg[3]. In fact the approximately exponential shape indicates that the brain mechanism involved in timing partakes of the nature of a simple analogue device, possibly of a decay time or delay line type.

I suggest that the above named arrangement of events in true time relationship occurs within this mechanism, displayed as it were against an exponentially decaying scale of the 3 s or so preceding the present instant; and that this, being man's only such arrangement, provides, in effect, our sensation of time.

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1 Classe, A., *The Rhythm of English Prose* (Blackwell, Oxford, 1939).
2 Neisser, U., *Cognitive Psychology*, 199–206 (Appleton Century Crofts, New York, 1967).
3 Lenneberg, E. H., *Biological Foundations of Language*, 107–120 (Wiley, New York, 1967).

matters arising

'Spatial oscillations' in the Zhabotinskii reaction

SIR,—In a recent study of temperature effects and the Zhabotinskii reaction, Thoenes[1] measured the speed of the reaction wave fronts and related it to the temperature distribution in the sample. A temperature gradient was artificially imposed from outside the system.

Thoenes' interpretation of the data is, however, either unclear or misleading on some points.

In the first paragraph Thoenes indicates that the theoretical analysis which he uses was suggested by Beck and Váradi[2]. It seems more probable, however, that the reference should be to Zaikin and Zhabotinskii[3]. These authors refer to the work of Prigogine and Nicolis[4], among others, as providing the theoretical foundation for their simplified analyses. Thoenes' implication that his theoretical analysis is independent of the work of Prigogine and coworkers is therefore misleading.

This analysis rests on the assumption that the frequency of the oscillations, ω, can be described as a function of the position, x:

$$\omega(x) = \omega^0 F(x) \qquad (1)$$

This is Thoenes' equation (1). He later identifies $F(x)$ with

$$\exp(-E/RT(x)) \qquad (2)$$

where E is an energy and $T(x)$ is the position dependent temperature. This is Thoenes' equation (5). Thoenes states that this treatment explains all the phenomena reported by Zaikin and Zhabotinskii[3]. This is not surprising as they had already shown this to be true. (In this context, recent work by Winfree[5] is also interesting.)

But equation (1) can also be misleading in the context in which it is given. The oscillatory behaviour which it implies is rather rare in chemical reactions and the Zhabotinskii reaction is a very special case. Several authors have discussed the the requirements for, and the characteristics of, such chemical reactions[6-8]. These works point out that equation (1) can only describe very special types of chemical reactions.

The interpretation of equation (2) may not be as simple as Thoenes suggests. The heat output and the redox potential step during the oscillations change according to the number of oscillations which have occurred[9]. Equation (2) would not therefore be expected to give a single datum, as Thoenes apparently assumes. Some of the effects of heat production in the Zhabotinskii reaction have already been noted by Busse[10], although his interpretations of some other points were incorrect[11]. The chemistry of the Zhabotinskii reaction has been discussed extensively[12,13], and there has been a theoretical study[14] of an ideal system of the type studied by Prigogine and coworkers but with heat production included. Beck and Váradi[2] also explicitly mention the effects of the container walls on the reaction characteristics.

Thoenes' statement "I conclude that spatial patterns in the Zhabotinskii reaction are not examples of dissipative structures as described by the theory of Prigogine" does not therefore seem to be correct. Thoenes' analysis is in fact based on Prigogine's work.

I conclude that Thoenes has reported very interesting experimental results on the effect of temperature on the Zhabotinskii reaction, and that in order to interpret this data he has also reformulated and enlarged upon a previous mathematical description by Zaikin and Zhabotinskii[3] which was in turn based on the theories of Prigogine and coworkers[4].

Yours faithfully,

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1. Thoenes, D., *Nature phys. Sci.*, **243**, 18 (1973).
2. Beck, M. T., and Váradi, Z. B., *Nature phys. Sci.*, **235**, 15 (1972).
3. Zaikin, A. N., and Zhabotinsky, A. M., *Nature*, **225**, 535 (1970).
4. Prigogine, I., and Nicolis, G., *J. chem. Phys.*, **46**, 3542 (1967).
5. Winfree, A. T., *Science*, **175**, 634 (1972).
6. Glansdorff, P., and Prigogine, I., *Thermodynamic Theory of Structure, Stability, and Fluctuations* (Wiley, London, 1971).
7. Prigogine, I., and Nicolis, G., *Q. Rev. Biophys.*, **4**, 107 (1971).
8. Prigogine, I., Nicolis, G., and Babloyantz, A., *Physics Today*, 23–28 November, (1972) 38–44 (December, 1972).
9. Körös, E., Orbán, M., and Nagy, Zs., *Nature phys. Sci.*, **242**, 30 (1973).
10. Busse, H. G., *J. phys. Chem.* **73** 750 (1969).
11. Gray, B. F., *Nature*, **242**, 257 (1973).
12. Field, R. J., and Noyes, R. M., *Nature*, **237** 390 (1972).
13. Noyes, R. M., Field, R. J., and Körös, E., *J. Am. chem. Soc.*, **94**, 1394 (1972).
14. Rosen, G., *Phys. Lett.*, **43A**, 349 (1973).

Structure of water in porous glass

SIR,—In an attempt to measure proton mobility, Roberts and Northey, used signal line broadening from steady state nuclear magnetic resonance (NMR) to study slices of sintered glass with a pore size of ~ 15 μm (ref. 1). They infer "long range modification of the water structure by sintered glass". Their conclusions do not necessarily indicate an ordering effect, and new evidence for the water–glass system casts doubt on their results[2,3].

Signal line broadening from steady state NMR has been used as an indicator of orientation effects for adsorbed systems, Interpretation of the data is, however, often ambiguous[4]. Pulse or spin echo techniques are considered more promising and have recently been applied to many systems[5], including water on porous glass[2,3].

Our results, using pulse NMR, indicate that for a specially prepared porous powdered glass at 100% relative humidity, with an average pore size of ~ 24 Å, about 80% (v/v) of the adsorbed water is motionally restricted, and the remaining 20% (v/v) is most probably bulk water[3]. As the average pore radius approaches 100 Å, the amount of motionally restricted water in the Vycor glass is reduced to below 10% (v/v), which is approximately one layer of surface-bound water about 3 Å thick (ref. 2). Thus, for very large average pore diameters, such as the 15 μm used by Roberts and Northey, with relatively small surface areas, it is probable that far less than 10% (v/v) of the occluded water is bound. In addition, because of the notoriously low sensitivity of NMR, detection of small percentages of bound water within very large percentages of bulk water is unlikely.

For the steady state NMR experiment, broadening which results from field inhomogeneities produced by the glass matrix would occur even if 100% (v/v) of the occluded water was free. Interpretation of the NMR data for adsorbed systems is far more complicated than the observation of the broadening effect[5]. For example, relaxation rates within, and exchange rates between, various environmental states (phases) critically affect the estimation

of the actual percentage of water within a particular state[6].

These limitations seriously challenge the validity of Roberts and Northey's conclusions and probably account for the discrepancy between their results and those of Belfort *et al.*, who used the preferred pulse NMR techniques.

Yours faithfully,

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1 Roberts, N. K., and Northey, H. L. *Nature*, **237**, 144 (1972).
2 Belfort, G., *J. Colloid Interface, Sci.* (in the press).
3 Belfort, G., *Nature phys. Sci.*, **22**, 60 (1972).
4 Kaufman, S., Stein, J., and Gibbs, J. H., *Nature*, **225**, 743 (1970).
5 Pfeifer, H., *NMR, Basic Principles and Progress*, **5**, (edit. by Dahl, P., Fleich, E., and Kosfeld, R.,) 53 (Springer Verlag, 1972).
6 Woessner, D. E., *J chem. Phys.*, **35**, 41 (1961).

DR ROBERTS REPLIES: We acknowledge that Belfort's criticism might be valid had we simply recorded the NMR spectrum of water sorbed in the sintered glass. As pointed out in our article[1], however, the spectrum was recorded after distilled water had been drawn through the porous glass membrane for 30 h—the time normally required for the determination of a diffusion coefficient by the diaphragm cell method. The initial NMR signal of the water sorbed in the sintered glass was broadened compared with that of bulk water, and it might be argued that this broadening was simply a consequence of "field inhomogeneities produced by the glass matrix."

When water was drawn through the sintered glass for 30 h, however, the NMR signal of the occluded water became still broader, and an increase in hydrodynamic resistance also occurred (see also ref. 2). This increased broadening, which amounted to approximately 8 Hz at half-height, cannot be explained by invoking field inhomogeneities.

Belfort's work on porous glass[3] does not discuss the effect of prolonged movement of water through glass pores. In this connection the observation[2] that the conductivity across a sintered glass disk immersed in dilute potassium chloride does not alter when the solution is drawn through the disk over an extended period, whereas the hydrodynamic resistance increases, suggests that the hydrodynamic resistance is not a result of obstruction of the glass pores by air bubbles or solid particles.

The purpose of our article[1] was not to argue that water in glass pores (5–15 μm diameter) is structurally modified. Rather, we argued that it becomes modified on prolonged movement through the pores, with a resultant decrease in the hydrogen ion diffusion (and most probably the diffusion of the hydroxide ion) but with no effect on the diffusion of other ions.

It would be interesting to be able to calculate the degree of broadening of the NMR signal of water which is compatible with a reduction in the value of the diffusion ratio, D/D°, of the hydrogen ion, from 1.0 to approximately 0.9. These were the values obtained respectively from the polarographic and the diaphragm cell methods.

Our recent work[4] on deuterium ion mobility in heavy water solutions of electrolytes confirms our original finding that the diaphragm cell method gives low results for the limiting diffusion coefficient of the hydrogen ion.

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1 Roberts, N. K., and Northey, H. L., *Nature*, **237**, 144 (1972).
2 Mysels, K. J., and McBain, J. W., *J. Colloid Sci.*, **3**, 45 (1948).
3 Belfort, G., *Nature*, **237**, 60 (1972).
4 Roberts, N. K., and Northey, H. L., *J. chem. Soc., Faraday Trans. I*, **70**, 253 (1974).

IQ and race

SIR,—In her recent communication Tizard[1] showed that the effects of environment on IQ are great but that racial differences are not significant. In fact, her data on children of 4.5 yr divided into those still in institutions, those returned to their natural mothers, and those adopted, show that the non-whites have a consistently higher mean score than the whites. The racial differences within each of the three environmental classes are, indeed, not significant but the probabilities may be combined, since the classes are independent, giving $P=0.04$. Taken alone, this result might not mean much, but in Tizard's other study non-white children have higher scores in each of three IQ tests than white children, the difference being significant for one test ($P=0.02$). It seems that the non-white children have higher IQs than the whites.

Unfortunately, this result is open to several interpretations, at least two of which seem probable. Non-whites may be better endowed genetically with regard to intelligence than are whites. On the other hand, however, the children tested might not have been random samples of the racial groups to which they belong: British blacks are of recent immigrant stock and probably of above average socioeconomic status in their homelands. (Tizard found no significant differences between fathers of different races in ratio of manual to nonmanual occupations but this is a crude test and, in any case, blacks will do less well than whites of similar intelligence in job competition, because of colour prejudice and educational differences.)

The question of 'nature and nurture' with regard to IQ is politically sensitive: the extremists of both left and right are seeking support for equally silly and wicked beliefs. It is, therefore, important that the scientific examination of the question should be scrupulous. I hope that these comments will help the truth to emerge, whatever it may be and to whomever it may be unpalatable.

Yours faithfully,

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1 Tizard, B., *Nature*, **247**, 316 (1974).

DR TIZARD REPLIES: Greenwood correctly states that the non-white children in our studies had consistently higher mean test scores than the white children. As I pointed out in my letter no definite conclusion can be drawn from this finding. Greenwood's suggestion that selective immigration by IQ has occurred from the West Indies is of course possible, but if true would constitute a powerful argument in favour of environmental influences on development, as it is known that the mean IQ of the school-age children of West Indian immigrants living with their families in London is significantly lower than that of white school children. An alternative 'genetic' explanation would be that West Indian immigrants who place their children in long-term care are of higher IQ than the corresponding group of white parents. 'Environmental' explanations of the finding could also be advanced; for example, small black children are often found particularly appealing and may receive more attention from their nurses.

The main argument of my letter, however, was that within each environment inter-racial differences in test scores were small, but differences in test scores which we could relate to measured aspects of the environment were large and significant.

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Is the slow worm a Batesian mimic?

SIR,—Smith[1] has suggested that juvenile slow worms (*Anguis fragilis* L.), and also those adult females which retain the dark vertebral stripe, are Batesian mimics of the adder (*Vipera berus* L.). His main evidence for this view is circumstantial; parturition occurs at the same time in the two species, but is usually about one week earlier in the viviparous lizard (*Lacerta vivipara* Jacquin). Smith suggests that parturition time in the slow worm has evolved such that the species gains maximum advantage from the mimicry.

There is, however, a simpler explanation of the time of parturition. The viviparous lizard is a strictly diurnal heliotherm, maintaining a 'preferred body temperature' of about 30° C whenever solar radiation is sufficiently intense to enable it to do so[2]; it can achieve this relatively high temperature rapidly[3]. Adders, in contrast, are both diurnal and crepuscular[4], and the slow worm, as Smith admits, is a burrowing animal which is not often seen in daylight. Body temperatures of the adder are consequently variable; they do not maintain a high temperature for long periods. Slow worms do not usually maintain a preferred body temperature at all; their body temperature is nearly always near that of the ambient air (unpublished observations).

It is not surprising, therefore, that embryological development in viviparous lizards should proceed more rapidly, and it is significant in this connection that the optimum temperature for development of viviparous lizard embryos *in vitro* is 27–28° C, whereas for slow worms it is 18° C (ref. 5).

I conclude that there is no justification for regarding the slow worm as a Batesian mimic of the adder, and adduce as additional evidence the fact that neither my children nor I—two predators with very different levels of experience—ever confuse the two species in the field.

Yours faithfully,

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1 Smith, R. H., *Nature*, **247**, 571 (1974).
2 Avery, R. A., *J. anim. Ecol.*, **40**, 351 (1971).
3 Avery, R. A., and McArdle, B. H., *Br. J Herpet.*, **5**, 363 (1973).
4 Smith, M. A., *The British Amphibians and Reptiles*, (Collins, London, 1951).
5 Maderson, P. F. A., and Bellairs, A. d'A., *Nature*, **195**, 401 (1962).

MR SMITH REPLIES:—Herpetologists have questioned my suggestion[1] that the dorsal zigzag of some female slow worms (*A. fragilis L.*) mimics that of adders (*V. berus L.*), since anyone familiar with the animals can easily distinguish them. Dr Bellairs (personal communication) notes the different behaviour of slow worms and adders when threatened, unlike other mimetic lizards[2], and Avery here suggests that the coincidence of parturition times is explained by body temperature and habits. The usual predators of slow worms are not humans, however, but are more likely to be small, colour-blind mammals which must decide quickly whether or not to attack. Thus a character associated with a previous unpleasant experience (an adder bite) may cause momentary hesitation, giving a slightly increased chance of escape and a small selective advantage.

Whether mimicry evolved in response to concurrent parturition times or *vice versa* is incidental to the increased advantage of a birth period around the peak of the adder's when gravid females are vulnerable to visual predation as they bask in the sun. Nevertheless, it is interesting that, although slow worms mate mainly in May, fertilisation does not occur until June[3] and embryonic development begins around the birth time of viviparous lizards (*L. vivipara* Jacquin), so that 'preferred body temperature' is only part of the explanation.

Thus, Batesian mimicry seems to be the best, current explanation of the dorsal zigzag pattern and its presence in some adult females only.

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1 Smith, R. H., *Nature*, **247**, 571 (1974).
2 Bustard, H. R., *Br. J. Herpet.*, **4**, 22 (1968).
3 Smith, M. A., *The British Amphibians and Reptiles*, (Collins, London, 1951).

Silent alleles and haploid expression in deficiency mapping

SIR,—If a portion of one of the chromosomes is missing, and a primary gene product is reduced in amount, this indicates that either one locus determining this enzyme is missing or that it is ineffective due to a silent allele[1].

The evidence can be evaluated in the conventional currency used in human linkage studies, the lod, or logarithm of the odds ratio[2,3], by the following simple procedure.

We suppose that some transformation of the enzyme level is normally distributed with unit standard deviation, and is d units in the diploid state and h units in the haploid state: this may be derived from data on 'silent' alleles, on the assumption that they are completely silent, or 'non-leaky', or inferred by halving the diploid level. If the parent conveying this hypothetical silent allele has a level a units, after transformation, the likelihood that this is due to haploid expression is the ordinate of the normal curve of unit variance with mean h or $\sqrt{(1/2\pi)}\exp[-\frac{1}{2}(h-a)^2]$ while the likelihood that it is due to diploid expression is $\sqrt{(1/2\pi)}\exp[-\frac{1}{2}(d-a)^2]$; the natural logarithm of the ratio of these ordinates is $\frac{1}{2}[(d-a)^2-(h-a)^2]$; and the logarithm of this ratio, or lod, is $0.217\ [(d-a)^2-(h-a)^2]$. The antilogarithm of this is the likelihood ratio or odds ratio.

On the data of Ferguson-Smith *et al.*[1] the mother had a mean level of 124 units; the mean level for controls with the same phenotype was 119 units and, taking the conservative estimate that a silent allele would reduce activity by 40%, we have $d' = 119$, $d = 15.4$; $h' = 71.4$, $h = 11.9$; $a' = 124$, $a = 15.7$ where the transformation $x = \sqrt{(2x')}$ is used. This gives an approximately unit standard deviation on the data of Spencer *et al.*[4]. The log of the likelihood ratio, or the lod, is then $0.217\ ((a-h)^2-(a-d)^2) = 3.1$ giving an odds ratio of about 1250:1 in favour of a nonsilent allele.

Supplementary information, such as the size of the deficiency, and the frequency of silent alleles, may also be incorporated. This is most simply done by working in logarithms throughout, so that the numerical results are additive.

The failure of the sister of the propositus to show evidence of a silent allele, which she would have a 50% chance of doing if one were there, provides a further lod of log ($\frac{1}{2}$), or 0.30, favouring deficiency.

I thank Professor M. A. Ferguson-Smith for discussions.

Yours faithfully,

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1 Ferguson-Smith, M. A., Newman, B. F., Ellis, P. M., and Thomson, D. M. G., *Nature new Biol.*, **243**, 271 (1973)
2 Barnard, G. A., *J. R. Stat. Soc.*, **B11**, 115 (1949).
3 Morton, N. E., *Am. J. Human Genet.*, **7**, 277 (1955).
4 Spencer, N., Hopkinson, D. A., and Harris, H., *Nature*, **201**, 299 (1964).

Muscle cross-reinnervation

SIR,—In a recent communication in *Nature*, Weeds *et al.*[1] described some modifications in the subunit constituents of myosin subsequent to surgical transposition of the nerves of two functionally disparate muscles (for example, a fast-twitch, glyocolytic muscle against a slow-twitch, oxidative muscle). The evident metabolic reprogramming of the cross-reinnervated muscles led them to conclude that "It would be interesting to know if the electromyogram activity of muscles does change following cross-reinnervation" (that is, is the alien-innervated muscle activated in a fashion comparable with that of the muscle for which the nerve was normally intended?).

Obviously, few if any efforts have been made to determine the actual pattern of utilisation of cross (and self) reinnervated limb muscles. Ideally such studies require precision monitoring of electromyogram activity (by chronic indwelling electrodes) or normal and alien-innervated muscles during the repertoire of physical activities of normal and experimental animals. There are, however, several less sophisticated observations available which indicate that cross-reinnervation by functionally dissimilar nerves does lead, at least partially, to the expected changes in patterns of activity of the affected muscles. Furthermore, an exchange of functionally similar nerves apparently allows some retention of the original manner in which a muscle was utilised.

In the course of several investigations on rats, I surgically rerouted either the phrenic nerve, or hypoglossal nerve, to a denervated ipsilateral sternomastoid muscle. Several months thereafter the animals were lightly anesthetised and the function of the sternomastoid muscle was observed directly through an incision in the neck. After phrenic reinnervation the sternomastoid muscle contracted rhythmically in concert with the intact contralateral hemidiaphragm (an activity that could be enhanced by temporarily occluding the air passages). Following hypoglossal reinnervation the sternomastoid twitched intermittently in conjunction with licking motions of the tongue. The extent to which this transposition of functional activity from diaphragm to sternomastoid, or tongue to sternomastoid is operative in the fully awake animal is uncertain. Nevertheless, the appropriate circuitry and functional capacity of the phrenic and hypoglossal motorpools apparently can be diverted through a cross-nerve anastomosis to a muscle (the sternomastoid) that is not primarily responsible for breathing or licking activities.

The functional consequences of self-reinnervation, rather than cross-reinnervation, have also been answered in part by Guth and coworkers[2] who demonstrated in the rat that the recurrent laryngeal nerve, some fibres of which normally transmit efferent bursts synchronous with those of the phrenic nerve, is often capable of restoring rhythmic diaphragmatic activity subsequent to a recurrent laryngeal-phrenic nerve anastomosis

Sperry[3], in still earlier studies, emphasised that a regenerated nerve tends to retain its original reflex activity.

Thus, in spite of the disparity in muscle nerves (with respect to quality and quantity of efferents and afferents, and the differences in central circuitry) there is good reason to assume that many of the nerves used in cross-reinnervation experiments continue to elicit a gross pattern of muscle activity quite similar to that of their original musculature. It is difficult, however, to envisage a precise replication of function in most cross-reinnervated muscles because of the extreme complexity and uniqueness of each nerve, each muscle, and the myriad factors interwoven in the nerve regeneration process.

Yours faithfully,

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1 Weeds, A. G., Trentham, D. R., Kean, C. J. C., and Buller, A. J., *Nature*, **247**, 135 (1974).
2 Guth, L., Soutter, L., Frank, K., Campbell, J. B., and Lloyd, J. B., *Expl Neurol.*, **2**, 251 (1960).
3 Sperry, R. W., *Q. Rev. Biol.*, **20**, 311 (1945).

THE AUTHORS REPLY:— Dr Yellin's observations are certainly pertinent to the question posed by us, and are also in line with expectation following the pioneering work of Sperry[2] to which Dr Yellin refers. The broader problem to which our query relates, however, is whether the altered patterns of motor nerve impulses which probably reach both fast-twitch and slow-twitch mammalian skeletal muscles following cross-reinnervation are by themselves sufficient to bring about the mechanical and biochemical changes which are now known to follow altered innervation. For this to be so at least four conditions must be met.

First, it must be established that alterations in the number, and or, pattern of nerve impulses reaching a normal muscle can alter that muscle's mechanical and biochemical characteristics.

Second, it must be demonstrated that following cross-reinnervation, there is an altered pattern of motor activity in the reinnervated muscles.

Third, it must be shown that the change in activity pattern following cross-reinnervation is quantitatively correct to account for the observed mechanical and biochemical changes.

Fourth, it is also necessary to demonstrate that the observed changes in the mechanical and biochemical characteristics of cross-reinnervated muscle are the direct result of the number, and or, pattern of motor nerve impulses, and are not due to some concomitant metabolic change occurring in the motoneurone as a consequence of its altered activity.

The first point is now established beyond doubt by the original experiments of Salmons and Vrbová[3], and the later work of Streter *et al.*[4] and Al-Amood *et al.*[5]. Dr Yellin's observations add weight to the expectation that the electromyographic activity of both fast-twitch and slow-twitch muscle is altered by cross-reinnervation, but, as he implies in his letter, the satisfying of the third requirement must await the quantification of the appropriate electromyograms from conscious, unrestrained, cross-reinnervated animals.

At the present stage of knowledge, consideration of the fourth requirement can only be speculative[5,6] but it is clear that if the changes following the cross-reinnervation of mammalian fast-twitch and slow-twitch muscles are to prove entirely explicable in terms of the pattern of motor nerve impulses reaching the muscles, all four criteria listed above must be satisfied. Only then can the additional possibility of another neural (trophic) factor operating in this situation be firmly rejected.

Yours faithfully,

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1 Weeds, A. G., Trentham, D. R., Kean, C. J. C., and Buller, A. J., *Nature*, **247**, 135 (1974).
2 Sperry, R. W., *Q. Rev. Biol.*, **20**, 311 (1945).
3 Salmons, S., and Vrbová, G., *J. Physiol., Lond.*, **201**, 535 (1969).
4 Streter, F. A., Gergely, J., Salmons, S., and Romanul, F., *Nature*, **241**, 17 (1973).
5 Al-Amood, W. S., Buller, A. J., and Pope, R., *Nature*, **244**, 225 (1973).
6 Buller, A. J., in *Modern Trends in Physiology*. (edit. by Downman, C. B. B.) (Butterworths, 1972).



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book reviews

Mathematics of the Universe

The Large Scale Structure of Space-Time. By S. W. Hawking and G. F. R. Ellis. Pp. xi+391. (Cambridge University: London, May 1973.) £10; $28.50.

THERE has been a considerable upsurge of interest in general relativity theory over the past ten years or so. This is undoubtedly partly due to several remarkable discoveries in observational astronomy in recent years (quasars, pulsars, the microwave background, explosions in galactic centres, and so on) which indicate the presence of gravitational fields in nature so strong that Newtonian theory seems unlikely to be completely adequate. But equally important to Einstein's theory, and largely independent of such observational considerations, have been a number of mathematical developments in the theory. The work of Bondi, Sachs and others on the positivity of energy carried by gravitational waves was an important landmark. So was the development of techniques for finding exact solutions of Einstein's field equations, leading to the discovery by Kerr of a solution representing the field of a rotating isolated source. More recently, topological methods have been developed and have proved to be increasingly valuable for the treatment of problems where an exact analysis is not yet feasible. It has been possible, in particular, to conclude that the presence of singularities in solutions of Einstein's equations is not just a feature of the high symmetry that had been normally assumed. It is now known, for example, that irrespective of symmetry the collapse of a massive star will, according to Einstein's theory (once a point of no return has been reached), result in a space–time singularity where it is to be expected that densities and curvatures will diverge to infinity. A similar singularity occurs in the initial stage of the expansion of the Universe—again irrespective of symmetry. In the case of a collapse, the resulting black hole can be studied by a variety of techniques, with topological and analytical arguments each enhancing the value of the other. These results have been the work of several different theorists, the name of S. W. Hawking being pre-eminent among them.

Until recently, no complete account of these later topological and related developments has been available, but the situation is now happily remedied with the publication of this book. It is an impressive *tour de force*. It starts with a detailed but rapid introduction to differential geometry; this is followed by a discussion of the physical basis of Einstein's equations; then there is an elaborate discussion of conjugate points on geodesics; next, many of the most important exact solutions of Einstein's field equations are studied in detail, including Kerr's solution, the frequent use of space–time diagrams being a most welcome feature; causal properties of space–times are then discussed and several useful theorems are proved; asymptotically simple space–times are analysed; then there is a detailed discussion of the Cauchy problem; the singularity theorems are next described and proved in detail; the Schmidt *b*-boundary is introduced to give a precise definition of a singularity; then the black hole theorems are discussed and proved; finally there is an analysis of the status (derived partly from observations of the microwave background) of the initial big bang singularity. Much of the later material is presented here for the first time; the book also is a remarkably complete survey of the relevant material. This book is clearly a 'must' for any advanced student who wishes to follow up these modern developments in any detail.

But he will have to work hard. The book is not, in my estimation, at all an easy one to study in detail. Much useful information can, no doubt, be obtained by browsing and the flavour of the arguments can be ascertained. But for a detailed appreciation of the techniques, there is a number of serious obstacles for the student. Some of these are intrinsic to the subject. The very breadth of knowledge involved, ranging from pure mathematics to observational astronomy, is in itself a serious hurdle. Most of the book is mathematical in nature. But it is relevant mathematics. Little concession is made to mathematical elegance for its own sake and this can make for difficulties in reading. I had the feeling also that in many places the arguments could have been simplified and greatly clarified by a certain amount of reorganisation. But it is perhaps unfair to ask of the authors that they should have produced a polished gem of clarity for work which is so new. One knows only too well the enormous extra labour that can be involved when one attempts to reduce a presentation to its best possible form.

Nevertheless, I feel that certain points of criticism may reasonably be made. There are several small errors and ambiguities present which can make reading difficult. (I noticed something like sixty of these but I found no serious errors. It should be pointed out that with the association of tensors with forms that is quite reasonably made, a factor $n!$ should occur with the definition of the integral of an n form; relation (3) on page 32 is wrong; proposition 4.5.12 seems not to be true as stated; some of the diagrams suffer from being inaccurately drawn, such as in Fig. 31, the "matter world-line" seems to be spacelike.) Also, key definitions are often given in the middle of paragraphs and can come upon one unexpectedly (for example on page 195 a local causality neighbourhood is defined in parenthesis). Perhaps this need not be a drawback if done systematically, but the concept of "Cauchy development" is essentially defined three times, on page 60, page 119 and page 201.

I should have been happier if the physical significance of the metric g had been made more clear cut, defined by the behaviour of clocks as emphasised by Synge and Bondi. I feel it is a pity that the causality and chronology symbols $<, \ll$ are not used, since they can greatly simplify the presentation of some arguments. The terminology 'closed trapped surface' is used frequently in the book, but I found no mention of a 'trapped surface'. A reversion to this latter (original) terminology would seem a worthwhile simplification. Whereas indexed symbols are used to denote sets of components rather than actual tensors, there are places where the notation seems, rather confusingly, to indicate the contrary. In the discussion of the Cauchy problem in chapter 7 there are many very detailed formulae but little explanation of the ideas involved. I found this very hard to follow. So also were a number of other proofs. The black hole collision depicted in Fig. 60, and in particular the behaviour of the apparent horizons, is not supported in the text by any formal statements. The conjectural aspects of this picture should have been brought out. I feel that credits are

sometimes given rather haphazardly (for example there is no mention of Newman in connection with the charged Kerr solution, nor of Bondi in relation to the mass carried by gravitational waves). Finally, the absence of any discussion of 'mixmaster' singularities or of any details of the Belinskii – Lifshitz – Khalatnikov work seems to me to be a serious omission.

I would like to emphasise that these criticisms are basically minor ones in view of the formidable and impressive task undertaken by the authors. They have succeeded admirably and their work is undoubtedly a landmark in the presentation of modern gravitational theory.

The question of where the universe comes from and where it is going cannot fail to be of interest to everyone. There is rather little in science which directly bears on this question, but if anything seems to, it is surely the subject matter so powerfully presented in this book.

ROGER PENROSE

Food manufacture

Biology and the Food Industry. By H. R. Barnell. Pp. 59. (The Institute of Biology's Studies in Biology, No. 45.) (Arnold: London, February 1974.) £1.50 boards; 75p paper.

THE story is told of an Oxford undergraduate who obtained consistently good grades for his essays by copying from the *Encyclopedia Britannica* which his tutor had never read. To help "students at school, college and university . . . to keep abreast of recent trends and know where the most significant developments are taking place", the Institute of Biology sponsors the preparation of a series of booklets on selected aspects of biology to supplement the more substantial but inevitably finite treatment of biology which a textbook could be expected to provide. The late Dr Rex Barnell has written such a booklet about the food industry. In it he has systematically described such salient features of manufacturing processes and methods of food preservation as could be expected within the constraint of 59 pages, bearing in mind that he had also to touch on food composition, nutrition and the future. He has performed his task well and both pupils and their teachers will be grateful whether or not there may be some readers who feel a sneaking sympathy for the Oxford tutor.

Here will be found the distinction between drying and dehydration, a definition of a convenience food, description of pasteurisation, canning, chilling, freezing, irradiation and four lines about pickling. Ten pages suffice for milling, baking and margarine making. Vegetable meats, leaf protein and micro organisms grown on hydrocarbons get seven pages. It is all very neatly done. The section devoted to nutrition and food quality sensibly draws attention to the contribution manufacturers make to an adequate diet by making their commodities readily available. In the breathless pace, however, figures for energy value seem to have slipped out of the table of "recommended intakes". The book ends on a high note with the promise that "it is by no means impossible that if groups of biological, physical and chemical research workers concentrate on the problems . . . virtually unlimited supplies of food . . . may be truly synthesised . . . from constituents of the earth's atmosphere and crust". Well, perhaps they may.

MAGNUS PYKE

Reactions at electrodes

The Chemistry of Electrode Processes. By Ilana Fried. Pp. x+225. (Academic: London and New York, January, 1974.) £4.90; $13.75.

RECENTLY, a number of books have appeared based on the study of electrode reactions or electrodics. According to the author, this monograph is designed as "an introduction to electrodics" for "the advanced student or the qualified chemist" who possesses "no familiarity with electrochemistry". The author has succeeded in achieving her goal and has produced a concise and very readable book. Every section is prefaced by a clear statement of the question to be answered and derivations have been kept simple by mixing rigorous treatments with comparisons and analogies. The SI unit system and IUPAC conventions have been adhered to throughout. Furthermore, the book contains a balance between theory, experimental details and areas of application.

The book is divided into six chapters; the first two are brief and introduce the area of interest, the fundamental concepts and definitions. The third chapter deals with the kinetics of electrode reactions by developing the relationships between current, potential and time for both reversible and irreversible electron transfer processes. Moreover, irreversible processes caused by slow chemical reactions preceding charge transfer and reactant adsorption are also considered. Finally, the influence of cell impedance on the current-potential behaviour is discussed. The fourth chapter which completes the theoretical part of the monograph is devoted to the electrode–solution interphase. Various models of the double layer are evaluated and the modern model which offers the best fit to the experimental results is described. The topic of specific adsorption is also examined because of its effects on the double layer. The final section discusses how electrode reactions are influenced by the electrode–solution interphase.

The fifth chapter introduces the methodology of electrode reactions. Included in this section are descriptions of cells, electrodes and apparatus; equilibrium measurements of capacitance and impedance; voltammetric methods; and potential, current and charge step techniques together with their limits of applicability. The chapter concludes by mentioning the techniques of reflection spectroscopy and transmission spectroscopy at optically transparent electrodes. One drawback to this chapter, in particular, is the lack of references to the literature. In fact, the bibliography contains only a selection of books that the author "found useful" and omits all references to recent reviews and original contributions.

The final chapter offers little more than an indication of the number of areas where electrode reactions are involved, for example, corrosion, electroplating, and fuel cells. This broad survey was too ambitious and the author would have been more successful in illustrating the applications of electrodics by examining, in detail, a limited number of solutions to problems related to the areas mentioned. Such an approach would have supplemented the material of chapter 5 and, by involving the reader in the problem-solving process, would have further familiarised him with the study of electrode processes.

C. M. ELSON

Cultured plants

Plant Tissue and Cell Culture. Edited by H. E. Street. Pp. viii+503. (Botanical Monographs, Vol. 11). (Blackwell Scientific: Oxford and London, 1973.) £12.50.

ALTHOUGH plant tissues were first cultured *in vitro* 35 years ago, the realisation of their potential has always seemed to remain tantalisingly in the future. The technical problems of initiating and maintaining cultures of all but a few particularly favourable plants have until quite recently seemed insuperable obstacles to achieving the ready answers to physiological questions that the tissue and cell culture approach seems to offer. This excellent book should completely dispel that notion. Professor Street has a knowledge and experience of the culture of plant tissues, and particularly plant cells, that is second to none, and with his cust-

omary energy and enthusiasm has produced a superb account of the technicalities and potentialities of treating plants as microorganisms. He has been ably assisted in this by first-class contributions from a group of experts in specific aspects of the topic, although the editor's organising and integrating influence can be discerned throughout the book.

Approximately half of the book is devoted to a careful and comprehensive description of the many different techniques available for the culture of plant tissues and cells, beginning with an extremely useful account of the basic essentials of a tissue culture laboratory written by the editor himself. Street also provides a chapter on the apparatus and methods necessary for the successful cultivation of plant cells as suspension cultures. This includes full descriptions of batch culture chambers and of automatic chemostat and turbidostat devices. The technicalities of initiating and maintaining callus cultures are admirably covered by M. M. Yeoman, from Edinburgh, whilst E. C. Cocking and P. K. Evans of Nottingham discuss the isolation, culture and potential of plant protoplasts. The exciting topic of the production of haploid cell lines, with their obvious implications for plant genetics, receives a scholarly review by N. Sunderland, from the John Innes Institute, in a chapter dealing with pollen and anther culture. The general cytology of cultured cells is described in a long, informative chapter jointly written by Yeoman and Street, and Sunderland separately discusses the vexing question of the karyological instability of plant cultures.

The growth pattern of callus cultures and cell suspension cultures is treated in depth in separate chapters by Yeoman and P. A. Aitchison, and by P. J. King and Street. The discussion on cell suspension cultures is particularly valuable since it not only provides information on the growth and physiology of isolated plant cells, but also shows in great detail how highly synchronous populations of cells may be established. Such cultures can exhibit a remarkable synchrony of cell division for up to eight generations and, with a cell cycle of around three days long, their potential for the intensive investigation of the control mechanisms of the cell cycle must surely be greater even than that of the yeasts and unicellular algae. The book is rounded off by discussions of organogenesis and embryogenesis (J. Reinert, Berlin), the culture of plant tumour cells with special reference to crown gall (J. N. Butcher, Cambridge), and the growth of plant parasites in culture (D. S. Ingram, Cambridge).

Plant tissue culture is no longer an end in itself; it has progressed to a point at which it can be considered to be a highly sophisticated technique capable of providing answers to problems throughout the whole of plant biochemistry, physiology, development, genetics and pathology. This beautifully produced book will be invaluable to the rapidly growing number of plant scientists who wish to use plant tissue or cell culture, but who do not wish to devote their time to becoming experts.

HARRY SMITH

Big game ecology

Wildlife Ecology. By A. N. Moen. Pp. xvi+458. (Freeman: San Francisco; 1973.) $17.50.

SYSTEMS analysis and predictive model building in ecology are at an interesting stage in their development. More and more autecological studies pour out increasing quantities of details for synecologists to work on. This book is a synecological study of north American big game. It is unique in that it attempts to describe the physical and biotic interactions of game with their environment and to assess the importance of these interactions in terms of overall productivity. Their influence on game and range management are clearly stated. The title of the book is misleading, for only a small amount of space is devoted to wild fowl, gallinaceous birds and other wild life. Most of the data and examples are taken from studies on elk (wapiti), mule and white tailed deer and caribou.

The chief value of this book is that it brings between two covers a multitude of details regarding methods of measuring environmental parameters. Working ecologists and game managers need to know these details, but cannot afford much time ferreting them out. They will find this aspect of the book most appealing. Particularly useful in this respect are the two chapters which deal with the effects of climatic conditions on physical characteristics of the environment and on large ungulates. The functional relationships between weather and organisms are minutely analysed from the viewpoint of transference of thermal energy. The distribution of thermal energy is the commonest bond in these relationships and from this is drawn a conceptual picture of the complex nature of heat exchange involving all the basic modes of heat transfer. Anyone actively involved in game management, as well as theoretical modellers, will find this section of the book of immense value.

Physiological and behavioural interactions between game and environment are covered in the first half of the book in thorough, but not long winded, detail. The second half deals with the analysis of interactions involving both these biotic interactions and the physical ones. Emphasis is laid on that essential statistic for range managers, the carrying capacity of the habitat. Effective calculation of this is possible only by computer simulation and integration of models of all aspects of the system being analysed. The author suggests a useful working format that such models should follow.

This book has, to my mind, two outstanding merits. The first is that it paints a detailed picture of the ecology of northern big game, in the style of Canaletto rather than that of the Impressionists. This alone should commend it to the attention of pure and applied ecologists alike. The second is that the book inspires the reader to think big; in terms of systems rather than species. For the development of ecology this seems like a good thing and for successful environmental manipulation an essential thing.

Good game management, like all ecosystem management, demands that decisions be taken on ecological bases. The benefit of step by step model building means that decisions taken can, before implementation, be tested by computer simulation. The degree of similarity between computer prediction and observed dynamic change depends on the realism of the biological models involved in the computation. No one must forget that this is the fundamental core of ecology which always has and always will depend on the skills of the individual field ecologist. As far as north American big game are concerned, this book brings us one step nearer rational management.

D. MICHAEL STODDART

Rho–sigma relations

The Hammett Equation. By C. D. Johnson. Pp. viii+196. (Cambridge University: London, October 1973). £4.50; $13.50.

TO L. P. Hammett is owed the recognition that polar effects of aromatic substituent groups on rate and equilibrium constants of side-chain reactions are quantitatively regular. This conclusion is formally expressed by Hammett's $\rho\sigma$ relation, to which the volume under review is devoted.

The author provides an elementary, yet fairly detailed account of the role of Hammett's treatment in organic chemistry. It is intended for senior undergraduate or first-year graduate students, a formidable aim since, even for the simplest cases, general statements have to be tempered by ifs and buts. The book describes the various developments of the original $\rho\sigma$ treatment and provides a guide to the brood of modified sub-

stituent constants (for example σ^+, σ^-, σ^*, σ°, σ_I, σ_D, σ_R, σ^n, σ^G) which, on the one hand, extend its practical usefulness and, on the other hand, rob it of its beguiling simplicity and blur its theoretical significance.

It can be argued that the two chief motive forces for the development of science are the establishment of unifying principles (which provide the landmarks in the history of science) and the destruction of these same unifying principles by the experimental falsification of their predictions. Although Dr Johnson would hardly present the issue in those terms, the Hammett equation provides an interesting illustration of the interplay of these forces in the current development of chemistry.

V. GOLD

Quantum mechanics

Principles of Quantum Mechanics: The Non-Relativistic Theory with some Illustrative Applications. By W. V. Houston and G. C. Phillips. Pp. xi+376. (North-Holland: Amsterdam, 1973.) Dfl.110; $44.

THIS book covers the standard undergraduate course in quantum mechanics. In the introduction it is suggested that students should already have met some of the physical ideas but in fact the book is self contained and would be a suitable textbook for a full year's course for students who have no previous knowledge of the subject. In a field which is already reasonably well populated with good textbooks this volume will not be out of place. It cannot, however, claim to have any significant new features.

It is hard to see how the chapter on classical mechanics fits into the general scheme of the book. Although it is sometimes claimed that Poisson brackets and the Hamilton–Jacobi equation are an aid to an understanding of quantum mechanics these topics do not appear to be used at all in the presentation of quantum mechanics given in this book so their presence in chapter 2 is an unnecessary hurdle for the student. Another slightly unsatisfactory feature is that a reader might be left with the impression that there are two rival 'quantum theories' which happen to give similar results, namely wave mechanics and formal quantum theory. Of course it is clearly stated that these are equivalent but the method of presentation, even involving two separate sets of axioms, is not conducive to making this obvious. The notation used in chapter 8 does not always help.

Several sections of the book, for example the chapter on scattering theory, do not make easy reading but this is partially due to the fact that more care is taken in presentation than in some similar books.

The many interesting examples in the book will be found useful, as will the brief mathematical appendix.

E. J. SQUIRES

Condensed matter

The Structure and Properties of Solids: The Crystal Structures of Solids. By P. J. Brown and J. B. Forsyth. Pp. 172. (Arnold: London, July 1973.) Boards £4.40; paper £2.20.

THIS book aims to give an understanding, at undergraduate level, of the important elements in the study of the crystal structure of solids. There are chapters on crystallographic geometry and symmetry, the production and properties of X rays, neutrons and electrons, and the theory of diffraction, including the scattering of the different radiations. The experimental study of diffraction is described, and is followed by three chapters describing, and to some extent rationalising, the structures of the elements, ionic compounds and binary alloys.

To attempt to describe all this body of work inside 172 pages is itself a major achievement, and the authors certainly have given the reader a large amount of information at a relatively low price. The book also contains much useful practical information not usually found in physics textbooks. For example, the use of the ASTM index for identifying powder patterns is described in detail, and the 'cross-multiplication' rule for calculating the zone indices of the intersection of two planes is given. Both of these aids are used frequently by crystallographers in practice, and it is good to see them (and many more) in a book of this kind.

It is perhaps not surprising that the authors have had some difficulty in coping adequately with the wide range of subjects in such a slim volume. For the book is really a distillation of three types of book: one on crystal symmetry, like Phillips' *Introduction to Crystallography*; one on diffraction theory and techniques; and one like Evans' *Crystal Chemistry*. Though it would be nice to think that an undergraduate could master all this information quickly because it is presented in a concise way, I must say I am sceptical. Much of the writing is almost in note form, and the level of explanation is often such that I can only understand the point made, because I, like the authors, have studied the subject before in a more leisurely fashion.

The list of topics to leave out is clearly the key decision in a book of this length, and although no two authors would agree on the list, the choice seems to have been exercised somewhat unevenly. Rather complex examples of non-crystallographic point groups and the idea of magnetic symmetry are introduced in chapter 1, and the complete derivation of the formula for the integrated diffracted intensity is in chapter 3. But space has not been found in chapter 5 for a description of how crystal structures are experimentally determined; after the formal theory, the reader could usefully be rewarded with at least a simple example of a structure determination.

Apart from this inexplicable omission, the book is a very good summary of the various aspects of the study of crystal structures. As such it will be useful for research workers who wish to survey the field quickly. It could also be usefully used as an undergraduate text; however, thought should be given to the speed at which the topics can be mastered, as there is as much material here as in most books of three times the length.

J. A. VENABLES

Dirty air

Fundamentals of Air Pollution. By A. Stern, H. C. Wohlers, R. X. Boubel and W. P. Lowry. Pp. xiv+492. (Academic: New York and London, September 1973.) $14.50.

THIS book represents a welcome addition to the literature on air pollution and will serve primarily as an introductory book to the field. It is divided into four parts respectively on the elements, effects, meteorology and control of air pollution. The authors intended to introduce the topic in part 1, which together with part 2 defines the problem, and to resolve it in parts 3 and 4. These objectives are not completely met and the book gives the impression of being four short books condensed into one. It is difficult for instance to understand why the sections dealing with sampling and analysis (chapters 14 and 15) have been included under "The Effects of Air Pollution" while the section dealing with isokinetic sampling techniques occurs in part 4.

Much of the analytical work is presented very poorly with undue emphasis on classical procedures. In contrast the meteorology section is well presented and informative. The section which must receive the greatest criticism is that on the control of air pollution. Here the authors go back to consider sources of air pollution and sampling techniques, devoting only twenty pages to the engineering control of emissions from both vehicles and industrial processes. This, in a book of 450 pages, is surely inadequate.

R. PERRY

WILEY INTERSCIENCE

Following the interest aroused by the Editorial in the April 26th issue we are pleased to provide further information on :

THE D.N.A. STORY

A colour film about one of the scientific dramas of the century : the race which ended in the discovery of the double helix as a workable structure for DNA. The film describes, in their own words, the interplay of the work of James Watson and Francis Crick at Cambridge and Maurice Wilkins at King's College London, and Linus Pauling at Caltech.
The four men prove to be engaging raconteurs as they take us through the events leading up to final success in 1953. With the edge of controversy now dulled we are offered through the scientists' laconic description, a fresh and entertaining study of the fascinating issues involved at many levels in this remarkable human achievement. On the technical level, the rise of molecular biology with its foundation in crystallography and the capacity of model-building to give tangible form to diffuse X-ray patterns are put in historical perspective. Scientific arguments are developed as needed for the narrative—as in Watson's book, *The Double Helix,* the scientists do not talk down to their audience. However, the film can profitably be shown to any level of student audience if it is preceded by discussion of the relevant fundamental biochemistry. (Wiley's prize-winning teaching film, *DNA Blueprint of Life,* might be ideal.) *The DNA Story* does not claim to teach the biology of DNA: rather it is a valuable illustration to supplement a biochemistry, biology or general science course and an excellent and enjoyable introduction to the exciting and usually remote world of scientific research. For the better informed the film should prove to be a most important historical document.
May 1974 Running Time: 45 minutes £175.00
A VSM production distributed by John Wiley & Sons

CORNELL SEMINARS IN NEPHROLOGY

Edited by **E. Lovell Becker,** *Cornell University, Medical College.*
Concise, expert discussions investigate the sources and manifestations of renal disease and nephrological disorders. (*Perspectives in Nephrology and Hypertension.*)
April 1974 272 pages £10.60

GENE EXPRESSION Volume 1. Bacterial Genomes

By **Benjamin Lewin,** *editor Cell.*
This volume discusses bacterial gene systems. The first part describes the breaking of genetic code, the mechanisms of protein synthesis, and the characteristics of the components of the protein synthetic apparatus. The second section deals with the control of gene expression, discussing the mechanism of RNA synthesis, the control of transcription and translation of the lactose, operon of E. coli, and the different forms which control systems may take in other bacterial operons and in bacteriophages. The final section discussed the systems responsible for the reproduction of DNA, including the replication process itself, the systems which modify, restrict, repair and recombine DNA, and the nature of the cell cycle.
June 1974 660 pages £10.00 (cloth)
600 pages £4.75 (paper)

BLOOD FLOW AND MICROCIRCULATION

By **Stanley E. Charm,** *Tufts University School of Medicine,* and **George S. Kurland,** *Harvard Medical School and Beth Israel Hospital, Boston.*
Assembles the available knowledge on rheology and flow in the microcirculation with emphasis on methods for quantitation of flow in a variety of circumstances. Illustrative numerical examples of calculation are presented to familiarize the reader with the mechanics of problem solving. Includes computer programs for the analysis of flow and a review of the clinical significance of altered viscosity.
March 1974 254 pages £10.20

FEMALE REPRODUCTIVE SYSTEM: Dynamics of Scan and Transmission Electron Microscopy

By **Alex Ferenczy, M.D.,** *College of Physicians and Surgeons, Columbia University,* and **Ralph M. Richart, M.D.,** *College of Physicians and Surgeons, Columbia University, Presbyterian Hospital, New York City, and International Institute for the Study of Human Reproduction.*
Gathers the best electron microphotographs of the female genital tract available and arranges them by organ system; vulva vagina, cervix, endometrium, myometrium fallopian tube, ovary and placenta. Textual discussion accompanies photographs. 143 slides will be available separately.
May 1974 414 pages £15.25

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The Physics of Liquid Crystals

P. G. de Gennes

The study of liquid crystals is complicated because it involves several different scientific disciplines, specialized tools such as nuclear magnetic resonance, and also a certain sense of vision in three-dimensional space in order to visualize complex molecular arrangements. Professor de Gennes' treatment of his subject will be interesting and useful to every research worker in the field, and to graduate students in chemical physics, solid-state physics, mechanics, and engineering. The book contains chapters on Anisotropic Fluids, Long- and Short-Range Order in Nematics, Static Distortion in a Nematic Single Crystal, Defects and Textures in Nematics, Dynamical Properties of Nematics, Cholesterics, and Smectics. £11·50 *International Series of Monographs on Physics 13 June*

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Oxford

Molecular Biology comes of age

This special **Nature** Supplement has been reprinted as a separate publication. First published in the issue of 26th April 1974, **'Molecular Biology Comes of Age'** looks back on the circumstances surrounding the original appearance in **Nature** of Watson and Crick's revolutionary paper on the double helix structure of DNA in 1953. It discusses the development in Molecular Biology up to the present day as well as prospects for the future. Among the distinguished contributors are Crick, Pauling and Chargaff.

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The Management Council of the Western Canadian Universities Marine Biological Society (WCUMBS) invites applications for the post of Director of its Marine Laboratory at Bamfield on the west coast of Vancouver Island, British Columbia, Canada. The position will be full-time but the incumbent will probably be associated in an honorary or part-time capacity with one of the member Universities of WCUMBS (Alberta, British Columbia, Calgary, Simon Fraser or Victoria). Salary will be negotiable.

Applicants should supply a brief biography including degrees, attainments and experience, a list of publications, the names of 3 referees and a statement of research interests. Views on the functions of a new marine laboratory serving a group of universities would be helpful to the Selection Committee.

Applications close on August 31, 1974 and should be sent to Dr. D. M. Ross, President, WCUMBS, c/o Faculty of Science, University of Alberta, Edmonton, Alberta, T6G 2E9, Canada, who will supply further particulars about the position, the Station and the Society upon request. (2131)

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Officers will advise and encourage those in the poultry industry to adopt methods and systems leading to increased productivity. Advisory visits to farms, preparing technical leaflets and local press articles and group discussion meetings. Ref: S/8647/3

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(2264)

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POSTDOCTORAL RESEARCH ASSISTANT
PROTEIN CHEMIST/BIOCHEMIST

Applications are invited for the post of Postdoctoral Research Assistant, funded by the Agricultural Research Council, for a period of three years, to work on cell interactions during the development of the graft union in plants, under the direction of Dr. M. M. Yeoman.

Salary within the range £2,025 to £2,346 (under review) with F.S.S.U. benefits.

Applications (2 copies) should be sent as soon as possible to the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh, EH8 9YL. Please quote reference 5023. (2199)

THE UNIVERSITY OF MANCHESTER
RESEARCH ASSISTANT IN THE DEPARTMENT OF CHILD HEALTH

Applications invited from graduates with experience or an interest in cell biology, endocrinology or electron microscopy to work on tissue culture of endocrine pancreas. Appointment for one year in first instance but possibility exists to proceed to Ph.D. for suitable candidate. Salary up to £3,048 p.a. F.S.S.U. Further information and application forms from Dr. R. D. G. Milner, St. Mary's Hospital, Manchester M13 0JH (061-273 3972). (2109)

FLINDERS UNIVERSITY OF SOUTH AUSTRALIA
LECTURER IN HUMAN BIOLOGY

Applications are invited for appointment to the above-mentioned position. A joint Lectureship in the School of Biological Sciences and Medicine has been established and is to be supported by a grant from the Institute of Aboriginal Studies for an initial period of three years, after which it will be funded by the University in the usual way.

The successful applicant must have research interests in the general field of genetics or demography of Australian Aborigines, and will be required to participate in teaching of Human Biology to Science and Medical students.

Informal enquiries may be directed to Professor A. M. Clark, in the University.

Salary Scale: $A8,698 to $A11,982.

Superannuation is on the F.S.S.U. basis.

Further information about the position, including conditions of appointment and details required of applicants, may be obtained from the Secretary-General Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H OPF. Applications should be lodged, **in duplicate**, with the Registrar, The Flinders University of South Australia, Bedford Park, South Australia, 5042, by **June 28 1974.** (2221)

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
(UNIVERSITY OF LONDON)
DEPARTMENT OF PHARMACOLOGY

Graduate Biochemist required to assist in research on muscle biochemistry. Biochemical experience would be an advantage. The successful candidate will be eligible to register for a higher degree. Starting salary within range £1,200 to £1,300 p.a. Applications should be sent to the School Secretary, 8 Hunter Street, London WC1N 1BP. (2225)

UNIVERSITY OF DURHAM
DEPARTMENT OF CHEMISTRY

Requires a Research Technician to work in the inorganic research group. Applicants should preferably hold a Higher National Certificate, a Final City & Guilds, a Degree or equivalent. Some experience in Chemistry is desirable.

Salary will be at an appropriate point on the University Scale for a Technician (£1,650 to £1,920) according to age, qualifications and experience. Applications in writing giving full details together with copies of two testimonials to the Personnel Office, Science Laboratories, South Road, Durham, by Friday, June 21, 1974. (2226)

UNIVERSITY OF LEICESTER
LECTURER IN BIOCHEMISTRY

Applications are invited for a Lectureship in the Department of Biochemistry (Professor H. L. Kornberg, F.R.S., and Professor W. V. Shaw, M.D.) from October 1, 1974. The Lecturer will teach in the Faculties of Science and Medicine, but preference will be given to applicants whose main experience and research interests are in fields relevant to the newly established Medical School (i.e. "Animal Biochemistry").

The salary scale for this appointment will be according to age and experience on the scale £2,118 to £4,896 a year with F.S.S.U. membership.

Further particulars may be obtained from the Registrar, University of Leicester, Leicester LE1 7RH, to whom applications should be sent by July 1, 1974. (2227)

THE UNIVERSITY COLLEGE OF WALES
ABERYSTWYTH
DEPARTMENTS OF PHYSICS AND CHEMISTRY
POST DOCTORAL RESEARCH ASSOCIATE

Applications are invited for the above post of work on an SRC supported programme on vibrational population distributions of the reaction products of shock-initiated reactions using infra-red spectroscopic techniques. Candidates are expected to hold a suitable Honours degree and have three or more years research experience. Appointment by October 1, 1974, or earlier by arrangement.

Salary: £1,929 to £2,058 plus F.S.S.U.

Further particulars and application forms from the Registrar, The University College of Wales, Aberystwyth. (2228)

UNIVERSITY OF OTAGO
LECTURERS IN BIOCHEMISTRY

Applications are invited, from young graduates, preferably with postdoctoral experience, for two position of Lecturer in Biochemistry. For one of these positions, preference will be given to candidates whose research interests lie in the field of enzyme structure and function.

Salary Scale: NZ$6,753 to NZ$8,568 per annum. Salary scales are subject to both triennial review and cost-of-living adjustments.

Further particulars are available from the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H OPF, or from the Registrar of the University.

Applications close in New Zealand and London on **July 15, 1974.** (2240)

OXFORDSHIRE HEALTH AUTHORITY
(TEACHING)
THE RADCLIFFE INFIRMARY
RENAL BIOCHEMISTRY

Biochemist or Laboratory Technician to collaborate in project on metabolic basis of renal function. Previous laboratory experience essential, experience in enzyme assays helpful. Post financed at present for 18 months (Salary £1,440 to £1,980 p.a.).

Apply in writing to Dr B. D. Rose, Nuffield Department of Clinical Biochemistry, Radcliffe Infirmary, Oxford. Quote ref. 185/74. (2234)

THE UNIVERSITY OF SHEFFIELD
STEROID BIOCHEMIST

Postdoctoral assistant required in the Department of Zoology from a date to be arranged until December 31, 1976 (with possibility of extension to three year appointment) to work with Professor F. J. Ebling on the responses of skin to androgens. Applicants should be chemists or biochemists with experience in field of steroid metabolism in vitro and in vivo. Starting salary £1,929 p.a. with F.S.S.U. provision. Further particulars from the Registrar and Secretary, the University, Sheffield S10 2TN, to whom applications (one copy) should be sent by June 20, 1974. Please quote Ref: R82/G. (2238)

THE GEOLOGICAL SURVEY OF GREENLAND
Geophysicist

A Geophysicist is required for the Geological Survey of Greenland. Candidates should have at least five years' experience in the various branches of oil exploration, geophysics, particularly seismic interpretation, and should have a good command of English. The person appointed will be based in Copenhagen and will be required to undertake periodic field work in Greenland.

The appointment will be on renewable contract and will be pensionable. Applications should be accompanied by a detailed curriculum vitae, an indication of the approximate salary required, and the names of two referees, and should be sent before August 1, 1974 to: The Director, The Geological Survey of Greenland, Øster Voldgade 10, DK-1350 Copenhagen K, Denmark. (2242)

THE ROYAL INFIRMARY OF EDINBURGH
Department of Clinical Chemistry

Applications are invited for the post of basic grade Biochemist. Candidates should have an Honours degree in Biochemistry or Chemistry, or one of the related biological sciences. The post will provide experience of routine hospital service in Clinical Chemistry, and opportunities for engaging in developmental and research work. A training programme exists for preparing basic grade Biochemists for further qualifications in Clinical Biochemistry.

Salary and conditions of service according to current Whitley Council agreements. Applications giving age, qualifications and experience, together with the names and addresses of two referees to Personnel Officer, Royal Infirmary, Edinburgh EH3 9YW by June 21, 1974.

Intending applicants may visit the Department by arrangement with Dr I. W. Percy Robb, telephone 031-229 2477, ext. 2319. (2214)

NATIONAL HEART & CHEST HOSPITALS
Brompton Hospital, London, SW3 6HP

The following vacancies exist in the newly re-organised Department of Pathology. Full training facilities are provided for ONC, IMLT and HNC examinations.

TECHNICIAN OR
JUNIOR TECHNICIAN 'B'
Biochemistry Department

TECHNICIAN
Virology Department

JUNIOR TECHNICIAN 'A'
All Departments

Qualifications required:
Technician — AIMLT or HNC in relevant subject
Junior Technician 'B' — ONC or B.Sc. in relevant subject
Junior Technician 'A' — GCE 'O' level passes in English, Mathematics and two science subjects.

Applications, with the names of two referees to:
F. J. Baker at the above address (2233)

A Professorship (AH 4) in the BIOLOGY FACULTY
of the
UNIVERSITY OF HEIDELBERG

is being re-advertised. The particular orientation in teaching in the sphere of neurosensory, and behaviour physiology should embrace research in one of these fields.

Please apply by letter, including a curriculum vitae, to the University of Heidelberg, 69 Heidelberg 1, Grabengasse 1, by September 30, 1974. (2286)

Ministry of Agriculture, Fisheries and Food
Food Science Division, London

Food Chemist/ Biochemist

■ **Advise on scientific aspects of manufacture and composition of foods and beverages ■ Liaise with other Government Departments ■ Participate in committee work in UK and abroad and in current awareness studies.**

☐ 1st/2nd hons degree in Food Science/Technology or related science ☐ At least 4 years' postgraduate experience in food industry or research on meat, fish, milk or protein foods ☐ Age under 32 ☐ Appointment as Senior Scientific Officer (£3026–£4123 under review) ☐ Ref. SB/8/AE.

Ministry of Agriculture, Fisheries and Food
Fisheries Laboratory, Lowestoft

Biologist

■ **To organise and execute fishery resource surveys using quantative echo-sounding methods.**

☐ 1st/2nd hons degree in Biology or related subject ☐ Understanding of fish population dynamics and working knowledge of underwater acoustics essential ☐ Knowledge of fish behaviour in response to capture and facility with mathematics required ☐ Age under 30 ☐ Appointment as Higher Scientific Officer (£2221–£2854) or Scientific Officer (£1435–£2329), according to age and experience ☐ Salaries under review ☐ Ref. SB/13/AD.

☐ Application forms (to be returned by 3 July 1974) from Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, telephone Basingstoke 29222 ext. 500 or London 01-839 1992 (24 hour answering service).

British Museum (Natural History)

Entomologists

■ **2 posts in Hymenoptera and Trichoptera sections ■ Post One: To carry out taxonomic research on Hymenoptera Parasitica ■ Post Two: To research taxonomy and biology of Trichoptera ■ Supervision of Curatorial work on the National Collection involved in both posts.**

☐ 1st/2nd hons degree or equivalent in Zoology, preferably with Entomology ☐ At least 2 years' postgraduate research experience ☐ Age under 30 ☐ Appointments as Senior Scientific Officer (£3026–£4123) or Higher Scientific Officer (£2449–£3082) depending on age and experience ☐ Salaries under review ☐ Ref. SB/26/DK.

☐ Application forms (to be returned by 3 July 1974) from Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, telephone Basingstoke 29222 ext. 500 or London 01-839 1992 (24 hour answering service).

Department of the Environment
Building Research Establishment
Princes Risborough Laboratory

Information Scientist

■ **In Application Services Section concerned with disseminating results of research ■ Organise courses, seminars, symposia and visits ■ Arrange presentation of Laboratory's work at exhibitions ■ Liaise with TV and film producers ■ Develop the use of Video Recording Equipment as visual aid.**

☐ Degree/HNC or equivalent in Science or Engineering ☐ Knowledge of timber technology an advantage ☐ Age under 27 ☐ Appointment as Scientific Officer (£1435–£2329 under review) ☐ Ref. SA/63/HA.

☐ Application forms (to be returned by 2 July 1974) from Establishment Office, Princes Risborough Laboratory, Aylesbury, Bucks, HP17 9PX.

Science group
CIVIL SERVICE

(2279)

GEOPHYSICIST

The Lamont-Doherty Geological Observatory of Columbia University announces a position with the seismology group as a Research Associate or Senior Research Associate. Candidates must have a strong background in seismic instrumentation and data analysis and will be expected to lead existing and initiate new programs of research involving observations of earth strain, tilt, and long-period seismic waves. Qualified candidates are invited to submit a résumé of their qualifications, publications, names and addresses of three references by August 15, 1974, to Dr. Lynn R. Sykes, Lamont-Doherty Geological Observatory, Palisades, New York 10964. Columbia University is an equal opportunity employer.

(2260)

TECHNICIAN

with biochemical experience required in Surgical Unit to provide a research analytical service. Commencing salary within scale £1,440 to £1,914 p.a. plus £126 L.W. Apply, The Secretary, St. Mary's Hospital Medical School, Paddington, London W2 1PG quoting Ref. SU1. (2255)

CAMBRIDGE UNIVERSITY

DEPARTMENT OF GENETICS

Application are invited from microbial or molecular geneticists for a University Demonstratorship that is expected to be available from October 1, 1974, or sooner.

The scale of stipends from October 1, 1974 will be £2,247 a year rising by annual increments to £2,931 or, if the person appointed is resident in College, £2,148 rising to £2,832.

Further particulars may be obtained from the Secretary of the Appointments Committee of the Faculty of Biology 'A', Department of Applied Biology, Downing Street, Cambridge CB2 3DX, to whom applications should be sent before June 21, 1974. (2254)

UNIVERSITY OF DUNDEE

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS

RESEARCH ASSISTANTSHIP

Applications are invited from University graduates or holders of equivalent qualifications for a Research Assistantship in the Department of Pharmacology and Therapeutics, located in the new Ninewells Hospital and Medical School complex, Dundee. The holder of this post, which is available immediately, will be required to provide a Gas-Liquid Chromatographic Service within the Department and to collaborate as required with other staff in research and teaching and will be afforded facilities to carry out his own research work on a part-time basis for a higher degree.

The salary attached to the post will be within the range £1,311 to £2,052 depending on qualifications and experience.

Applicants should apply, giving their qualifications, the names of two referees and quoting Reference Est/39/74J by June 21, 1974 to The Secretary, The University, Dundee DD1 4HN, from whom further particulars may be obtained. (2213)

UNIVERSITY OF HULL
POSTDOCTORAL FELLOWSHIP

Applications are invited for a Postdoctoral Fellowship (SRC) to work in collaboration with Dr R. Aveyard on the effects of electrolytes on aqueous solutions of surface active non-electrolytes. Research experience in thermo-dynamics, physical chemistry of electrolyte solutions, or colloid and surface chemistry would be an advantage.

The appointment which is for two years, is immediately available in the salary range, £2,037: £2,151 per annum rising to £2,226: £2,340 per annum on October 1.

Applications giving details of qualifications, research experience, and the names of two referees should be sent before June 30, 1974, to the Registrar, University of Hull, Hull HU6 7RX, from whom further particulars may be obtained. (2209)

UNIVERSITY OF NEWCASTLE UPON TYNE
DEPARTMENT OF PHYSIOLOGY
JUNIOR RESEARCH ASSOCIATE

Junior Research Associate required from September 1974 for one year in the first instance to work on gastrointestinal hormones. Applicants should have or expect to obtain at least a second class degree and have an interest in biochemistry and cell physiology.

Salary scale (effective from October 1, 1974) £1,569 to £1,818 p.a. or the equivalent of a post-graduate grant if registered for a higher degree.

Applications, enclosing curriculum vitae and naming two referees, should be sent as soon as possible and within three weeks of the date of this advertisement to Dr. C. N. A. Trotman, Department of Physiology, The Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU. (2210)

THE UNIVERSITY OF LEEDS
DEPARTMENT OF BIOCHEMISTRY

A post-doctoral appointment is to be made for research concerned with the control of triglyceride and lipoprotein release from the liver and with the regulation of the enzyme, lipoprotein lipase. Previous experience in either of these areas, or in work on the plasma lipoproteins, would be useful, though not essential.

The research has Medical Research Council support and, though the appointment will be for one year in the first instance, there is provision for extension for up to five years. Salary according to age, qualifications and experience. FSSU benefits will be available. The starting date will be October 1st or as soon thereafter as possible.

Applications, together with the names of three referees and curriculum vitae, should be sent not later than 30 June 1974 to Professor D. S. Robinson, Department of Biochemistry, The University, Leeds, LS2 9JT, from whom further particulars are available. (2237)

UNIVERSITY OF LONDON
CHAIR OF MINERAL TECHNOLOGY AT IMPERIAL COLLEGE

The Senate invite applications for the Chair of Mineral Technology tenable in the Department of Mining and Mineral Technology at Imperial College with effect from October 1, 1974 or as soon as possible thereafter. Initial salary to be agreed but not less than £5,973 plus £162 London Allowance. Applications (10 copies) must be received not later than July 26, 1974 by the Academic Registrar (N), University of London, Senate House, London WC1E 7HU, from whom further particulars should first be obtained. (2222)

UNIVERSITY OF SOUTHAMPTON
FACULTY OF MEDICINE—MICROBIOLOGY

Applications are invited from suitably qualified science graduates for the post of LECTURER IN MICROBIOLOGY. The person appointed will be expected to undertake teaching and research duties in the field of microbial infections. Preference will be given to candidates with an interest in immunochemistry, virology or the chemistry of microbial structure.

Salary scale: £2,118 to £4,896. The initial salary will depend on qualifications and experience. Further particulars may be obtained from the Deputy Secretary's section (Ext. 353), The University, Southampton SO9 5NH to whom applications (7 copies from United Kingdom applicants) should sent not later than June 22, 1974, quoting reference be sent not later than June 22, 1974, quoting 58/74/L. (2249)

ICI Central Toxicology Laboratory

Administrative Assistant

We are looking for a person with a good biological background who will be responsible for the administration and planning of long-term toxicological studies. The work also involves reporting the findings of scientific committees, compiling data and editing technical documents, extensive liaison with staff throughout the Laboratories and a miscellany of administrative tasks necessary for the effective working of a group of 30 laboratory staff.

Applicants should either be graduate biologists or people with experience in laboratory administration. The ability to type would be an advantage.

Applications and enquiries to:

Miss A. Waring
Personnel Officer
ICI Central Toxicology Laboratory
Alderley Park
Nr. Macclesfield, Cheshire SK10 4TJ
Tel: Alderley Edge 2711, Ext. 156

(2248)

Department of the Environment
Building Research Establishment
Princes Risborough Laboratory

Technical Editor

■ **Assist in editing publications** ■ **Draft articles on timber and related technology** ■ **Prepare press releases and liaise with Press** ■ **Arrange dissemination of research results through scientific and technical journals.**

□ Degree/HNC in Science or Engineering □ Editing and technical writing experience and knowledge of timber technology an advantage □ Age under 27 □ Appointment as Scientific Officer (£1435–£2329 under review) □ Ref. SA/62/HA.

□ Application forms (to be returned by 28 June 1974) from Establishment Office, Princes Risborough Laboratory, Princes Risborough, Aylesbury, Bucks, HP17 9PX.

Science group CIVIL SERVICE

(2280)

THE GERMAN CANCER RESEARCH CENTER

REQUIRES

FOR THE INSTITUTE OF IMMUNOLOGY AND GENETICS

QUALIFIED SCIENTISTS

AS HEADS OF THE FOLLOWING GROUPS

I. CELLULAR IMMUNOLOGY	area of research : cell mediated immunity against malignant cells. Experience regarding the following areas would be required : cellular immunity, transplantation immunology and development and growth of tumors.
II. IMMUNOCHEMISTRY	area of research : Characterization of tumor antigens, factors involved in differentiation.
III. IMMUNOGENETICS	Experience regarding the following areas would be required : Histocompatibility antigens, genetic basis of cellular susceptibility to tumor viruses, variability of antibodies, and, genetics of mice. General research area : problems of carcinogenesis.

It is anticipated, that another group "SOMATIC GENETICS", will be established at a later time.

REQUIREMENTS: Recognized scientific ability in the above areas as well as the readiness to cooperate with groups that are already established at the German Cancer Research Center and that are concerned with related subjects.
OFFERS: Salary group AH 3, provided the applicant fulfils the conditions of German Civil Service, or an otherwise appropriate grade of employment. Funds for scientific and technical assistants are available. The space in the institute available for each group will be 300 qm. Facilities including an animal colony, computerised data processing, modern literature search service, central photography laboratory, central spectroscopy laboratory, and central workshop, are available to all scientists at the German Cancer Research Center.

Only written applications will be considered. They should be submitted with a complete list of publications and curriculum vitae to the administration of the "Deutsches Krebsforschungszentrum, D-69 Heidelberg, Kirschnerstraße 6", by September 30, 1974, and should be marked "Berufungsausschuß Immunologie und Genetik".

(2207)

THE UNIVERSITY OF LEEDS
ASTBURY
DEPARTMENT OF BIOPHYSICS

Applications are invited for 3-year post-graduate awards financed by the S.R.C. and M.R.C. for research in the fields of

STRUCTURAL STUDIES OF ENZYMES, POLYSACCHARIDES AND LIQUID CRYSTALS

by means of X-ray diffraction, spectroscopy, chemical sequencing and solution studies.

Candidates are expected to obtain a first or upper second class degree in Physics, Chemistry, Biochemistry or a related subject.

Applications, including the names of two referees, should be sent to Professor A. C. T. North, Astbury Department of Biophysics, The University, Leeds, LS2 9JT. (2236)

THE ROYAL HORTICULTURAL SOCIETY

Applications are invited from graduates in Horticulture or Biological Sciences for the Emmie Clough Scholarship to be awarded by the Royal Horticultural Society for postgraduate research at the R.H.S. Garden, Wisley, Woking, Surrey, on an approved horticultural project.

The scholarship is intended to lead to a higher degree at the University of Reading. Applicants must satisfy the University's entry qualifications, and the research project will be under University supervision.

The subject of research will be approved jointly by the Society and the University and must be concerned with a problem of practical horticultural importance. Possible research topics could be the host relations and control of rusts affecting ornamental plants; sweet pea scorch; the biology and control of lichens. Other topics put forward by applicants will also be considered.

The scholarship of not less than £750/annum is tenable for a period of two years and may be extended.

Applications for the scholarship which will run from October 1974 should include full details of the applicant's experience and qualifications together with the names and addresses of two academic referees and should reach the Secretary, Royal Horticultural Society Offices, Vincent Square, London SW1P 2PE, not later than June 30, 1974. (2253)

SURREY EDUCATION COMMITTEE
EWELL COUNTY TECHNICAL COLLEGE
Reigate Road, Ewell
DEPARTMENT OF BIOLOGICAL SCIENCES

Required as soon as possible

LECTURER 1 OR 2 IN BIOCHEMISTRY

To teach at Higher National Diploma and Certificate and at Membership of the Institute of Biology levels.

An interest in protein biochemistry and the application of physical techniques to biomacro molecules would be an advantage. The successful candidate would also be involved in teaching areas of metabolic biochemistry.

Candidates should have a First Degree in Biochemistry plus industrial and/or reesarch experience. Only those candidates with appropriate teaching experience, in addition to the above, will be considered for the Lecturer 2 grade.

Salary: Lecturer 1—£1,660 to £2,685 p.a.—for Good Honours Graduate the scale is extended to £2,847 p.a.

Lecturer 2—£2,515 by 8 increments to £3,243 p.a.

£118 p.a. London Allowance payable.

Generous relocation expenses and assistance with house purchase in approved cases.

Stamped addressed foolscap envelope, please, for further particulars and application forms from the Vice-Principal. Applications to be returned within 4 days. (2252)

EARTH SCIENCE—QUATERNARY

Applications are invited for a permanent position ubject to availability of funds) effective July 1, 75, at Scarborough College in the University of ronto. Duties—undergraduate instruction in oductory Earth Science and advanced (graduate) ching and research in Quaternary geology or sical geography. Applicants must have a com- ed Ph.D. and postdoctoral experience is pre- d. Salary and rank commensurate with ex- nce. Closing date for applications—January 1, , or when filled. Application, including a com- curriculum vitae and the names of three ees to Dr J. C. Ritchie, Chairman of the h Committee, Scarborough College, University Toronto, 1265 Military Trail, West Hill, rio. (2258)

Cardiovascular Research

Section Head, £4000+

Our cardiovascular teams have proved themselves capable of identifying new approaches to the treatment of heart disease. It is intended that these teams will form the basis of a new section in the department of Pharmacology and Biochemistry.

We need an established pharmacologist, someone with a minimum of 4 years' post-doctoral experience, capable of giving excellent leadership to this section. Good managerial potential is expected since the section will probably expand.

Future growth of cardiovascular research in Fisons will depend largely on your drive and creative ability. A contribution to other fields of drug research will also be expected through interaction with senior scientists in other departments.

Starting salary will be over £4000; benefits, including contributory pension, welfare schemes and expenses towards relocation are excellent.

Please write or telephone for an application form, quoting reference 355R:
John Mills, Personnel Manager, Fisons Limited, Pharmaceutical Division, Bakewell Road, Loughborough, Leics.
Tel: Loughborough 66361

FISONS

(2

Science Graduates interested in the administration of Medical Research

If you are a Science Graduate with a first or second class honours degree, aged 32 or over, and would like to make a career in the administration of research, a post of Scientific Administrative Officer in the MRC Headquarters Office in London should be of interest to you. Research experience in fields associated with medicine—including social science—and a research degree would be an advantage.

The Medical Research Council is the main Government agency responsible for the promotion of medical research and sponsors an extensive programme, some of which is abroad. The vacant post will be concerned primarily with part of the Council's now clinical activities which includes such subjects as physiology, psychology, genetics, immunology, microbiology and molecular biology. The work will include liaison with members of the Council's scientific staff and others active in the relevant research field and participation in scientific committee and conference activities.

Salary will be in the range of £3,943 to £5,123 per annum, with superannuation provision. Appointments will be to the Council's established staff, subject to satisfactory completion of a probation period of two years.

Application forms may be obtained by writing to or telephoning Mrs. Anne Edwards, Medical Research Council, 20 Park Crescent, London, W1N 4AL. Tel. 01-636 5422 (Ext. 300). Closing date for applications June 26, 1974. Please quote E2/584. (2266)

MRC
Medical Research Coun

TURAL ENVIRONMENT RESEARCH COUNCIL
TITUTE OF GEOLOGICAL SCIENCES

Hydrogeologists

pplications are invited for Senior Scientific Officer, Higher Scientific Officer and ientific Officer vacancies which exist in small teams in the Hydrogeological De- rtment of the Institute, working on basic and applied research projects.

ost 1 Duties are primarily concerned with overseas groundwater modelling projects. Candidates are expected to have a good honours degree or equivalent in Geology or Physics and experience in Fortran programming. Scientific Officer Band I, Higher Scientific Officer Band I, or Senior Scientific Officer.

Post 2 Duties are concerned with overseas hydrogeochemical problems. A good honours degree or equivalent in Geochemistry is required and a background in physical chemistry; some sedimentary or aqueous geochemistry is desirable. Scientific Officer Band I, Higher Scientific Officer Band I, or Senior Scientific Officer.

Post 3 Duties are concerned with hydraulic analysis relating mainly to regional groundwater research studies in the UK. A good honours degree or equivalent in Geology is required while some knowledge of hydraulic engineering or mathematics would be an advantage. Scientific Officer Band I, Higher Scientific Officer Band I, or Senior Scientific Officer.

Posts 4 to 7 Duties are concerned with applied studies overseas. Candidates are expected to have a good honours degree or equivalent in Geology or Environmental Science, with Mathematics or Physics as ancillary subjects. Scientific Officer Band II, Higher Scientific Officer Band I, or Senior Scientific Officer.

Posts 8 and 9 Duties relate mainly to regional research studies in the UK. A degree, HNC or equivalent in Geology or Environmental Science is required; cartographic ability advantageous. Scientific Officer Band III, or Higher Scientific Officer Band II.

For all posts some postgraduate experience in hydrology and/or hydrogeology is desirable.

SALARY (currently under review)

Scientific Officer	£1,663-£2,557
Higher Scientific Officer	£2,449-£3,082
Senior Scientific Officer	£3,026-£4123

Starting salaries may be above the minima.

The staff of the Council are not Civil Servants although their pay and conditions of service are similar to those of scientists in the Civil Service.

Application forms available from Establishment Section, Institute of Geological Sciences, Exhibition Road, London SW7 2DE, quoting Ref. SO/HYD/74/2.

CLOSING DATE FOR RECEIPT OF APPLICATION FORMS JUNE 21, 1974.

(2218)

RESEARCH SCIENTIST (ARMYWORM ECOLOGICAL GENETICIST)
Ref. Res/74/11

Applications are invited from candidates of Ph.D. status to fill the post of Ecological Geneticist in the Armyworm Programme at the I.C.I.P.E. Research Centre.

Responsibilities: The appointee will be required to investigate the ecological genetics of the Armyworm moth, *Spodoptera exempta* (Agrotidae), especially its polymorphism. This must include cryptic polymorphism, ascertained by means of electrophoresis.

Salary: Will depend on qualification and experience, but not less than K.Shs.44,000 per year.

Other benefits include ten per cent gratuity of basic salary earned, a housing allowance, and medical insurance.

Applications, in six copies, should give the following information: General education, Professional qualifications, experience, marital status, age, present salary and terms of service, and names and addresses of four referees (including a personal reference). Photostat copies of relevant transcripts, thesis abstracts, and certificates should be enclosed.

Applications should be addressed to:

The Administrative Officer,
I.C.I.P.E. Research Centre,
P.O. Box 30772,
NAIROBI

to reach him on or before July 1, 1974. (2259)

Kings
HEALTH DISTRICT (TEACHING)
Lambeth, Southwark and Lewisham
Area Health Authority (Teaching)

Research Graduate

Graduate required to work on the Biochemistry of various hormones in women with benign or malignant tumours of the breast.

Application forms obtainable from the Personnel Office, King's College Hospital, Denmark Hill, London, S.E.5. Tel: 01-274 6222 Ext. 2726 (Miss Millwood) should be completed and returned by two weeks after date of insertion. (2251)

UNIVERSITY OF EAST ANGLIA
SCHOOL OF CHEMICAL SCIENCES
RESEARCH IN SPECTROSCOPY

Applications are invited by June 28 for a Postdoctoral Research Associateship available from September 1974 to work with Professor N. Sheppard, F.R.S., on the infra-red spectra of chemisorbed molecules using interferometric techniques. Salary within the range £1,929 to £2,223 p.a., according to age and experience. F.S.S.U. benefits. School of Chemical Sciences, University Plain, Norwich NOR 88C. (2250)

UNIVERSITY OF KENT AT CANTERBURY
RESEARCH POSTS IN MICROBIOLOGY

Applications are invited for the following research appointments in the Biological Laboratory for work on phage and bacterial plasmids.

(a) A Postdoctoral Fellow to work on new methods for determining the kinetics of bacterial growth in infected animals using temperate phage and colicin factors. Salary initially £2,118 in the range £2,118 to £2,412.

(b) A graduate Experimental Officer to assist in physiological and molecular studies on colicin factors. Salary initially £1,686 in the range £1,686 to £1,947.

Microbiological training is essential for both posts and a knowledge of bacterial genetics and its techniques would be an advantage. Application forms and particulars may be obtained from the Assistant Registrar, Natural Sciences, Chemical Laboratory, The University, Canterbury, Kent CT2 7NH to whom completed applications should be returned by June 21. Please quote ref. A55/74. (2215)

UNIVERSITY OF SOUTH AFRICA
DEPARTMENT OF MATHEMATICS AND APPLIED MATHEMATICS
VACANCIES FOR SENIOR LECTURER OR LECTURER IN MATHEMATICS AND APPLIED MATHEMATICS OR ASTRONOMY

Applications are invited for the above vacancies. Candidates must state in which topics in one or more of the subjects they have specialised.

Qualified academics are invited to apply to:

The Registrar,
University of South Africa,
P.O. Box 392,
Pretoria,
South Africa.

Applications close on June 30, 1974.

The salary scales are:

Senior Lecturer R6,300 by R300 to R8,100 plus 15%.

Lecturer R4,800 by R300 to R6,900 plus 15%. (2257)

IMPERIAL COLLEGE
DEPARTMENT OF ZOOLOGY AND APPLIED ENTOMOLOGY

Postdoctoral Research Assistant, financed by the S.R.C., required to work on the rôle and nature of insect neurosecretion. Candidates should possess or expect to obtain shortly a Ph.D. and have ability in either Physiology or Biochemistry. The position is for two years and could be taken up as soon as possible. Starting salary £2,094 (under review) including London allowance, with F.S.S.U. A full time Research Technician is available.

Applications by letter, naming two referees, to Dr W. Mordue, Department of Zoology and Applied Entomology, Imperial College, London, S.W.7, by June 28. (2277)

UNIVERSITY OF SYDNEY
FIXED TERM LECTURESHIP IN THE DEVELOPMENT OF CONTINUING EDUCATION IN VETERINARY SCIENCE

Applications are invited for the abovementioned position. The appointee will be involved in the development of continuing education in Veterinary Science and will be expected to work in close liaison with the Postgraduate Committee in Veterinary Science and other appropriate scientific bodies.

The initial appointment will be for two years with the possibility of an extension beyond that period.

Salary: $A8,698 to $A11,982 p.a.

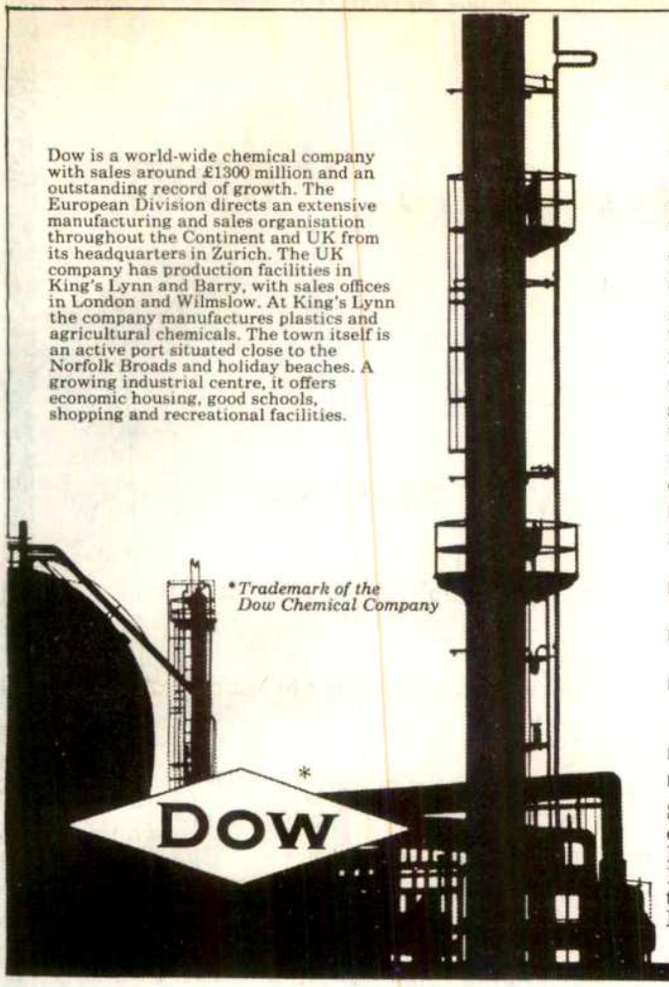
Further information available from the Dean of the University. Applications including curriculum vitae, list of publications and names of three referees to the Registrar, University of Sydney N.S.W. 2006, Australia, by **June 28, 1974,** or as soon as possible thereafter. Information also available from Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1 0PF. (2220)

FEDERAL INSTITUTE OF TECHNOLOGY
ZÜRICH, SWITZERLAND

Applications are invited from physical chemists for the position of postdoctoral research assistant to work with Prof Urs P. Wild in the field of photochemical and photophysical primary processes. Experience in the field is required. The appointment will be for 1 year, renewable for a second year, commencing not later than October 1974. Salary sfr. 30,000 per annum.

Detailed application including curriculum vitae, list of publications, and names of three referees should be addressed to:

Prof Urs P. Wild,
Physical Chemistry Laboratory,
Universitätstrasse 22,
CH-8006 Zürich, Switzerland. (2



Dow is a world-wide chemical company with sales around £1300 million and an outstanding record of growth. The European Division directs an extensive manufacturing and sales organisation throughout the Continent and UK from its headquarters in Zurich. The UK company has production facilities in King's Lynn and Barry, with sales offices in London and Wilmslow. At King's Lynn the company manufactures plastics and agricultural chemicals. The town itself is an active port situated close to the Norfolk Broads and holiday beaches. A growing industrial centre, it offers economic housing, good schools, shopping and recreational facilities.

*Trademark of the Dow Chemical Company

Entomologist

for Products Screening

to join our recently formed and rapidly expanding Agricultural Research Centre in King's Lynn.

At the Centre, Dow's first major research establishment outside the USA, we are looking for screening specialists to play a key role in its future growth.

Their initial task will be to develop a systematic approach to screening for biologically active compounds, leading eventually to the function of Product Specialist Insecticides.

Candidates, male or female, should have the following qualifications:

- University degree, preferably Ph.D. in Entomology.
- Extensive experience in screening of biologically active compounds.
- A wide knowledge of the crop insecticide markets and their requirements, particularly in the area Europe/Africa.
- A good basic knowledge of organic and biochemistry and of structure-activity relationships.
- Experience in organising biological screens and experience in developing and maintaining laboratory cultures and colonies.
- Experience of working in Continental Europe.
- Fluency in a European language, other than native tongue.

Starting salary will be negotiable depending on experience and qualifications. Benefits include contributory pension scheme, BUPA membership and comprehensive removal allowances. Please write for an application form, quoting reference U.166, to: A. Clapham, UK Personnel Manager, Heathrow House, Bath Road, Hounslow TWS 9QY.

(2247)

RESEARCH TECHNICIAN

required to assist in exciting current project in an expanding field of research for the development of suitable in vitro immunological techniques related to the understanding and treatment of allergy. Salary on Whitley Council scales. Enquiries and applications in writing stating age, qualifications, experience and names of two referees to Mr K. C. Huggins, Department of Immunology (N), The Middlesex Hospital Medical School, Arthur Stanley House, 40/50 Tottenham Street, London W1P 9PG. (2274)

QUEEN ELIZABETH COLLEGE

(UNIVERSITY OF LONDON)
CAMPDEN HILL ROAD, W8 7AH

Applications are invited for the post of Lecturer in the Microbiology Department. Candidates should be postdoctoral or about to complete Ph.D. thesis; they should be active research workers and versatile as teachers. Further particulars from the College Secretary (N), to whom applications should be sent by July 1. (2275)

RESEARCH ASSISTANT

Applications are invited for a Research Assistantship to work on problems concerned with mammalian fertilisation. Applicants should be of at least graduate status with an interest in the physiology and biochemistry of reproduction. The appointment would be for one year in the first instance with the possibility of an extension for a further two years. Salary around £1,850 plus F.S.S.U.

A curriculum vitae and names of two referees should be sent immediately to Dr D. B. Morton, Strangeways Research Laboratory, Worts Causeway, Cambridge CB1 4RN. (2282)

REQUIRED

Female Graduate Geologist with secretarial training. Good shorthand and typing necessary as well as ability to assess and assemble scientific material. Apply: Personnel Officer, Robertson Research International Limited, Ty'n-y-Coed, Llanrhos, Llandudno LL30 1SA, Caernarvonshire, North Wales. (2283)

DUBLIN INSTITUTE FOR ADVANCED STUDIES

SCHOOL OF COSMIC PHYSICS

Applications are invited from persons suitably qualified in the field of Solid Earth Geophysics for a permanent appointment in the Geophysical Section of the School of Cosmic Physics.

Appointment will be made at the level of either Professor or Assistant Professor.

Salary scales: Professor, £4,185 by £188(3) by £187 to £4,936; Assistant Professor, £3,092 by £123(6) by £128 to £3,958. (Both scales under review at present).

Applications, accompanied by a curriculum vitae, list of publications and the names of two referees, should be sent so as to reach the undersigned (from whom further particulars may be obtained) on or before July 8, 1974.

The Registrar,
Dublin Institute for Advanced Studies,
10 Burlington Road,
Dublin 4. (2278)

THE FREE UNIVERSITY OF BERLIN

has an opening at the Assistant Professor level for a MEMBRANE BIOCHEMIST with interests in the membrane transport of yeasts.

The appointment will be for four years (salary about 30,000 DM per annum) commencing to August 1, 1974, or later.

Demands are experience in the isolation and characterisation of cell membranes as well as a good knowledge in biochemical methods. The candidate agrees to perform courses on problems of biochemistry and molecular biology.

Applications giving full details of education and qualifications should be sent within four weeks after publication of this advertisement to Dr Uwe Reichert, Freie Universität Berlin, Zentralinstitut für Biochemie und Biophysik, D-1000 Berlin 33, Ehrenbergstraße 26-28. (2291)

CENTRAL BIRMINGHAM HEALTH DISTRICT

THE CHILDREN'S HOSPITAL

PRINCIPAL AND SENIOR GRADE BIOCHEMIST

The Clinical Chemistry Department at the Children's Hospital gives a Regional Paediatric service and offers experience in a wide range of Biochemical techniques, including screening and research into inherited metabolic disease. The appointment of the successful candidate will depend on previous hospital experience, qualifications, and ability to deputise for the Consultant Chemical Pathologist.. Salary:

Principal £3,969 to £5,175.

Senior £2,964 to £3,843.

For further information please contact Dr D. N. Raine (telephone 021-454 4851), to whom applications giving personal details and the names of two referees should be sent. The Children's Hospital, Ladywood Middleway, Fiveways, Birmingham B16 8ET. (2267)

IMPERIAL COLLEGE

DEPARTMENT OF ELECTRICAL ENGINEERING
ENGINEERING IN MEDICINE LABORATORY

BIOLOGICAL SIGNAL ANALYSIS

A Postdoctoral RESEARCH ASSISTANT to Professor B. McA. Sayers is required for a continuing research programme supported by the Medical Research Council, on the exploratory analysis of signals of neural, physiological, clinical, or epidemiological origin.

Candidates should have a Ph.D. and a good knowledge of computer programming, and preferably be familiar with signal analysis techniques. The appointment will be initially for one year, renewable annually subject to satisfactory progress up to a maximum period of 5 years. The starting salary is up to £2,385 including London allowance (under review) depending on age and experience, and carries membership of F.S.S.U. Applications, with the names of two referees, should be sent to The Secretary, Engineering in Medicine Laboratory, Department of Electrical Engineering, Imperial College, SW7 2BT as soon as possible. Tel. 01-589 5111, Ext. 1409. (2276)

vrije universiteit amsterdam

The Netherlands

The Central Interfaculty of Philosophy has a vacancy for a full

professor of philosophy of science

who should be qualified in the physical sciences and in philosophy and is expected to have a doctor's degree in one of the two.

He should give lecture courses on philosophy of science in general and philosophy of the physical sciences in particular and conduct research in this field.

Teaching and research will be mainly in the Faculty of Mathematics and Natural Sciences, which has departments of mathematics, physics, chemistry, geology and biology and numbers appr. 30 full professors and 1.750 students.

The Central Interfaculty numbers 10 professors and 150 students.

The Free University was founded as a private non-denominational Christian Institution and is now a fully state subsidized private university. The preamble to its charter speaks of the Christian idea of service, in obedience to the Gospel of Jesus Christ, as the University's guiding principle. It will be appreciated if candidates would express their attitude towards this principle in their application.

Gross salary offered: about D.fl. 95.000,- per annum.

Both applicants and those who would like to draw the attention to possible candidates are requested to write within two months from the appearance of this issue to the Chairman of the appointment committee, Dr. J. v.d. Hoeven, Central Interfaculty, Vrije Universiteit, De Boelelaan 1105, Amsterdam, The Netherlands.

Candidates should mention referees when applying.

(2261)

THE HANNAH RESEARCH INSTITUTE

DEPARTMENT OF APPLIED STUDIES

CARBOHYDRATE BIOCHEMISTRY

Applications are requested from persons having experience of carbohydrate biochemistry to assist with work on the starch-degrading enzymes of bovine serum as an index of genetic potential. The appointment will be made at the H.S.O. level (£2,221 to £2,854, with an allowance of 5½% to offset superannuation contributions); applicants should possess an honours degree and a minimum of two years' relevant postgraduate experience. Applications, giving a full curriculum vitae and the names of two referees, should be sent to the Secretary, The Hannah Research Institute, Ayr KA6 5HL to whom further particulars may be obtained from by June 21, 1974. (2284)

UNIVERSITY OF TORONTO

INSTITUTE OF BIOMEDICAL ENGINEERING

DIRECTOR

Applications are invited for the above position in Ontario, Canada. The applicant should have distinction as an investigator in the biological engineering field, with academic experience, leadership, and administrative ability. The Director of the Institute will also be appointed to appropriate Departments in Engineering and/or Medicine.

Nominations and applications should be directed to the Chairman of the Search Committee, Professor B. Etkin, Faculty of Applied Science and Engineering, University of Toronto, Toronto, Ontario, Canada.

(2290)

Ph.D. PSYCHOLOGIST

Ph.D. Psychologist to be responsible for carrying out specific phases of research with nonhuman primates. The applicant should be competent in telestimulation and telemetry of discrete brain areas, have a thorough knowledge of the neuroanatomy of Macaca speciosa, experience in the recording of single unit activities in the awake animal, and must know how to suppress stimulus artifacts. Other duties involve biochemical assays of perfusates obtained from push-pull cannula and iontophoretical studies of neuronal activities in CNS.

Assistant Professor level. Send applications to Professor James Maas, Department of Psychiatry, Yale University School of Medicine, 333 Cedar Street, New Haven, Ct., 06510, U.S.A.

AN EQUAL OPPORTUNITY EMPLOYER

(2285)

POLYTECHNIC OF THE SOUTH BANK
DEPARTMENT OF PHYSICS
GRADUATE SCIENTIFIC ASSISTANT

Applications are invited for a Graduate Scientific Assistant to work with Dr Haque on an M.R.C. supported project to study the radiation dose to the respiratory system due to radon and its daughter products using experimental and computer methods. Candidates should possess a good Honours degree in Physics or equivalent qualifications and preferably postgraduate experience. The successful applicant may register for a Ph.D. degree.

Salary offered is up to £1,400 per annum, depending on age and experience.

Applications by letter, naming two referees, should be sent to the Secretary, Physics Department, Polytechnic of the South Bank, Borough Road, London SE1 0AA, from whom further particulars may be obtained. (2268)

UNIVERSITY OF LONDON GOLDSMITHS' COLLEGE
NEW CROSS, LONDON SE14 6NW
CHEMISTRY DEPARTMENT

Required, as soon as possible, Lecturer in Chemistry with good academic qualifications, able to teach inorganic and/or physical chemistry up to B.Ed. and B.Sc. Standard II.

Salary on Pelham scale for teaching staff in College of Education, plus London Allowance.

For further particulars write, enclosing a stamped addressed A4 envelope, to the Personnel Officer, to whom applications should be sent by June 14, 1974. (2263)

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY
DEPARTMENT OF BIOCHEMISTRY
POSTGRADUATE RESEARCH

Research activities open to graduates wishing to work for the M.Phil. or Ph.D. degree include:

Yeast viruses: host-virus relationships.

Mycoviruses: characterisation and biochemistry of virus replication cycle.

Grants will be available to suitable candidates.

Enquiries should be made to:

Dr K. W. Buck,
Department of Biochemistry,
Imperial College,
London SW7 2AY. (2262)

INSTITUTE OF ARCHAEOLOGY
UNIVERSITY OF LONDON

Lecturer/Senior Lecturer in Archaeological Conservation. Salary within the scale £2,118 to £5,844. The person appointed will be required to organise teaching and research relating to the conservation of archaeological materials, in which subject new First Degree and Higher Degree courses are being established. Qualifications should include a good Honours Degree in a science subject (preferably Chemistry). Practical experience in the techniques of archaeological conservation would be an asset. The successful applicant will be expected to take up his post on, or as soon as possible after, October 1, 1974. Applications (2 copies) giving full details of qualifications, experience and publications should reach the Director, University of London Institute of Archaeology, Gordon Square, London WC1H 0PY by June 30, 1974. (2292)

UNIVERSITY OF THE SOUTH PACIFIC
FIJI

Applications are invited for LECTURESHIP IN CHEMISTRY in the School of Natural Resources, tenable from the beginning of 1975. Applicants should have interests in Inorganic Chemistry and should have a strong motivation to developing courses appropriate to the South Pacific context. Teaching duties will be assigned by the Head of Chemistry and may be at all levels of the B.Sc. course. Research involving the natural resources of the Region is encouraged and there are unique opportunities in areas such as Marine Chemistry and Soil Science. Salary scale (under review): $F4,700 to $F6,500 p.a. (£1 sterling = $F1.94). 10% annual gratuity for contract appointment; superannuation contributions; partly furnished accommodation at rental of 15% of salary; appointment allowance. Contracts may be for two, four or six years. Formal applications (6 copies) should contain full name, date and place of birth, nationality, marital status, educational qualifications, employment history and experience, names and addresses of three referees, general statement of physical fitness, estimated starting date. A passport-size photograph should also be sent. Further particulars, including an outline terms of service, are available from the Registrar (Post 74/16), University of the South Pacific, P.O. Box 1168, Suva, Fiji. Closing date: as soon as possible. (2306)

Agro Chemical Formulation

Development of our activities has created the need for a graduate to work in our Development Laboratories concerned with Animal health, pet, crop protection and amateur garden products. The position, tenable at Beeston near Nottingham, involves the design and investigation of formulations, including appropriate stability testing. Particular emphasis will be placed on investigational work.

Applications are invited from graduates in Chemistry, Pharmacy or an allied subject and although experience in Product Development would be an advantage it is not essential.

Conditions of employment are first class. Some help with relocation expenses will be given where appropriate.

Please write for application form, quoting ref. U.198, to J. F. Pattison, Employment Manager.

Boots

The Boots Company Ltd.
Nottingham NG2 3AA.

(2336)

THE GEOLOGICAL SURVEY OF GREENLAND
Stratigrapher/Palynologist

A position is available in the Oil and Gas Section of the Geological Survey of Greenland for a geologist with a background in stratigraphy and in particular palynology. The successful candidate will be required initially to work on Mesozoic-Tertiary sediments of central West Greenland, but will later assist in building up knowledge of the West Greenland shelf on the basis of deep well samples.

The person appointed will be based in Copenhagen and will be required to undertake periodic field work in Greenland.

Applications should be accompanied by a detailed curriculum vitae, and indication of the approximate salary required, and the names of two referees, and should be sent before August 1, 1974, to: The Director, The Geological Survey of Greenland, Oster Voldgade 10, DK-1350 Copenhagen, Denmark. (2243)

Graduate Required

Physics/Chemistry Graduate required for interesting research post within our Central Laboratories Division. The job is concerned with the creepage along and wetting of metal surfaces by liquids. The position is new and offers excellent scope for originality and innovation.

Undergraduates qualifying this year will be considered.

Research facilities and future prospects are good.

Location: North London.

Hours: 9 am to 5.15 pm (Mon to Fri) 36¼ hr. week.

Apply:

EVER-READY®

Administration Manager,
Ever Ready (Holdings) Limited,
Central Laboratories,
St. Ann's Road,
London, N15 3TJ. Tel. No. 01-800 1101. (2244)

Beecham Pharmaceuticals

Biochemist

A Biochemist is required for the Fermentation Development Department to investigate the metabolic control of penicillin production.

Ideally applicants should be post doctoral workers with experience of the biosynthesis of secondary metabolites or the control of metabolic pathways, although a good new graduate with a keen interest in these areas could be considered. Excellent facilities exist for studying Laboratory, Pilot Plant and large-scale production fermentations.

We offer competitive salaries, four weeks holiday, excellent Sport and Social facilities and an attractive South Coast location. Benefits also include a non-contributory pension and life assurance scheme.

Please write giving brief details of age, qualifications and experience to Miss C. Turnbull, Personnel Officer, Beecham Pharmaceuticals, Clarendon Road, Worthing, Sussex.

THE QUEENS AWARD TO INDUSTRY 1966 1970 1971 1972.

(2281)

Lister Institute of Preventive Medicine

(University of London)

Elstree, Herts.

PRODUCTION MANAGER

The Lister Institute requires a Manager to take charge of the production for human use of bacterial vaccines, virus vaccines and therapeutic sera at its Elstree Laboratories. Applicants must have considerable experience in the organisation and day to day running of a unit producing biological materials; and the person appointed will be expected to take an active interest in the expansion of production. The salary will be in accordance with experience and qualifications and will be appropriate to a post of this seniority. Superannuation under F.S.S.U.

Applications to: Secretary, Lister Institute of Preventive Medicine Elstree, Hertfordshire.

(2245)

NATIONAL INSTITUTE FOR MEDICAL RESEARCH

MILL HILL, LONDON

ELECTRON MICROSCOPY TECHNICIAN

familiar with thin tissue sectioning is required to assist in the examination of cells and sub-cellular components during animal development. Experience in electron microscopy would be an advantage. Initial salary, according to age, qualifications and experience, will be on scales £1,566 to £2,418 p.a. or £2,202 to £2,949 p.a. Please write, quoting ref. EM/DB to J. H. Woodcock, Personnel Officer, N.I.M.R., The Ridgeway, Mill Hill, London NW7 1AA. Tel: 01-959 3666. (2232)

BIRKBECK COLLEGE
(UNIVERSITY OF LONDON)
DEPARTMENT OF ZOOLOGY
M.Sc. DEGREES FOR PART-TIME STUDENTS
IN
ENTOMOLOGY
AND IN
COMPARATIVE PHYSIOLOGY

Each Course extends over a period of two calendar years and involves attendance on three evenings each week during term. Examination is by written papers and course assessment.

Full details may be obtained from The Secretary, Department of Zoology, Birkbeck College, Malet Street, London WC1E 7HX, to whom application should be addressed. (2293)

WYE COLLEGE
(UNIVERSITY OF LONDON)
PLANT BREEDER

Applications are invited for a temporary appointment as Assistant Plant Breeder in the Hop Research Department.

The Department undertakes research on most aspects of the hop crop and the plant breeding section is engaged in meeting the urgent needs of the industry for varieties of improved brewing value combined with disease and pest resistance.

The successful applicant will be expected to assist with all aspects of the breeding programme but to concentrate on the nature and inheritance of disease and pest resistance and to undertake some cytological work. There will be every opportunity for undertaking a higher degree.

The appointment will be in the S.O. (£1,707 to £2,329) or H.S.O. (£2,221 to £2,854) grade according to experience, and will be for four years. There will be a non-pensionable allowance of 4½% of gross salary to offset contributions to the Superannuation Scheme operated under the F.S.S.U.

Candidates should have, or expect to have, a good Honours degree but postgraduate experience is not essential.

Applications, naming two referees to the Secretary (N), Wye College, Wye, Ashford, Kent TN25 5AH, by June 30, 1974. (2294)

UNIVERSITY COLLEGE, CARDIFF

RESEARCH ASSISTANTSHIPS IN THEORETICAL ASTRONOMY

Applications are invited from suitably qualified candidates for two S.R.C. Research Assistantships: one for work on Relativistic Astrophysics and one for work on Interstellar and Intergalactic Grains. Salary Range: £2,118 to £2,247. Duties to commence as soon as possible. Closing date is two weeks from the appearance of this advertisement.

Applications, together with the names and addresses of two referees, should be forwarded to The Registrar, University College, P.O. Box 78, Cardiff CF1 1XL. Please quote ref. 0582. (2288)

UNIVERSITY OF IBADAN
NIGERIA

Applications are invited for the following posts in the DEPARTMENT OF AGRICULTURAL ECONOMICS AND EXTENSION:—

1. SENIOR LECTURER IN RURAL DEVELOPMENT AND AGRICULTURAL POLICY. Applicants should be Agricultural Economists with emphasis on Rural Development and Agricultural Policy. They should have a Ph.D. in Agricultural Economics with a sound background of economic theory and its application to rural development and agricultural policy. They should also possess proven ability to undertake and direct comprehensive research in rural integrated development.

2. SENIOR LECTURER IN AGRICULTURAL PRODUCTION ECONOMICS AND ECONOMETRICS. Applicants should specialise in the field of Agricultural Production Economics and Econometrics. They should have a Ph.D. with a major in Production Economics and a minor in Agricultural Statistics and/or Econometrics. The appointee should be able to supervise experimental designs for graduate research in the Faculty.

Salary scale: N5,030 to N5,750 p.a. (£1 sterling =N1.47). The British Expatriates Supplementation Scheme is unlikely to be applied to these appointments. F.S.S.U. Family passages; various allowances; regular overseas leave. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by air mail, not later than 1st July, 1974, to the Registrar, University of Ibadan, Ibadan, Nigeria. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London, W1P 0DT. Further particulars of these appointments may be obtained from either address. (2312)

UNIVERSITY OF KHARTOUM
SUDAN

Applications are invited for (a) READERSHIP or (b) SENIOR LECTURESHIP IN THE DEPARTMENT OF PHARMACEUTICS. This post is tenable for one year only. Salary scales: (a) £S3,000 p.a. (b) £S2,400 to £S2,800 p.a. (£1 sterling=£S0.77). The British Government (or similar arrangement may be made) may supplement salary of Senior Lecturer by £1,600 p.a. (sterling) for married appointee or £900 p.a. (sterling) for single appointee (normally free of all tax). The supplementation for Reader's salary will be determined when an appointment is made at that level. These supplementation rates are currently under review. There may also be provision of children's education allowances and holiday visit passages. Family passages; various allowances; superannuation scheme; annual overseas leave. Detailed applications (2 copies), including a curriculum vitae nd naming 3 referees, should be sent by air mail, not later than June 27, 1974 to the Personnel Secretary, University of Khartoum, P.O. Box 321, Khartoum, Sudan. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address. (2305)

UNIVERSITY OF KHARTOUM
SUDAN

Applications are invited for the post of SENIOR TECHNICIAN IN THE DEPARTMENT OF PHYSIOLOGY. Candidates should hold A.I.M.L.T. Salary scale: £S1,250 to £S1,500 p.a. (£1 sterling=£S0.77). The British Government may provide a supplement to the salary, the amount to be determined when an appointment is made. This supplementation is normally free of all tax. There may also be provision of children's education allowances and holiday visit passages. Family passages; various allowances; superannuation scheme; annual overseas leave. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by air mail, not later than June 27, 1974 to the Personnel Secretary, University of Khartoum, P.O. Box 321, Khartoum, Sudan. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London, W1P 0DT. Further particulars may be obtained from either address. (2304)

NEW ZEALAND
UNIVERSITY OF CANTERBURY
CHRISTCHURCH
VISITING LECTURER IN CHEMISTRY

Applications are invited for a Visiting Lectureship in Chemistry tenable for one year. The appointment is to be taken up as early as possible in 1975. It is anticipated that the appointee will have completed a Ph.D., and show evidence of strong research interest in some particular field of Chemistry. The emolument for the position is in the range of NZ$6,753 to NZ$8,568 per annum. (Subject to regular cost of living adjustments.) When the salary is being decided, due account will be taken of the appointee's travel costs.

Further particulars and Conditions of Appointment may be obtained from the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London, WC1H 0PF.

Applications close on **September 1, 1974.** (2311)

UNIVERSITY OF OTAGO
DUNEDIN, NEW ZEALAND
LECTURER OR SENIOR LECTURER IN PHYSIOLOGY

Applications are invited from Medical or Science Graduates for appointment as Lecturer or Senior Lecturer in Physiology. Preference will be given to candidates with experience in renal and electrolyte physiology, but those with interests in other fields will be considered.

Medical Graduate: Lecturer—A scale from NZ$9,172 to NZ$13,107 per annum with a bar at NZ$10,836 per annum; Senior Lecturer—A scale from NZ$13,485 to NZ$15,528 per annum.

Science Graduate: Lecturer—A scale from NZ$6,753 to NZ$8,568 per annum; Senior Lecturer—A scale from NZ$8,718 to NZ$11,139 per annum.

Further information may be obtained from the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University. Additional detailed information may be obtained from Professor J. R. Robinson, Department of Physiology, University of Otago Medical School, P.O. Box 913, Dunedin, New Zealand.

Applications close in London and in New Zealand on **July 31, 1974.** (2308)

SWISS FEDERAL INSTITUTE OF TECHNOLOGY, ZURICH (SWITZERLAND)
(EIDGENÖSSICHE TECHNISCHE HOCHSCHULE)

Professor of Experimental Nuclear Physics

Applications are invited for the above appointment which is to commence on April 1, 1975 or by arrangement. The professor will be expected to carry out teaching and research in experimental nuclear physics and to take part in the general teaching programme of the Physics Department.

Candidates are requested to send their applications with curriculum vitae, and list of scientific publications (and names of references) not later than June 30, 1974 to: The President, Eidgenössische Technische Hochschule Zürich, CH-8006 Zürich/ Switzerland. (2216)

Australian Academy of Science

Executive Secretary

The present Executive Secretary of the Australian Academy of Science, Mr J. Deeble, shortly takes up the post of Director of Special Projects. The Academy therefore invites applications for the post of Executive Secretary. The appointee will be responsible to the Council, through its Officers, for the conduct of the business of the Academy. Applicants should have a background in science and ability and experience in administration. General information is available from the Year Book of the Academy. Supplementary information will be furnished by the Secretaries on application to the address below.

Salary will be in the range Reader to Professor in Australian Universities (currently $A16,389 to $A19,614 per annum).

Applications should be forwarded to the **Secretaries, Australian Academy of Science, P.O. Box 821, Canberra City, A.C.T. 2601, Australia.** They should include a detailed curriculum vitae and the names of at least three referees. The closing date is **July 5, 1974.** (2322)

ST. MARY'S HOSPITAL MEDICAL SCHOOL
(University of London)
Paddington, London W2 1PG

Applications are invited from candidates with 1st or 2nd Class Honours Degree in Biochemistry or related discipline wishing to work for a Ph.D. for three studentships for training in research methods in Pharmacological Biochemistry. These awards will be governed by the M.R.C. regulations. Apply with curriculum vitae and names of two referees to Professor R. T. Williams, Department of Biochemistry. (2326)

WESTFIELD COLLEGE
(UNIVERSITY OF LONDON)
LECTURESHIP IN ZOOLOGY

Applications are invited for a lectureship in the Zoology Department which it is anticipated will become established with effect from October 1, 1974. The post will be for three years in the first instance and an appointment will be made on one of the three lowest points on the lecturer scale £2,118, £2,247, or £2,412 plus £162 London Allowance. Applicants should have an experimental approach involving the study of whole animals in relation to their environment & should be capable of contributing to the teaching programmes in Cell-Biology or environmental science. Applications (6 copies) should be sent as soon as possible and not later than **June 18, 1974,** giving the names of two referees to Dr J. Buckingham, Personnel Officer, Westfield College (N), Kidderpore Avenue, Hampstead, London NW3 7ST. **Applicants quoting overseas referees should ask them to submit references immediately and without further intimation.** (2302)

UNIVERSITY OF KHARTOUM
SUDAN

Applications are invited for the following posts in the Department of Clinical Studies, Faculty of Veterinary Science:—

1. SENIOR LECTURER OR LECTURER IN VTERINARY OBSTETRICS AND GYNAECOLOGY. Candidates should hold a first in Veterinary Medicine and a higher degree in Obstetrics and Gynaecology, and have at least three years' teaching and practical experience. Appointee will teach Veterinary Obstetrics and Gynaecology to students and participate in hospital work and research.

2. SENIOR LECTURER OR LECTURER IN VETERINARY SURGERY. Candidates should hold a higher degree in Veterinary Surgery and at least two years' teaching experience at university level. Duties will be: teaching Veterinary Surgery, including Anaesthesia, to final and subfinal year students; doing research in the Department; supervision of postgraduate students for higher degrees; participation in clinical and surgical work in connection with the University Veterinary Clinic.

Safary scales: Senior Lecturer £S2,400 to £S2,800 p.a.; Lecturer £S1,500 to £S2,300 p.a. (£1 sterling=£S0.77). The British Government may supplement salaries in range £1,300 to £1,600 p.a. (sterling) for married appointees or £800 to £900 p.a. (sterling) for single appointees (normally free of all tax) and provide children's education allowances and holiday visit passages. These supplementation rates are currently under review. Family passages; various allowances; superannuation scheme; annual overseas leave. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by air mail, not later than June 27, 1974 to the Personnel Secretary, University of Khartoum, P.O. Box 321, Khartoum, Sudan. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London, W1P 0DT. Further particulars may be obtained from either address. (2303)

MASSEY UNIVERSITY
PALMERSTON NORTH, NEW ZEALAND
SENIOR LECTURER/LECTURER IN VETERINARY PUBLIC HEALTH AND MEAT HYGIENE

Applications are invited from veterinary graduates for this new post in the Department of Veterinary Pathology and Public Health. Applicants should have an interest in this challenging and increasingly important sphere of veterinary science. Although experience in some facet of this discipline, which includes meat hygiene as an important component, is desirable, a recent graduate would be considered. Such a latter appointment would be in the lower scale of the lecturer range and the appointee would be given the opportunity to undertake further study.

The successful applicant will be required to assist in the teaching of veterinary public health and meat hygiene to both undergraduate and postgraduate students, and to develop research in appropriate fields of study. The position involves close cooperation with workers in related disciplines both within and outside Massey University. The particular interests of the department include basic epidemiology, meat hygiene and food born diseases and all aspects of zoonoses and general public health.

Present salary scales are: Lecturer NZ$6,753 to NZ$8,568; Senior Lecturer NZ$8,718 to NZ$10,232 (bar) $NZ11,139.

Further details of the position and of the University, together with the general conditions of appointment may be obtained from the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University.

Applications close on **July 15, 1974.** (2309)

THE BRITISH NUTRITION FOUNDATION

An independent advisory and grant-giving body, invites applications from graduates in Food Science or Technology or Nutrition for the post of Scientific Information Officer. Salary negotiable, but up to £2,750 depending on experience. Particulars of the duties may be obtained from the Director-General of The British Nutrition Foundation, Alembic House, 93 Albert Embankment, London SE1 7TY, by whom applications, including particulars of career and the names and addresses of two referees, should be received not later than June 28, 1974. (2300)

AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited for the following:

RESEARCH SCHOOL OF SOCIAL SCIENCES
RESEARCH FELLOW
DEPARTMENT OF STATISTICS

The successful applicant will be expected to engage in research in probability and statistics. The interests of present members of the department are in time series, gometrical probability, random processes and statistical inference. Head of the Department is Professor P. A. P. Moran. Closing date: **July 5, 1974.**

RESEARCH SCHOOL OF BIOLOGICAL SCIENCES
DEPARTMENT OF DEVELOPMENTAL BIOLOGY

Appointees to the following posts will work in a research group headed by Professor Brian Gunning.

RESEARCH FELLOW

The proposed field of study is the analysis of developmental processes in plant cells, especially algae, using ultrastructural methods in conjunction with additional experimental approaches.

RESEARCH ASSISTANT

Applicants should have a degree in a life science, an interest in plant cell biology, and preferably some experience of electron microscopy. The work will involve assistance in the work of the group by means of laboratory and library research. Closing date: **July 12, 1974.**

Salaries: Salary on appointment to the posts will be in accordance with qualifications and experience within the ranges: Research Fellow $A8,698 to $A11,901 p.a.; Research Assistant Grade 1 $A4,394 to $A6,250 p.a.; Research Assistant Grade 2 $A6,247 to $A7,846 p.a. Current exchange rates are approximately $A1=$US1.49.

Other Conditions: Tenure: Research Fellow normally for three years in the first instance with the possibility of extension to a maximum of five years; Research Assistant initially for a period of two years.

Reasonable travel expenses are paid and assistance with accommodation is provided for an appointee from outside Canberra. Superannuation (where applicable) is on the F.S.S.U. pattern with supplementary benefits.

Travel expenses from overseas (except New Zealand) are not paid for posts at the level of Research Assistant but assisted passage can be considered for a person offered appointment from the United Kingdom who intends to settle permanently in Australia.

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should write to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, for further particulars before applying. (2310)

UNIVERSITY OF EAST ANGLIA
SCHOOL OF CHEMICAL SCIENCES

Applications are invited for a S.R.C. Post-Doctoral Research Assistantship for one year in the first instance from 1 October 1974, to work on the magnetic circular dichroism of matrix-isolated molecules. A novel apparatus for this purpose has recently been constructed and is now operational. The investigation is to be extended to the study of the magnetic and optical properties of unstable molecules generated photochemically. Salary will be on a scale starting at £1929 per anunm plus F.S.S.U., according to previous experience.

Applications as soon as possible either to Dr. R. Grinter or Dr. A. J. Thomson, School of Chemical Sciences, University of East Anglia, Norwich, NOR 88C. (2296)

UNIVERSITY OF STRATHCLYDE
DEPARTMENT OF NATURAL PHILOSOPHY
LECTURESHIP

Applications are invited from experimental physicists preferably with research experience in plasma/laser physics.

The successful candidate will be required to take part in undergraduate teaching and to pursue research in his field of interest.

Salary scale £2,118-£4,896 (1st October, 1974) with placing according to qualifications and experience. F.S.S.U. benefits.

Application forms and further particulars (quoting 23/74) may be obtained from the Registrar, University of Strathclyde, Royal College Building, 204 George Street, Glasgow, G1 1XW with whom applications should be lodged by 17 June, 1974. (2316)

UNIVERSITY OF GLASGOW
DENTAL HOSPITAL AND SCHOOL
RESEARCH ASSISTANTSHIP IN BIOCHEMISTRY

Applications are invited for a RESEARCH ASSISTANTSHIP in BIOCHEMISTRY, tenable for a period of three years from September or October 1974.

Candidates should have an Honours Degree in Biochemistry or an allied subject. There will be an opportunity to apply to register for a higher degree.

The work will involve a study of salivary proteins with a view to correlating abnormalities with pathological disorders. The project is financed by a grant from the Scottish Home and Health Department.

Salary Scale £1,500 to £1,650 per annum. F.S.S.U.

Applications, including curriculum vitae and names of two referees, should be sent to Dr. J. A. Beeley, Department of Dental Biochemistry, University of Glasgow Dental School, 378 Sauchiehall Street, Glasgow, G2 3JZ, from whom further information may be obtained. (2298)

UNIVERSITY OF IBADAN
NIGERIA

Applications are invited for the post of READER IN PHYSICAL BIOCHEMISTRY OR MICROBIOLOGICAL CHEMISTRY in the Department of Biochemistry. Applicants should hold a research doctorate and have several years academic experience. Salary: N6,100 p.a. (£1 sterling=N1.47). The British Expatriates Supplementation Scheme is unlikely to be applied to this appointment. F.S.S.U. Family passages; various allowances; regular overseas leave. Detailed applications (2 copies) including a curriculum vitae and naming 3 referees, should be sent by air mail, not later than 1st July 1974 to the Registrar, University of Ibadan, Ibadan, Nigeria. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London, W1P 0DT. Further particulars of these appointments may be obtained from either address. (2307)

UNIVERSITY OF MANCHESTER
DEPARTMENT OF PHYSICS

Eighteen months postdoctoral appointment for research on photophysics of aromatic hydrocarbons at Synchrotron Radiation Facility, Daresbury. Apply Dr S. Hamilton, Manchester M13 9PL (Phone 061-273 3333) as soon as possible. (2329)

UNIVERSITY OF CAPE TOWN
SOUTH AFRICA
SENIOR LECTURER IN DEPARTMENT OF MICROBIOLOGY

Applications invited for above post at University of Cape Town. Salary commensurate with Senior Lectureship status and local conditions. Starting salary dependent on qualifications, which should include experience in Virology or Immunology.

Full details on request.

Submit resumé and names and addresses of references to:

Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa.

Closing date July 31, 1974. (2317)

THE UNIVERSITY OF SHEFFIELD
UNIVERSITY DEPARTMENT OF MEDICINE, THE ROYAL HOSPITAL
RESEARCH ASSISTANT

required for investigation concerning regulation of gastrointestinal fluid transport in experimental animal and man, financed by The Wellcome Trust. Applicants should be science graduates with some postgraduate laboratory experience. The post is tenable for up to three years from a date to be arranged. The work would be suitable for a higher science degree. Initial salary £1,929 a year, rising to £2,223 a year, subject to limitation if the person appointed registers for a higher degree. Further particulars from the Registrar and Secretary, The University, Sheffield S10 2TN to whom applications should be sent by June 21, 1974. Please quote reference R85/G. (2328)

BEDFORD COLLEGE
(UNIVERSITY OF LONDON)
RESEARCH ASSISTANT
in
ZOOLOGY

Research Assistant required to work under a three year N.R.C. grant on the neurophysiology and behaviour associated with limb shedding in crabs.

Applications are invited from recently qualified, or soon to be qualified graduates, who have had experience in neurophysiology and routine microscopy. The work will involve visits to a marine laboratory. The appointment will date from October 1, 1974. Salary scale £1,500 by 2/87 to £1,674 p.a.

Applications quoting names of two referees, to: Assistant Secretary (Personnel), Bedford College, Regent's Park, London NW1 4NS. (Tel. 01-486 4400, Ext. 312). (2327)

THE ANIMAL HEALTH TRUST
EQUINE RESEARCH STATION, NEWMARKET
CHEMIST/BIOCHEMIST

Applications are invited for the post of research assistant in the laboratory for chemical pathology to undertake research into the basic chemical pathology of the thoroughbred racehorse.

The post is for three years in the first instance and is suitable for a graduate in biological sciences wishing to work for a higher degree. Salary on scale £1,560 to £1,704 per annum with F.S.S.U. benefits.

Further details and application forms are available from the Chief Executive Officer, The Animal Health Trust, 24 Portland Place, London W1N 4HN. (2330)

ROYAL STATISTICAL SOCIETY
LIBRARIAN

The Royal Statistical Society invites applications for the post of Librarian. Candidates should be Chartered Librarians, will be expected to have had considerable experience of library work, preferably in the statistical or analogous fields and will be required to take over the duties of the present Librarian within a year.

The Library is situated near Manchester Square and other staff are employed.

Starting salary in accordance with age and experience within a competitive salary scale.

Applications in writing to the Secretary, Royal Statistical Society, 21 Bentinck Street, London W1M 6AR. (2331)

MEDICAL RESEARCH COUNCIL

An adequately qualified technician required to work in the Virology Laboratory of the

Laboratory Animals Centre
Woodmansterne Road
Carshalton, Surrey

The position calls for an experienced person with the ability to adapt and/or devise techniques for incorporation into a comprehensive routine for the diagnosis of disease in a wide range of laboratory animals, mainly using serological procedures.

The ability to work on his own initiative and with a minimum of supervision calls for a high level of self-starting and self-maintaining potential.

For the right person, this could be a most rewarding position in a little known but expanding field.

Salary scale ranging from £1,440 to £2,292 plus London Weighting.

Application forms available from the Chief Technician's Office or telephone 01-643 8000.

(2333)

LIVERPOOL POLYTECHNIC
DEPARTMENT OF BIOLOGY
LECTURER GRADE II IN PHYSIOLOGY

Applications are invited for the post of Lecturer Grade II in Animal Physiology. Applicants should have an interest in the Environmental Aspects of Animal Physiology, have a good Honours Degree and have pursued a research problem to the level of Ph.D. Teaching or Industrial/Research station experience would be of advantage. The successful candidate will be expected to teach to Honours Degree level, to contribute to the post-graduate teaching programme and to undertake research.

SALARY: £2,700 to £3,474 p.a.

Details available from Staff Office, Dept. N, Liverpool Polytechnic, Richmond House, 1 Rumford Place, Liverpool, L3 9RH. (2321)

THE QUEEN'S UNIVERSITY OF BELFAST
SENIOR EXPERIMENTAL OFFICER IN ELECTRON MICROSCOPY

Applications are invited for the post of Senior Research Officer in Electron Microscopy in the Department of Pathology. Candidates should have a University Degree or equivalent qualification in a relevant scientific subject. Extensive experience is required in the conduct and management of an Electron Microscope Laboratory including all aspects of maintenance and service. Research experience in the structure of biological materials would be an advantage and the appointee will be expected to conduct research on the nature of connective tissue and to collaborate in other University research programmes. The appointee will take part in the provision of diagnostic services to the Royal Victoria and City Hospitals under the direction of Professor Gardner. The post will carry a limited undergraduate teaching commitment. The salary scale is £2,736 to £3,708 (under review) plus F.S.S.U. Initial placing on the scale will depend on qualifications and experience. Application should be made in writing, giving details of qualifications and experience and the names of two referees, to the Personnel Officer, The Queen's University of Belfast, Belfast BT7 1NN, Northern Ireland, by June 30, 1974. (2325)

NATIONAL INSTITUTE OF AGRICULTURAL ENGINEERING
SCOTTISH STATION
ABSTRACTOR/LIBRARIAN

Abstractor/Librarian required by Agricultural Research Institute to run small library, provide scientific information and abstracting service to staff, arrange translation of scientific papers, etc.

The appointment will be on Higher Scientific Officer Grade, salary for which is £2,221 to £2,854 by eight annual increments.

Further details and application form from the Secretary, N.I.A.E., Scottish Station, Bush Estate, Penicuik, Midlothian, EH26 0PH, to whom completed forms should be returned by June 21, 1974. (2320)

OXFORD UNIVERSITY
DEPARTMENT OF ZOOLOGY

Applications are invited for the post of Research Assistant to work under Dr Malcolm Coe on the reproductive biology of Giant Tortoises on Aldabra Island in the Indian Ocean. The appointment will be for a period not exceeding three years, of which two years will be spent carrying out field work on Aldabra and one year in Oxford.

Applicants should be available to take up this post by the beginning of August 1974. Preference will be given to candidates with an interest or experience in the field of Herpetology. Salary will be by arrangement according to age, qualifications and experience.

Applications with a full curriculum vitae and the names of two referees should be sent immediately to the Secretary, Animal Ecology Research Group, Department of Zoology, South Parks Road, Oxford OX1 3PS. (2334)

F.R.A.M.E.

which is concerned with disseminating information on alternatives to laboratory animals seeks science graduate with initiative to assist with P.R. work, literature searching and abstracting. The post is challenging and offers great scope to anyone seeking a rewarding job with a real sense of purpose. For further details write to: The Secretary, F.R.A.M.E. (Fund for the Replacement of Animals in Medical Experiments), 312a Worple Road, London SW20 8QU. (2332)

FELLOWSHIPS AND STUDENTSHIPS

UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF ANATOMY
BEHAVIOUR OF NON HUMAN PRIMATES

RESEARCH STUDENTSHIP available for studying the effects of hormones on the behaviour of non-human primates. Acceptance subject to the University's regulations for research students. Applications (with the names of two referees) to Dr J. Herbert, Department of Anatomy, Downing Street, Cambridge CB2 3DY. (2127)

RESEARCH STUDENTSHIP

Applications are invited for a three-year research studentship to study aspects of the vitamin requirements of the Golden hamster (**Mesocricetus auratus**). Applicants should have or expect to attain this summer, first or upper second class honours degree in nutrition, or a relevant biological discipline. The successful candidate who will register for further degree, will receive a grant plus a small travel and book allowance, and fees will also be paid.

Applications with names of two referees and short curriculum vitae should be made to Dr. D. J. Ford, Medical Research Council, Laboratory Animals Centre, Woodmansterne Road, Carshalton, Surrey SM5 4EF, from whom further details may be obtained. (2019)

THE SCHOOL OF PHARMACY
UNIVERSITY OF LONDON
DEPARTMENT OF PHARMACOLOGY
BRAIN RESEARCH STUDENTSHIP

Applications are invited from students graduating in biochemistry, physiology or pharmacology for an M.R.C. Research Studentship. The successful applicant will work with Dr M. J. Neal on various aspects of transmitter substances in the brain.

Applications, with the names of two referees should be sent to Dr M. J. Neal, Department of Pharmacology, The School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX. (2217)

UNIVERSITY OF QUEENSLAND
AUSTRALIA
POSTDOCTORAL FELLOWSHIP

Solid state physicist with experimental or theoretical experience in semi-conductors to join solar energy project on photovoltaic cells using organic materials.

Salary in range $A8,698 to $A14,308 per annum, plus 10% superannuation.

Start early 1975. Appointment 1 year, renewable, maximum 3 years.

Write to Professor L. E. Lyons, Department of Chemistry, University of Queensland, St. Lucia, Brisbane, Queensland 4067, Australia. (2219)

The POLYTECHNIC WOLVERHAMPTON

Faculty of Science:

Research Studentships

Applications are invited for studentships tenable from September 1974 on the following research projects:

SRC Case Studentships
(with Shell Research)
Insect acetylcholine receptor molecules.
Plant cell plasma membranes.

(with Koch-Light Ltd)
Non-aqueous applications of water-insoluble enzymes.
Biospecific absorbents.
High speed gel permeation chromatography.

Industrial Studentships
Affinity chromatography.
Solid phase synthesis.

Applicants should normally hold by September appropriate honours degrees or equivalent professional qualifications. Applications (curriculum vitae and names of 2 referees) or requests for further information to the Establishment Officer, The Polytechnic, Wolverhampton WV1 1LY.

(2212)

THE QUEEN'S UNIVERSITY OF BELFAST
DEPARTMENT OF INDUSTRIAL CHEMISTRY

Post Graduate Studentships

Applications are invited for the above studentships which are tenable for three years. The topics of research include: Studies in the crosslinking reactions of proteins related to nutrition; Oxidation reactions in the bleaching of cellulosic fibres; Studies in the depolymerisation of polypropylene in the recycling of waste materials; Chemical engineering studies related to the drying of fabrics; Physico-chemical studies of the dyeing mechanisms of protein fibres.

Post graduate studentships are normally valued at about £700 p.a. plus fees.

Applicants should have an honours degree in chemistry, biochemistry or chemical engineering (or equivalent professional qualifications). Applications should be received by August 1, 1974. Further details from Professor R. S. Asquith, Department of Industrial Chemistry, The Queen's University of Belfast, Belfast 9.

(2324)

UNIVERSITY OF SALFORD
Department of Pure and Applied Physics

Research Studentships

Applications are invited from candidates holding or expecting to obtain shortly an honours degree or equivalent qualification in an appropriate discipline, for research studentships leading to higher degrees. The current value of the awards, normally tenable for 3 years, is £695 p.a. plus approved fees. Research studentships are available for:

1. An investigation of thermal characteristics of transition metal oxide switching glasses, in collaboration with Standard Telecommunication Laboratories Ltd., Harlow. This is an S.R.C. (C.A.S.E.) award.
2. Investigations of thermoelastic properties of carbon fibre reinforced composites, in collaboration with the Royal Aircraft Establishment, Farnborough.

Further details of these and other research awards may be obtained from the Registrar, Room 102, University of Salford, Salford M5 4WT, quoting reference Nat/86. Informal enquiries may be made to Professor R. R. Birss, Chairman of the Department of Pure and Applied Physics, University of Salford, Salford M5 4WT. (2239)

UNIVERSITY OF GLASGOW
Department of Natural Philisophy
RESEARCH FELLOWSHIP
Solid State Physics Group

A postdoctoral research fellow is required to join a group concerned with structural studies of the surface of liquids by high energy reflection electron diffraction. At a later date this work may be extended to investigations of solid state surface phenomena. Experience of U.H.V. and/or electron diffraction methods would be desirable but not essential.

Salary scale for the post will normally be within the range £1,929 to £2,223 per annum according to age and experience. Tenure two years on appointment with possible renewal for a further year. F.S.S.U. benefits.

Applications stating age, academic qualifications, research experience and the names of two referees should be submitted not later than June 28, 1974, to Professor R. P. Ferrier, Department of Natural Philosophy, University of Glasgow G12 8QQ. In reply please quote Ref. No. 3438M.

(2231)

UNIVERSITY OF LIVERPOOL
RESEARCH STUDENTSHIP IN BIOCHEMISTRY

Applications are invited for an S.R.C. Research Studentship to study some aspects of Affinity Chromatography. Applicants should hold or expect to receive a good honours degree in Biochemistry and will be encouraged to submit for a higher degree.

Applications, stating age, qualifications and experience, together with the names of two academic referees, should be sent to the Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote ref. RV/N/276050. (2265)

UNIVERSITY OF SOUTHAMPTON
DEPARTMENT OF PHYSIOLOGY AND BIOCHEMISTRY
RESEARCH STUDENTSHIPS

Applications are invited from graduates, or from those who expect to graduate this summer, for Research Studentships to undertake research work for higher degrees. The Department has a wide research interest in several areas of physiology and biochemistry.

Further details and application forms obtainable from Postgraduate Admissions Office, Department of Physiology and Biochemistry, Medical and Biological Sciences Building, The University of Southampton, Bassett Crescent East, Southampton SO9 3TU. (2211)

UNIVERSITY OF DUBLIN
Trinity College

Post-Doctoral Fellowship in the Department of Genetics

As part of the Commission of the European Communities Environmental Research Programme a team is being assembled to develop a European Mutagenicity and Carcinogenicity Test System using mice and micro-organisms.

A post-doctoral fellow is required to develop suitable strains of mice and to use these for testing the mutagenicity of actual and potential carcinogens: a knowledge of mouse genetics is desirable.

The starting salary will not be less than £2,000 per annum and applications, naming 2 referees, should be sent to:

Professor G. W. P. Dawson,
Department of Genetics,
Trinity College,
DUBLIN 2.

(2319)

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
DEPARTMENT OF BIOCHEMISTRY AND CHEMISTRY

Applications are invited from graduates with a 1st or upper 2nd class Honours degree in Biochemistry or other relevant subject, or from those graduating this year, for an S.R.C. Research Studentship tenable from October 1, 1974, leading to a higher degree in the University of London. The person appointed will be able to study the biochemistry of vitamin E, selenium, and membrane systems with Dr A. T. Diplock. Applicants should send a Curriculum vitae and the names of two referees, as soon as possible, to Professor J. A. Lucy, Royal Free Hospital School of Medicine, 8 Hunter Street, London WC1N 1BP. (2223)

UNIVERSITY OF GLASGOW
DEPARTMENT OF CHEMISTRY
POSTDOCTORAL FELLOWSHIP

Applications are invited for a POST-DOCTORAL FELLOWSHIP to investigate the mechanism and stereochemistry of ENZYMIC REACTIONS by chemical and computational studies of model systems. Applicants should have experience of organic synthesis but previous experience of computing is not essential. The Fellowship is sponsored by the Enzyme Chemistry and Technology Committee of the Science Research Council. Appointment will be for two years from October 1, 1974, on the salary scale £2,118 to £2,247 to £2,412 per annum.

Applications, including the names of two referees, should be sent as soon as possible to Professor K. H. Overton, Department of Chemistry, The University of Glasgow, Glasgow, G12 8QQ, Scotland.

In reply please quote Ref. No. 3471M. (2229)

POLYTECHNIC OF THE SOUTH BANK
FACULTY OF ENVIRONMENTAL SCIENCE AND TECHNOLOGY
S.R.C. RESEARCH STUDENTSHIPS

Applications are invited for research studentships in Environmental Science and Technology.

Projects, suitable for Higher Degrees, are available in the following areas:

I. Thermal Comfort.
II. Control of Noise.
III. Ventilation of Buildings.
IV. Two phase flow (Liquid/gas; Solid/gas).
V. Heat Pumps.

Candidates should either have (or expect to acquire in 1974) a good honours degree in Science or Engineering.

Further particulars from Dr D. R. Scott, Head of Faculty of Environmental Science and Technology, Polytechnic of the South Bank, Borough Road, London SE1 0AA. Tel. 01-928 8989. (2318)

UNIVERSITY OF BIRMINGHAM

Department of Pharmacology (Preclinical) and MRC Neuropharmacology Unit

POSTGRADUATE RESEARCH STUDENTSHIPS

Applications are invited for 2 research studentships supported by the Medical Research Council, commencing on 1 October 1974. The research work of the Department and Unit covers various aspects of the pharmacology of the central nervous system with special reference to the mode of action of drugs, and there are opportunities for learning new techniques such as microiontophoresis, radiochemical assay and zonal centrifugation.

Applicants should have a 1st or upper 2nd Class Honours degree in pharmacology, biochemistry, physiology or related biological subjects.

Applications, in writing to:

Professor P. B. Bradley,
Dept. of Pharmacology (Preclinical),
Medical School,
Birmingham,
B15 2TJ (2273)

UNIVERSITY COLLEGE CARDIFF
DEPARTMENT OF BIOCHEMISTRY
RESEARCH STUDENTSHIPS AND TUTORIAL FELLOWSHIP

Two C.A.S.E. postgraduate studentships are available from October 1 for the following projects: (a) Biosynthesis and function of hexadecanoic acid in higher plant chloroplasts—supervised jointly by Dr J. L. Harwood (Cardiff) and Dr A. T. James (Unilever Ltd.); (b) Techniques for the study of organ specificity as applied to the metabolism of drugs—supervised jointly by Drs A. H. Olavesen and C. G. Curtis (Cardiff) and Dr R. F. Collins (May and Baker Ltd.). Ref. 0580.

Tutorial Fellowship available as from October 1. The successful applicant will be expected to participate in some of the teaching activities of the department and to undertake research on either enzymology or on dopamine metabolism. The appointment will be for three years and it will be possible to register for a higher degree. Salary for this appointment on the scale £1,311 to £1,392 to £1,473 (under review). Ref. 0580A.

For all the above appointments an Upper Second Class Honours Degree or better will be required.

Applications as soon as possible (and not later than June 30) to The Registrar, University College, P.O. Box 78, Cardiff CF1 1XL. Further information can be obtained from Professor K. S. Dodgson, Department of Biochemistry. (2289)

UNIVERSITY OF DURHAM RESEARCH STUDENTSHIPS IN CHEMISTRY

Funds are available to finance research studentships in chemistry in the following areas:

(i) Studies of some cyclic sulphonyl imides. An investigation of the reactivity of some sulphur-nitrogen ring systems and their conversion to compounds with potential application in bio-degradable agricultural products and polymers.
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(iv) Inelastic neutron scattering in molecular spectroscopy. An investigation of some librational and vibrational transitions of molecular systems, which are not infrared and Raman active, by inelastic neutron scattering spectroscopy.

Project (i) is supported by a C.A.S.E. studentship. The equivalent of an upper second class honours degree or better will be required for all projects. For project (iv) a degree in physics or a joint honours degree in physics/chemistry or maths/chemistry is as acceptable as one in chemistry.

Applications, with class of degree, or approximate date of degree results, together with the names of two referees should be sent as soon as possible to Professor T. C. Waddington, Department of Chemistry, Science Laboratories, South Road, Durham DH1 3LE. (2208)

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Applications are invited from recent graduates in Veterinary Medicine, Medicine or holding a good honours degree in Science, and from final-year students expecting to graduate this year, for M.R.C. and S.R.C. Studentships for Training in Research Methods, tenable at the Department of Veterinary Physiology in the University of Edinburgh. The major areas of research are the neurosciences, especially sensory neurophysiology. An M.Sc. course is run in neurophysiology.

Applications to Professor A. Iggo, Department of Veterinary Physiology, University of Edinburgh, Edinburgh, EH9 1QH (2313)

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In reply please quote Ref. No. 3470M. (2230)

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Further particulars from the Unit of Microbiology, Department of Biochemistry, University of Aberdeen, Marischal College, Aberdeen, AB1 9AS.

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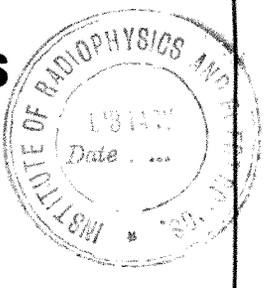
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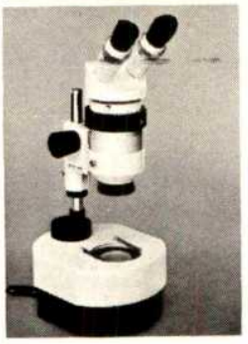
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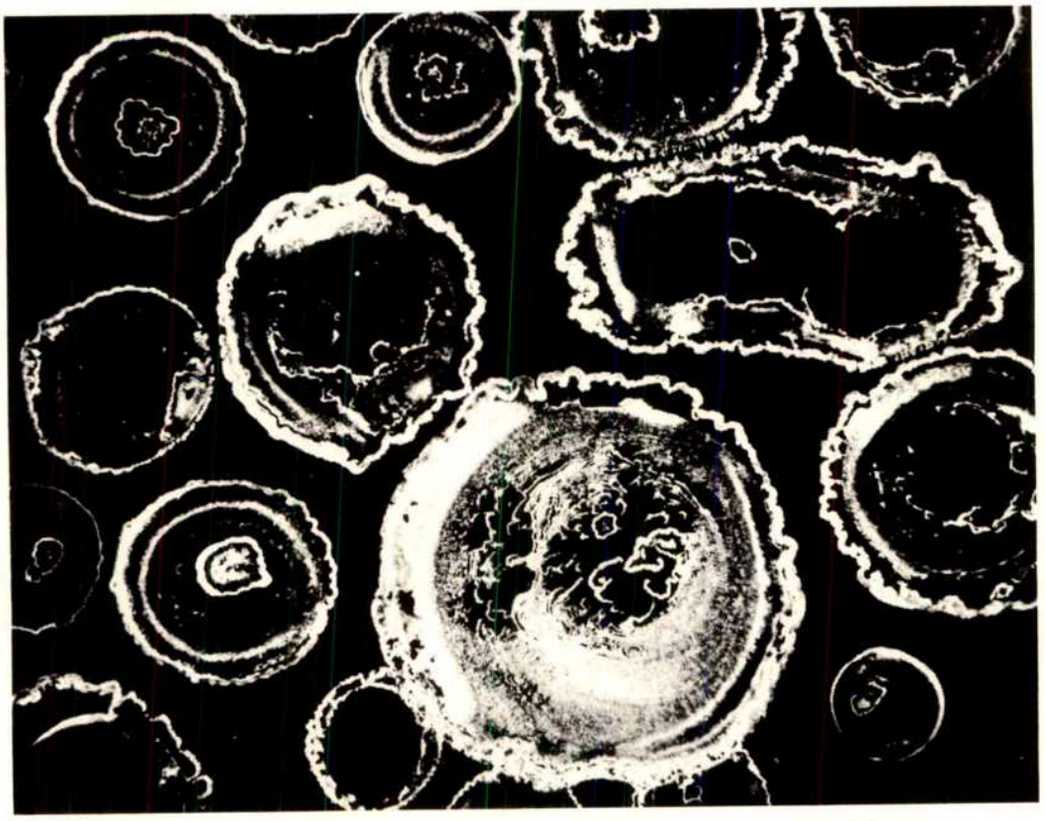


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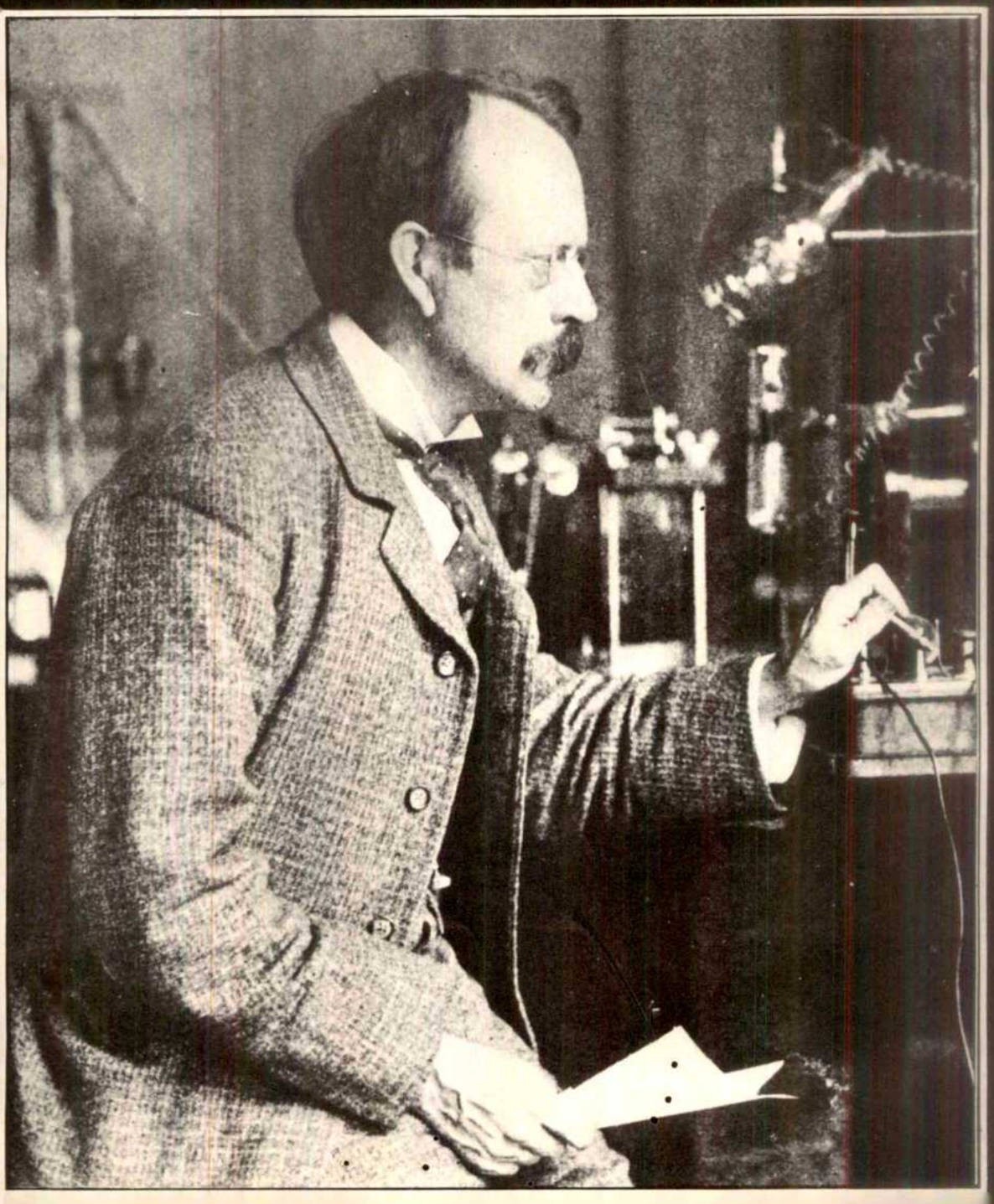
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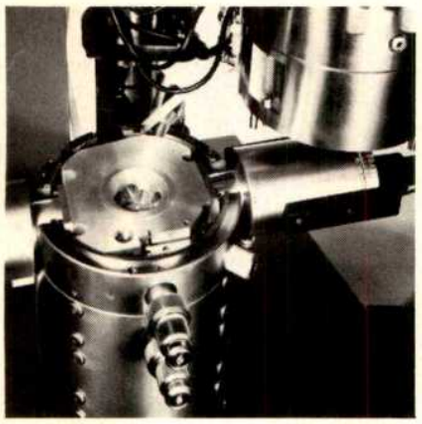
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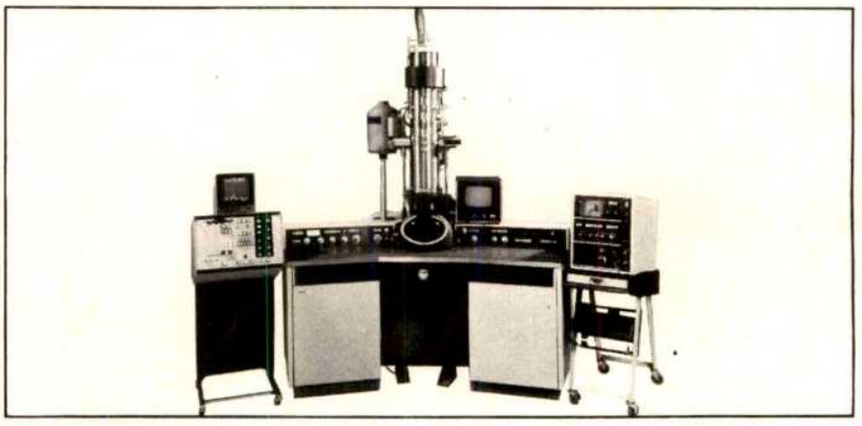
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Cover picture

Professor Sir J. J. Thomson working in the Cavendish Laboratory, Cambridge. The Cavendish is 100 years old this week and on page 502 Professor A. B. Pippard expresses some views about the laboratory.

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The June issue of Cell will include the following articles, reviews, and book reviews:

The Gene Numbers Game: J. O. Bishop

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Increased Protease Levels in Transformed Cells: A Casein Overlay Assay for the Detection of Plasminogen Activator Production: A. R. Goldberg

Changes in Adenylate Cyclase, Cyclic AMP, and Protein Kinase Levels in Chick Myoblasts, and Ther Relationship to Differentiation: R. J. Zalin and W. Montague

Induction of a Deoxycytidineless State in Cultured Mammalian Cells by Bromodeoxyuridine: M. Meuth and H. Green

Expression of Thymidine Kinase Variants is a Function of the Replicative State of Cells: R. Adler and B. R. McAuslan

Book Reviews

Among the Protozoologists: S. H. Hutner
Cell Culture—the State of the Art: J. Bard
Ribonucleic Acids in Review: E. M. Scolnick

The July issue of Cell will include the following reviews and articles:

A Unified Theory of the Control of Actin and Myosin in Nonmuscle Movements: A. C. H. Durham

The Role of Protein Factors in the Biosynthesis of Proteins: H. Weissbach and N. Brot

An Electron Microscope Study of the Effects of Cyclic AMP on Chinese Hamster Ovary Cells: K. R. Porter, T. T. Puck, A. W. Hsie, and D. Kelley

The Morphology and Growth of a Pluripotent Teratocarcinoma Cell Line and its Derivatives in Tissue Culture: G. R. Martin and M. J. Evans

Isolation and Biochemical Characterization of Folate Deficient Mutants of Chinese Hamster Cells: M. W. McBurney and G. F. Whitmore

Characterization of a Chinese Hamster Cell with a Temperature-Sensitive Mutation in Folate Metabolism: M. W. McBurney and G. F. Whitmore

The Degradation of Ribonucleic Acids Injected into Xenopus Laevis Oocytes: C. C. Allende, J. E. Allende, and R. A. Firtel

Poly Adenylic Acid Synthesis In Vitro in Isolated HeLa Cell Nuclei and Whole Cell Homogenates: W. R. Jelinek

The August issue of Cell includes the following articles:

Cytosol Androgen Receptors from Kidney of Normal and Testicular Feminized (Tfm) Mice: B. Attardi and S. Ohno

Isolation of Lymphoma Cell Variants Resistant to Killing by Glucocorticoids: C. H. Sibley and G. M. Tomkins

Mechanisms of Steroid Resistance: C. H. Sibley and G. M. Tomkins

Effect of Cyclic Nucleotides on the Induction of Ornithine Decarboxylase in BHK Cells by Serum and Insulin: B. Hogan, R. Shields, and D. Curtis

A Direct Stimulating Effect of Cyclic GMP on Purified Phosphoribosyl Pyrophosphate Synthetase and its Antagonism by Cyclic AMP: C. D. Green and D. W. Martin

Electrophoretic Characterization of Shuttling and Nonshuttling Small Nuclear RNAs: L. Goldstein and C. ko

Host Restriction of Friend Leukemia Virus: M. N. Sveda, B. N. Fields, and R. Soeiro

Poly (A) in mRNA Does Not Contribute to Secondary Structure Necessary for Protein Synthesis: R. F. Muñoz and J. E. Darnell

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A fuller guide appeared in *Nature* (**246**, 238; 1973).

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Volume 249 June 14, 1974

INSTITUTE OF ASTROPHYSICS AND GEOPHYSICS Date

In defence of the anonymous referee

SINCE Professor Fraenkel-Conrat's plea for an end to anonymity in the evaluation of scientific manuscripts three months ago we have received several more letters on the subject. One or two have been published, but the majority have said little more than 'me-too' in tones ranging from the reasonable to the outraged and lengths ranging from one paragraph to ten pages. The question of anonymity in refereeing appears regularly in the correspondence columns of scientific journals, usually associated pejoratively with a cloak, and it is desirable that scientists give more than a passing thought to it every few years. In most other fields the arbitrator is not anonymous—justice, sport, criticism of the arts, industrial relations all maintain highly visible decision-makers. Can science alone remain aloof from requiring personal accountability in its judgements?

Those who advocate the removal of the cloak point to two benefits which, it is claimed, would immediately accrue. Referees would have to take their job more seriously since their judgement would be semi-public, and the stab-in-the-back opportunity for the malevolent referee would disappear. There cannot be an editor who wouldn't be glad to eliminate careless or mischievous refereeing, although its incidence may well be greatly exaggerated. On the other hand the debit side of removing anonymity is rarely mentioned.

One of the commonest misconceptions about refereeing is that it is done by a small elevated community. A careful study by Zuckerman and Merton (*Minerva*, **9**, 66–100; 1971) showed very clearly that this was not true for *The Physical Review*, and although we have not the same impressive statistics to parade, the file of referees for *Nature* in 1973 contained more than a thousand names. Could we call on such numbers if the identities of referees were revealed? Only, we maintain, when the job was going to be the agreeable one of recommending the acceptance of excellent manuscripts. Large numbers of neither careless nor mischievous referees would send back, unreviewed, manuscripts which they believed to be inadequate and with those authors they had no desire to engage in combat.

Three points need emphasising.

First, referees should not be asked to endure face-to-face or even telephone encounters with irate authors. The sort of verbal roughing-up that even *Nature's* office staff occasionally have willed on them makes it clear that referees should not be placed in a position such that they can be got at by authors. The presentation of science is and must remain ultimately a written activity. This is not to deny the obvious power of the spoken word in education, exposition and elucidation. It is to deny the use of verbal battles, in which forcefulness, slick presentation and quick wittedness are prized assets in resolving scientific disputes. It would be unfair to ask a referee to engage in conflicts on our behalf, and in any case most would quite reasonably decline to do so. This would result in the decision-making process falling back on a smaller core of conflict-hardened reviewers.

Second, not all the unpleasant things that are said from behind the cloak of anonymity are said from a desire to do mischief. The rather common home truth 'this is the tenth paper based on the same data that the author has attempted to get into the literature in the last year' may be motivated less from malice than a genuine dislike of the practice of multiple publication. Such things would be less easy to say without anonymity—some forms of truthfulness would be positively impaired by identification.

Third, the anonymous opinion, to the conscientious author, cannot easily be discounted. The referee serves more than as just the expert vouching for technical plausibility. He is also the potential reader vouching for relevance, wide appeal and readability. He is the one sample of 'readership' opinion that an author will normally go on. The value of these functions can be diminished by knowing the referee's identity and therefore dismissing his critique on purely personal grounds.

Would identification even do what its advocates claim: bring more conscientious and honest reviewing and deter mischief-making? The latter, probably yes, to a certain extent, although most of those already involved in mutual back-stabbing are well enough known to each other not to worry much about anonymity. Some of these combatants are also well enough known to us not to be allowed too frequent a battle. The former, probably no. The tendency would be just as much towards pulling punches and being less critical.

The tradition of depersonalisation in the assessment of manuscripts has, we believe, served scientific communication well. There are obviously flaws in the system, but radical steps to remove these flaws might remove instead a lot which is good.

100 years ago



THE NEW PHYSICAL LABORATORY OF THE UNIVERSITY OF CAMBRIDGE

ON the 16th inst., at a congregation held in the Senate House, Cambridge, the Cavendish Laboratory was formally presented to the University by the Chancellor. The genius for research possessed by Prof. Clerk Maxwell and the fact that it is open to all students of the University of Cambridge for researches will, if we mistake not, make this before long a building very noteworthy in English science.

From *Nature*, **10**, 139, June 25, 1874.

The Cavendish tradition

Professor Brian Pippard, present Director of the Cavendish Laboratory looks at its rôle in the forefront of physical research during the century of its existence.

ON June 16, 1874, the doors of the Cavendish Laboratory were formally opened. A hundred years later, the last members of the Department of Physics left in those buildings are preparing to move to new quarters, still to be called the Cavendish Laboratory, on Madingley Road.

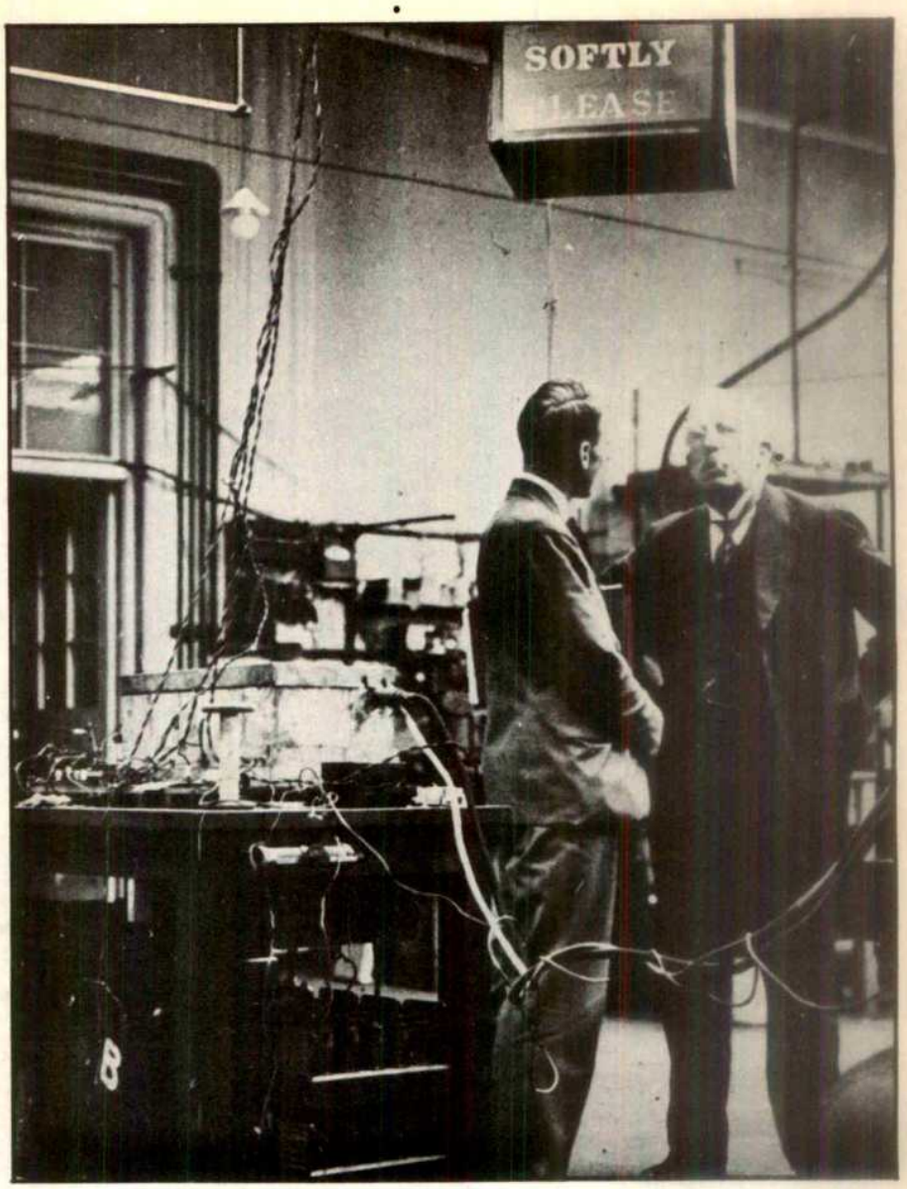
Cambridge was not the first university in the British Isles to encourage experimental physics, and if in its earliest years the Cavendish stood out from the rest, it was more because of its first two professors, Maxwell and Rayleigh, than for any notable difference in outlook. Only after this, with the inspired appointment of J. J. Thomson at the age of 27 (not without some grumbling about mere boys being made professors), did the work begin to develop a special character. Of course the change was not sudden, but it is clearly revealed by comparing the publications in Rayleigh's day (before 1885) with those of 15 years later. Rayleigh at Cambridge is a different man from the gentleman scientist of Terling; he maintains the academic tradition of what we may call Natural Philosophy, undertaking with his students careful measurements to illustrate and guide mathematical reasoning, and to provide values of the constants of nature that pure reason alone cannot determine. By 1898 one has to search hard to find any trace of the earlier attitude; all is qualitative, the laborious search for patterns in phenomena beyond the reach of mathematical prediction. The names are revealing — Thomson, Rutherford, C. T. R. Wilson, Townsend, Zeleny—the roll-call of early investigators of electrical conduction in gases. Thomson had started to work in this field as soon as he was appointed, had discovered the electron in 1897, and had built up a small team ready for action when the discovery of X rays and radioactivity swept away the old world of physics and inaugurated the golden age of string and sealing-wax.

Once more, let us remember that it was no sudden change; indeed, so far as the Cavendish was concerned these new effects principally gave new strength to the attack on gaseous conduction. But Rutherford in McGill was developing that technique which is now looked on as the epitome of the Cavendish tradition. How much was his own natural gift, and how much he picked up of what was congenial in J.J.'s approach, is hard to resolve; at all events, by the time he returned to the Cavendish in 1917, with his finest achievements behind him, he found there an outlook compatible with his own, and proceeded within the next few years to develop the laboratory to a world-commanding eminence.

This is the accepted version of the myth, though one wonders if it is seen in the same light in Manchester, where Schuster was succeeded by Rutherford, and he in turn by Bragg and then by Blackett. It was in Manchester that the atom was born and first disintegrated, yet to the world at large it is the Cavendish that stands for British physics in that period. This is not the only example of the compelling hold that Cambridge exerts on the emotions of its alumni, and it is a self-perpetuating process whereby in due course the old boys send their best students back to seek their fortunes. The reputation of the Cavendish rests partly on what its professors have done, but more securely on what its students and junior staff are doing. So many of the country's most talented students are attracted to Cambridge by its reputation, to be joined, those who stay for research, by so many equally promising graduates from elsewhere, that the head of department's task is to encourage and to criticise, not to dominate. The conventional picture of Rutherford as something of a one-man band is quite misleading. It ignores the contributions of Chadwick and many others too numerous to mention, who proceeded to chairs and spread the Cavendish gospel of nuclear physics to the wide world; it also ignores the presence of Appleton, Bernal, Kapitza and G. I. Taylor, some of those who benefited from Rutherford's support without being drawn into his orbit.

Nevertheless, in comparison with the laboratory we now know, Rutherford's was an archaic monolith that could not survive the expansion of physics after the second world war, and Bragg soon recognised that the time had passed when one man could oversee the whole research effort. Inevitably he was conscious of stepping into a giant's shoes, for it was many years before the Cavendish could free itself of the private feeling, and the world's expectation, that nuclear physics was the field it ought to cultivate above all others. But Bragg seized on the essential point that ideas are not the privilege of the leader but spring from the community of workers, and his decision to share responsibility among a number of virtually autonomous groups led to such successes as ultimately laid the ghost of the past. It was the seeds planted in the thirties by Kapitza, Bernal, Appleton, and Ratcliffe that grew in those years, so that by the end of Bragg's tenure Low Temperature Physics, Crystallography (especially what was to become Molecular Biology) and Radioastronomy were vying with Nuclear and Particle Physics to attract the best students. Devolution was complete; the laboratory was three times larger than at Rutherford's death, and its ability to contribute at the frontiers of research was now extended over a much broader field.

At the same time a certain weakness was becoming apparent. The experimental approach which we have traced to J. J. Thomson had developed into something of an antimathematical attitude; the followers of the prewar physicists, who had been amateurs in the best sense, were in danger of becoming merely amateurish in an increasingly professional world. And not only through disregard of essential theoretical techniques, but equally through nostalgia for the string and sealing-wax days when experiments were cheap and quick and, above all, for individual efforts. Thus, though some might have found a paradox in Mott's election to an experimental chair, and others welcomed it as a return to the ideals of Maxwell and Rayleigh, it is better seen as the recognition of the need to abandon parochial attitudes, such as the hope that physics will remain accessible to intuition and robust commonsense, with mathematics as an optional extra. Equally it was essential to realise that modern problems demanded modern equipment. Simplicity is for the man of genius; most of us have to do it the hard way. However much it may have wounded the pride of those who saw the Cavendish as something apart, it could only have been harmful to pretend that any single laboratory can go it alone nowadays. There will be local



Lord Rutherford talking softly to J. A. Ratcliffe in the 1930s.

traditions and national tendencies, but only as minor variants of an international style with, for example, British physics still leaning towards the experimental approach in contrast to the more mathematical tendency of the United States and Japan.

In keeping with this general trend many of the best contributions of the Cavendish in the last 20 years have been founded on technical innovations. The invention and exploitation of aperture synthesis for radiotelescopes, and the design and marketing of Sweepnik, the automatic scanner, are two very different examples of recent work in the old Cavendish style. Nor are there lacking descendants of the pre-war amateurs, who eschew the beaten track and find more scope in opening up new lines, where observation and measurement get little guidance from established formalism. If we ask which type of work seems to be more highly regarded, the answer is rather disconcerting. For we smile at Rutherford's division of science into nuclear physics and stamp collecting, not recognising all round us the modern counterpart in the esteem we accord to mathematical analysis and, at best, the grudging respect for the accurate description of phenomena.

In looking to the future we could do worse than ponder for a moment the danger that always theatens physics of degenerating into scholasticism, of valuing the formal structure above all, especially if it can be disguised as ultimate truth. No one, of course, will dispute the mastery of those who have contributed to our deeper knowledge of the fundamental laws, and there is no physics laboratory but is richer for the presence of a leader in these studies. It is another matter, however, for students to be seen switching off their attention when the lecturer embarks on a discussion of observations that do not fit into any tidy pattern; and we are all too familiar with the way in which, given the choice, the brightest students choose theoretical physics, leaving the more experimental options for the less gifted. This is the legacy of a generation of professional research workers whose collective learning has established a standard which demands of all recruits such detailed formal knowledge as will leave them no time and, worse, no desire to tread paths where the signposts are few and uncertain.

This is one of the challenges which a laboratory like the Cavendish should attempt to meet. It has enough of a reputation to justify minor eccentricities of philosophy, and because of this, as well as the quality of its students, perhaps it has a greater responsibility than most to transit its version of the real nature of the subject. Its reputation never rested on knowing what everyone else had done, but on what its own members did, and how they inspired their students with the excitement of being a physicist. To carry on doing good research should not be too much of a problem, given that it is essentially a matter of catching and keeping as many as one can of the most ardent spirits, and providing the opportunity and encouragement for them to show what they are made of. But to carry this inspiration to the undergraduate level, where the need is greatest, is much harder than research—the urge is less, the praise fainter—and it is here that effort is really needed. To take a detached view, it could not be said to matter very much if most of the outstanding problems of physics were to remain unsolved. Yet it matters that we should continue to try to solve them, and discover new realms to explore, if only to renew in ourselves, and pass on to others, the assurance that physics is much more than a collection of laws whose application is a matter of knowledge and routine. For physics is, besides, a living craft of hand and mind which is transmitted more by example than by rote. Embodying as it does a technique essential to fight the material problems of the world, it must continue to be taught, and taught as a craft. There are countries whose universities teach that science is what the textbooks say, and reduce their students to intellectual sterility; and there are not lacking in this country influential men who would promote the same disaster here, believing innocently that academic research is an expensive luxury. The challenge of our times is not to assert, but to prove, that this is a dangerous illusion. If the Cavendish has a role to play, it is once more its traditional role—to be in the forefront of the battle.

international news

Counting the cost at Flixborough

by John Wilson

TWENTY-EIGHT people killed, more than one hundred injured and upwards of £70m damage done to plant and surrounding property are the results of the most devastating explosion in peacetime Britain. It happened on May 4, at the 60-acre Nypro chemical factory in the village of Flixborough, Lincolnshire, which produces caprolactam—used in the manufacture of Nylon 6—and was caused by the sudden escape of a large quantity of cyclohexane vapour. The plant, owned jointly by Dutch State Mines (DSM) and the National Coal Board (NCB) was the sole producer of caprolactam in the United Kingdom. Its closure will affect 30,000–35,000 jobs throughout British industry to varying degrees.

A most disturbing aspect of the disaster is the damage outside the factory gates—thirty families have had to be rehoused—and questions are being asked in parliament and elsewhere about the safety of other large chemical installations in Britain, and indeed throughout the world (Nypro owns similar plants in Holland and Augusta, Georgia). The Secretary of State for Employment, Mr Michael Foot, has ordered a public inquiry into the disaster and the British Chemical Industry Safety Council are to review its findings. Meanwhile, Mr Jack Jones of the Transport and General Workers Union has said that the union will hold a separate inquiry of its own. Twenty-one of the dead were members of the union and Mr Jones believes that the survivors will talk more freely to their own union-appointed investigator.

But more than a week after the disaster, explosions were still taking place on the site and firemen had not penetrated through the pools of dangerous chemical to the control room where many vital records were kept. It will probably be a long and difficult task to reconstruct exactly what happened.

What is now almost certain, however, is that the escape of cyclohexane vapour occurred at that part of the plant concerned with the oxidation of cyclohexane to cyclohexanone oxime, an intermediate product. High temperatures and pressures are involved in this process. One of the units was under repair at the time and a bridging pipe is thought to have fractured, although how the gas ignited is not yet known. But Mr Leslie Grainger, NCB board member for science, says that the way the gas escaped is more important than its ignition. In spite of searches for cigarettes and matches, the banning of motor vehicles and the issue of special anti-static clothing, most experts are agreed that the risk of a random spark cannot be entirely eliminated. The effects of any explosion, however, can be minimised by the design of the plant. The established practice of piping volatile material in the open, in the hope that any leak will be dispersed, must now be revised.



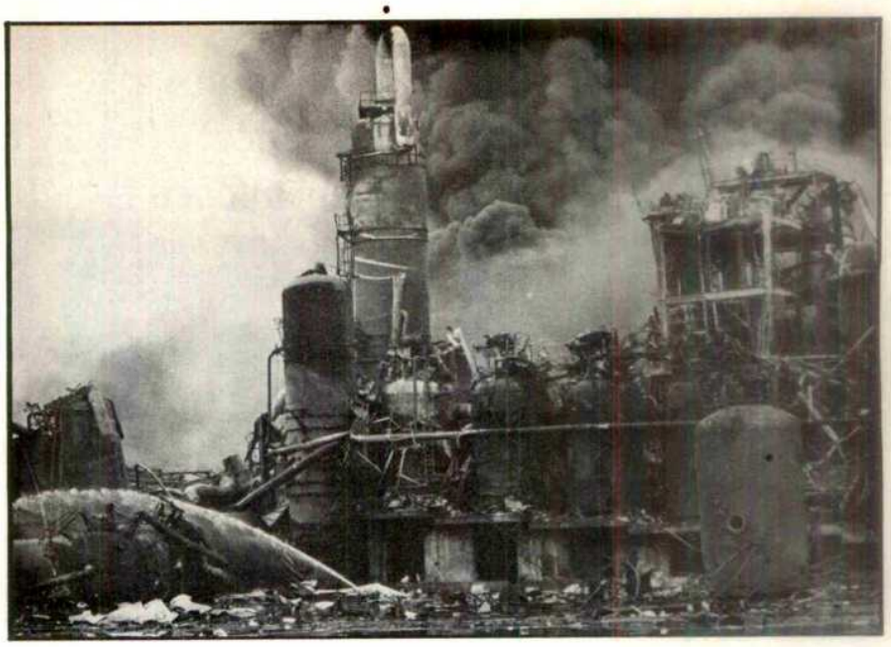
Flixborough: no rebuilding?

The greater the degree of physical connection between various processes at risk, the greater the danger that more than one unit will become involved in an accident. Even the siting of other processes, the manufacture for hydrogen, for example, may be vital in an emergency. The Nypro control room and laboratories were built in the centre of the site, between the oxanone processes and the hydrogen plant. Obviously some degree of connection is necessary—that is what a petrochemical complex is all about—but there must come a point when safety factors outweigh engineering expediency. The difficulty, says Mr Grainger, is to maintain the economic links without the physical ones.

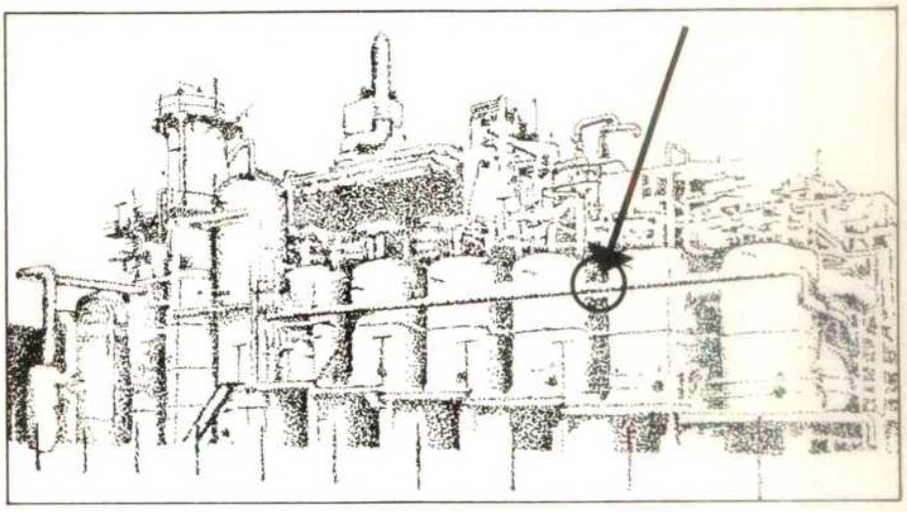
The NCB became interested in the Flixborough venture when it was seeking an outlet for its crude benzol (a source of cyclohexane) produced at coke ovens up and down the country. Its opportunity came when Fisons Ltd, the fertiliser manufacturer, withdrew from partnership with DSM in 1971. By December 1972 a new £15 million extension to the plant was completed, producing caprolactam from cyclohexane; up to that time phenol had been used as a raw material.

The cyclohexane was oxidised to cyclohexanone and the oxime formed by the addition of ammonia and sulphuric acid. Ammonium sulphate used to be a principal by-product of this reaction—which is why Fisons were involved—but was later recycled as phosphoric acid. The oxime was finally converted to caprolactam by Beckmann isomerisation.

A third phase of expansion was to have been commissioned later this year but whether that expansion would have used phenol or cyclohexane had not been decided at the time of the explosion. Mr Grainger says that there is now an emotional argument against the cyclohexane technique but hopes that any further decision will be based on rational grounds. Whatever process might be used in the future, there is strong local opposition to rebuilding the site at Flixborough. Comparisons are inevitably drawn in any industrial disaster of this size with the safety of nuclear installations. These have greater safety restrictions placed upon them and in Britain they need the approval of the Nuclear Inspector, whose decision is final. But a chemical plant may be built without consulting anyone except the local planning authority, who usually have little expert opinion to draw on. The Factory Inspectorate has no say in the matter until the plant is erected. At a time when the safety of nuclear power plants is in question, this seems to be a double standard.



THE oxanone plant at Flixborough, where the leak which caused the fatal blast is thought to have occurred. The picture above shows the cyclohexane containers after the explosion. One tank had been removed from its place for repair, and in the space it had formerly occupied a bridging pipe was used to connect the remaining tanks (location arrowed in drawing below). Nypro Ltd refused to comment on the suggestion that temporary piping was the source of the leak. They are waiting for the report of the Factory Inspector which will be published later this year.



Teaching for safety in technology

by Roger Woodham

WHATEVER the precise cause of the Flixborough disaster may turn out to be, Sir Brian Flowers, Rector of Imperial College, London, and Chairman of the Royal Commission on Environmental Pollution has set wheels in motion within Imperial College to find out whether teaching there places sufficient emphasis on reducing technological hazards of all kinds to an absolute minimum. Sir Brian expressed concern in a speech in April that "what is regarded as an acceptable disaster is increasing all the time, whether it is a hijacking or a motorway pile-up . . . It seems strange that increasing acceptance of technological failures should seem to coincide with increasing intolerance of environmental pollution." So the Flixborough disaster was the catalyst but not the cause of the setting up of a working party under Professor A. R. Ubbelohde, Head of the Department of Chemical Engineering and Chemical Technology at Imperial College.

Professor Ubbelohde said last week that the ultimate objective must be to imbue engineers—be they recent graduates or those with industrial experience returning to Imperial College for post-experience courses—with the "concepts of safe design". He also predicted that the monitoring of potential hazards, preferably by two independent methods, will receive much more attention in the future.

Sir Brian says that all too often "clever economic solutions have come before the safe solution which respects life." Modern technology should be fail-safe (so that alterations in any parameter of the system make it safer) but it is not, he said.

The working party has three months to submit a report and it seems unlikely that the document will stay internal to Imperial College for very long.

Gloomy outlook for Project Independence

Colin Norman, Washington



EVER since Arab oil producers shut off oil supplies to the United States late last year, President Nixon has been telling the American public at every opportunity that his Administration is committed to a pell-mell drive to make the United States self-sufficient in energy. Called Project Independence, the programme was launched with a flood of rhetoric in November 1973, and President Nixon still maintains in his public announcements that the goal will be met by 1980. But last month, two independent studies concluded that the effort will be extremely costly, and that self-sufficiency will probably be unattainable—even by 1985.

With much of the press distracted by more topical matters, the studies drew little public attention but they provide the strongest evidence so far that the United States will have to rely on imported oil to meet its energy demands for at least another decade and probably for long after that, in spite of massive investment in research and development and capital equipment.

Prepared by the Massachusetts Institute of Technology's Energy Laboratory and the National Academy of Engineering (NAE), the studies analysed Project Independence from two different standpoints but both reached essentially the same conclusion. The MIT study argued that energy self-sufficiency can only be bought with another sharp increase in the price of domestic oil which, in the short term, "would yield little more than an income transfer from consumers to producers", while the NAE study concluded that it will take capital expenditure amounting to about $600,000 million over the next 10 years and government intervention in the energy industry to make the United States independent of foreign energy supplies by 1985.

If anything, the NAE study paints the bleaker picture, for it simply examines what will be needed from each energy sector between now and 1985 to give Project Independence a chance of success, and it comes up with some pretty sobering conclusions. The burden of the study's message is perhaps best summed up by the following statement in the NAE report: "Achieving the complete range of programs described in this report by 1985 is not considered . . . to be of high probability. Even if it is accomplished, the United States would be buying time. For beyond 1985 there looms the ominous prospect of even greater demands for energy from ever-increasing and ever-rising expectations from home and abroad".

Although the Administration's statements about Project Independence have so far been unencumbered by a definition of what actually constitutes self-sufficiency, and President Nixon seems to pay little attention to advice from his top energy officials that 1980 is a totally unrealistic date to expect the United States to become independent of foreign energy supplies, the effort required will be staggering.

For a start, energy supply and demand predictions made before the energy crisis began to bite indicated that by 1985, Americans would be consuming the equivalent of 58 million barrels of oil every day, while domestic supplies would amount to little more than 40 million barrels. Somehow, therefore, Project Independence will have to contend with a shortfall of about 18 million barrels of oil equivalent a day within the next 10 years.

According to the NAE study, this could be achieved by an aggressive conservation programme and a crash effort, beginning this year, to exploit all domestic energy resources in the United States. The conservation effort could be expected to reduce demand by about 8 million barrels a day, while the development efforts would entail doubling coal production, massive strip mining in the Western United States, increasing nuclear power output so that fission reactors generate a third of the total electricity output in the country (up from less than 1 per cent last year), increasing oil and gas production by 25%, and establishing entire new industries to produce synthetic petroleum and natural gas from coal and oil from shale.

Apart from the $600,000 million price tag, there are several major obstacles which are likely to prevent Project Independence from being a success, however its goals are defined. For one thing, such a massive development of energy resources is likely to be achieved at the expense of considerable destruction of the environment, and for another, the NAE study suggests that there is likely to be a considerable shortage of skilled manpower, materials and equipment. In short, Project Independence is likely to be an expensive failure when measured against President Nixon's public announcements.

As for each energy sector, the NAE study comes up with the following conclusions:

- Coal. The United States has about one third of the world's coal reserves, and these are likely to provide the bulk of any increase in domestic energy output in the next decade. According to the study, output must be doubled by 1985, chiefly by opening up large federally owned areas of Montana and Wyoming for strip mining and expanding underground mining in the Eastern United States. By itself, an increase from 660 to 1,260 million tons a year over the next decade doesn't sound too daunting a prospect, but when translated in terms of the physical facilities which will have to be added to the coal industry, the undertaking is staggering.

"Stated another way", the report says, "on the average one new deep mine and one new surface mine must be brought into production every month for 10 years. In contrast, only 13 mines of greater than 2-MTPY (million tons per year) production were opened in the 10 years from 1960 to 1969".

Equally startling are implications for transporting coal to power stations and other users if output is doubled. The NAE study suggests that 60 new rail-barge systems, between 100 and 150 miles long and each capable of handling 2 MTPY, will have to be constructed in the eastern United States, and 70 3-MTPY systems of between 1,000 and 1,200 miles will have to be constructed in the West. To haul the stuff, 8,000 locomotive units and 150,000 hopper cars, each of 100 tons capacity will have to be built. At present there is a severe shortage of hopper cars.

- Oil and Gas. Last year, more than 75% of energy requirements in the United States were supplied by oil and gas, but increases in domestic production will only come from very costly new sources such as offshore deposits in the Atlantic and in Alaska, and the NAE study predicts that at best an increase of about 25% can be expected from United States sources. To achieve even that increase would cost about $180,000 million over the next decade: it would require an average of 58,000

new wells to be drilled each year—twice the rate in 1973—new methods for recovering oil from marginal wells would have to be developed and environmental problems associated with offshore production would have to be resolved. But even if all those programmes are successful, domestic oil and gas production would reach only about 27 million barrels per day equivalent in 1985, which is about 1.6 million barrels short of the demand in 1973.

- Electricity production. The total generating capacity of the United States electrical industry in 1973 was about 435 million kW and the NAE study concludes that this could be doubled over the next 10 years with capital investment of about $300,000 million. First, nuclear power would have to be expanded greatly above present projections, to reach about one third of total electrical capacity by 1985. This will require between 2 and 3 new nuclear units to be built every month, uranium production would have to be expanded by a factor of 6, between 30,000 and 40,000 plant operators and maintenance personnel would have to be trained, 30 reactor pressure shells and turbine generators will have to be manufactured and delivered each year, and the time it takes to license and construct nuclear plants will have to be greatly reduced from the present 10-year average.

Another key programme in the electrical energy sector is the expansion of coal-fired power station capacity. This, the NAE study suggests, will require five oil-fired power stations to be reconverted to coal every month for the next four years, two new 700 MW stations will have to be brought on line each month for the next decade, and 10 new plants will have to be constructed to produce medium-BTU gas from coal to fuel 50 power stations which are now fired with natural gas. All of that, however, would have to rely on the development of new technologies to control the emission of sulphur dioxide from power plants, or alternatively, environmental regulations would have to be greatly relaxed.

- Synthetic petroleum, natural gas and shale oil. In order to meet the predicted shortfall in oil and gas, it is estimated that new technologies must be developed to produce the equivalent of 1.1 million barrels of oil per day from coal gasification, 0.6 million barrels per day of synthetic petroleum from coal, and 0.5 million barrels per day of shale oil. Apart from a massive research and development programme, it will take a capital investment of between $16,000 and $22,000 million for the coal conversion projects, and between $3,000 and $5,000 million for the shale oil production.

The NAE analysis points out that the cost of those processes is likely to deter early production, and constraints such as the availability of water and environmental regulations are likely to set a limit on the ultimate capacity of some of the processes. And the MIT study is even more pessimistic, for it states that at present oil prices, there is insufficient financial incentive for the energy industry to move into those fields. Both studies therefore recommend that the federal government should provide a variety of incentives such as guaranteed prices, tax breaks and joint financing of development plants to bring the technologies on line as early as possible.

- Conservation. The prospects for the success of Project Independence hang to a great extent on whether or not the United States' insatiable appetite for energy can be curbed. Although the rapidly increasing cost of energy will work to reduce demand through the normal market forces, it is clear that the federal government must take a strong lead in encouraging conservation by a variety of measures such as allocation of funds to mass-transit systems, promoting research and development on more efficient ways to produce and transport energy, and by continuing such measures as speed limits on highways and encouraging car pooling.

The NAE study identifies four chief constraints which lead to its prediction that it is highly unlikely that all the projects necessary to achieve energy independence will be carried out. First, the capital investment required—$600,000 million over 10 years—is almost double the present investment rate in the energy industry, and it should be compared with the total investment in all industrial plant and equipment of about $100 million in 1970. Second, the availability of water is "a serious concern for producing oil from shale or synthetic fuels from coal". Third, environmental damage resulting from strip mining—particularly in the remote Western coalfields—and from burning large quantities of coal will have to be overcome by the development of new technologies, otherwise environmental regulations will have to be relaxed. And finally, a huge manpower problem could arise since several hundred thousand people will have to be recruited and trained in the energy industry during the next decade; in particular, the NAE study points out that enrolment has been dropping in university engineering courses.

One thing is clear from the study—a number of complex decisions must be taken by the federal government within the next year, and a coherent national energy policy must be formulated. So far, however, neither the Administration nor Congress has matched Presidential rhetoric with concrete action.

ESRO awards Spacelab contract

THE European Space Research Organisation has decided to award a contract worth approximately 180 million accounting units* for the design and development of the manned orbital laboratory Spacelab to VFW-Fokker/Erno of Germany, as prime contractor for a European industrial team whose composition reflects the financial contributions to the Spacelab programme by participating ESRO member countries. The unanimous decision was made by ESRO's administrative and finance committee, composed of member country delegates, at a meeting in Paris. It followed six weeks of evaluation by ESRO officials of two industrial proposals of high technical quality.

This six-year contract provides for the delivery of one Spacelab flight unit, fully qualified and ready for the installation of experiments, by April 1979. Other main deliverable items are two engineering models, three sets of ground support equipment and initial spares.

The Spacelab project is the most important cooperative programme between ESRO and NASA. ESRO is responsible for the design, development and construction of the laboratory, which NASA will launch in its space shuttle and operate. The first Spacelab launch is scheduled for early in 1980. NASA will soon commit itself to purchase a second Spacelab unit from ESRO and has indicated its intention to purchase such additional Spacelabs as may be required.

Unlike earlier space laboratories, Spacelab will be reusable. It will be designed for 50 missions of a week to a month, or a nominal life of 10 years. It will enable, for the first time, scientists, engineers and technicians to travel and work in Earth's orbit without undergoing intensive astronaut training. Spacelab is designed to reduce significantly the cost and time needed for space research and its applications, and to provide to a large number and wide variety of American and European users a versatile laboratory in which experimenters can work in comfort.

* At mid-1973 price levels 180 MAU is equivalent to approximately £95 million, FF 1,000 million, DM 578 million and $226 million.

correspondence

Earth scientists

SIR,—'European earth scientists disunite', an article by Peter J. Smith in your issue of April 12 is erroneous and misleading; we are concerned that such an article should have appeared in *Nature* without an attempt to check the facts.

The main tenor was implication of lack of coordination between the organisers of a forthcoming meeting in Reading of European Geological Societies (the Geological Society and the University of Reading) and the European Geophysical Society. In fact the minutes of the first meeting of the Policy Committee for the Meeting of European Geological Societies which is to take place from September 8-12, 1975, were sent in July 1973 to the Secretary of the European Geophysical Society at the Royal Society. A letter with his comments was received. It was certainly not "more or less by accident" that he discovered that there would be such a meeting. In December 1973 a special letter was sent to the President of the European Geophysical Society, with a copy to the Secretary, inviting participation of members of EGS and requesting their views and suggestions for topics for discussion. The letter was acknowledged by President Morelli welcoming the chance for cooperation, and expressing the hope that many members of the EGS would be interested in attending the Reading meeting.

For one whose professed anxiety is about the unity of earth science (that is, of geology) Mr Smith lays a surprising amount of emphasis on a distinction between geophysics and geology. We would expect him to recognise, as we have for many years, that geophysics is a necessary and important part of geology. Phrases such as "there being no such thing as the geology of the core" make semantic nonsense to stratigraphers, mineralogists and sedimentologists like us. Of course we agree that geophysicists, tectonicians, geochemists, palaeontologists, petrologists and the rest need to talk to one another: that is the object of the projected Reading meeting in 1975; we do not, however, see it as a matter for geophysicists on the one hand and "geologists" on the other. The theme chosen for the 1975 meeting, 'Europe from Crust to Core' is designed to secure the widest possible interdisciplinary discussion. As to the formation of a Pan-European Society of Earth Scientists, that is not the immediate object of the exercise, but if some of those attending wish to discuss such a possibility, so well and good.

PETER KENT
The Geological Society of London

KINGSLEY DUNHAM
Institute of Geological Sciences, London

PERCIVAL ALLEN
University of Reading

Reply from Chile

SIR,—In your journal (March 29) you published a letter sent to you by well known scientists who referred to the "dramatic conditions of the Chilean academic community". As these scientists have great influence in the scientific community, I must reply to such a letter.

I was in fact nominated as Rector of Universidad Católica de Chile by the Junta Militar de Gobierno, which designation was confirmed by a Decree of the head of the Catholic Church in Chile and Gran Cancillar of this University, Cardinal Raúl Silva Henríquez. Nothing said in the letter referred to, corresponds to the real situation in this University since the day I assumed office, early in October, 1973.

Contracts of academics are not provisional as the letter suggests. It is true that some of them have been dismissed although no special Commission has existed for this purpose. They were dismissed because they simply abandoned their job or because they never anticipated in the academic life of this University or any other but preferred political activities instead of doing the work they were paid for.

In any case, the number of academics dismissed from this University is less than the figure of 65 quoted in the letter. This is out of a total of over 2,000 teachers. In all cases of dismissal full legal compensation has been paid. None are natural scientists; they all belong to the social sciences, mainly sociology and political science.

None of the 11,500 students in this community has been dismissed and so there has been no need of reregistration.

With regard to the staff in prison or executed, some teachers and administrators have indeed been questioned. Very few are still in prison (only 5) and legal procedures are followed to clarify their responsibilities for the crimes of which they are accused, according to traditional Chilean law. They have been allowed legal defence, as it is a long standing custom in Chile which nobody here has forgotten. As to the executions which they claim have happened, I would like to hear of one member of this University who has suffered, for I know of none.

As to the departments which have been dismantled, none of the examples given in the letter has occurred here. No Faculty or Institute has been closed except for two political centres whose Marxist political inspiration was dedicated to public indoctrination.

Nevertheless, I can say that those who judge us seem to ignore the facts and to forget our previous history. They have not considered at all what has really happened in the University. They have not considered that the Supreme Court and the Congress had previously declared the former government illegal, nor that our Armed Forces have historically been politically neutral, nor the people's claim over the last two years to remove the Marxist régime which was destroying all our rights and most beloved traditions. Scientists particularly, should be aware that proof is required before any decision. Nobody should say anything which he may regret the next day; nobody should allow themselves to be the instrument of others hidden intentions.

In this sense, I trust that time and history will establish what really happened in this country, despite all intentional and international campaigns against Chile.

JORGE SWETT MADGE
Universidad Catolica de Chile, Santiago, Chile

Penicillin

SIR,—Your article (*Nature*, May 24) on cancer research by Brian Ford contains the statement that it was the war effort and directed research which gave us penicillin. This statement is incorrect. The reinvestigation of penicillin which led to the discovery of its curative properties was started in 1938, well before the outbreak of the war, and its aim was not the practical one of discovering a new chemotherapeutic agent.

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news and views

Rabbit–scallop transplants

COMPARING and contrasting animals of widely different form is a common pastime for muscle specialists, since in their field similarities generally outweigh differences and there is much common ground for detailed correlation. In general, muscle contraction takes place by some kind of sliding filament mechanism, that is, by relative movement of actin and myosin filaments, and regulation of the system is mediated by the level of calcium ions. Within this fundamental framework, however, there is considerable variation of detail in the Animal Kingdom—in the way, for example, the calcium control is exercised. In vertebrate skeletal muscle, changes of calcium level affect the muscle activity by modifying the troponin and tropomyosin components of the actin filaments, whereas in the muscles of molluscs and some primitive invertebrates myosin and not actin is controlled. In some invertebrates there is dual control, that is, both types of filament are involved in regulation. Evolutionary forces are clearly at work here, and the understanding of problems of animal specialisation and adaptation provides an interesting background to the more fundamental task of elucidating the molecular basis of muscle contraction.

Myosin is a protein with a long tail portion and two globular heads. From the heads may be dissociated polypeptides of relatively low molecular weight, the so-called light chains. In rabbit skeletal muscle myosin there are two classes of light chains, one class released by alkali and the other by DTNB (5,5′-dithiobis-(2-nitrobenzoic acid)). The alkali light chains have been shown to be involved in the enzymatic activity of the myosin, but the function of the DTNB light chain has long remained a mystery. Scallop muscle myosin, on the other hand, has light chains released by EDTA, and without these chains the splitting of ATP by the myosin–actin system is not susceptible to changes of calcium, that is, the myosin becomes 'desensitised'. Is it possible, therefore, that the DTNB light chain in the rabbit has a role analogous to that of the EDTA chain in the scallop? If it does play a part in calcium regulation, how significant is its function relative to the actin-linked control? Perhaps the existence of the DTNB light chain is vestigal, an evolutionary relic of the more primitive scallop type of myosin regulation that has been usurped by sophisticated actin control? To test these ideas, Kendrick-Jones (see page 631 of this issue of *Nature*) has carried out molecular transplants in which the DTNB light chain from rabbit is grafted onto desensitised scallop myosin. Sure enough, the sensitivity is retrieved. This is a most remarkable result, and a triumph for advanced protein techniques which, together with the investigator's skill, can bridge a gap that was many millions of years in the making.

The DTNB light chain restores the calcium sensitivity more fully to the intact scallop myofibrils that have been treated with EDTA than to preparation of pure scallop myosin similarly treated. There is no evidence that the DTNB reacts directly with any protein other than the myosin, however, and it is possible therefore that actin in the myofibrils may have an indirect effect by stabilising the myosin in some way. Experiments with radioactivity labelled DTNB light chains show that one mole is bound per myosin molecule. Since the myosin molecule normally releases one mole of EDTA light chain, and this must be removed before the DTNB chain will attach, the binding sites for the two polypeptides are likely to be essentially the same. The binding of the DTNB light chain is tight and specific, and the other rabbit light chains—the ones released by alkali—cannot be made to bind to scallop myosin or to affect its calcium sensitivity.

Since rabbit turns on scallop, what can the latter do in return? Kendrick-Jones has not omitted this intriguing alternative, but, disappointingly, the full reciprocal relationship is not established. Although radioactive labelling shows that the scallop EDTA light chain will bind to rabbit myosin that has been treated with DTNB, the myosin does not then become calcium sensitive. Still, the fact that the two chain types are interchangeable is interesting enough, and the interest is intensified by the knowledge that their chemical structures have no obvious similarities.

There is little direct evidence for myosin playing a part in the regulation of vertebrate skeletal muscle activity by calcium. X-ray diffraction of stretched living muscle has indicated that the positions of the myosin cross-bridges alter when the calcium level is changed. When, however, the myosin is extracted from the muscle, its ability to split ATP in the presence of pure actin is unaffected by the calcium level, though it does bind small amounts of calcium. The fault may lie here in the extraction and purification procedure.

Some living organisms have myosin-linked regulation, some actin-linked, and some have both. The transition and gradation between these regulatory mechanisms, their evolutionary significance and what particular physiological advantages are conferred by the possession of one or other or both remain fascinating questions. It could be argued, however, that the scope at least of the problem has been encompassed by Kendrick-Jones's successful matching of the light chains separated by aeons, and union of two animals sufficiently dissimilar to startle even Edward Lear.

E. J. O'BRIEN

Aspects of the asthenosphere

IT may be a cliché to say that the Earth's asthenosphere exerts a profound influence on the crust and lithosphere above, but it is neither less true nor less important a statement for that. Irrespective of whether convection currents are limited to it or go deeper, the asthenosphere clearly plays a crucial part in the horizontal motion of continents and ocean floors; it is the source of at least some of the material which finds its way from the mantle to the Earth's surface, and is closely related in composition to that part of the mantle which provides the rest; and it is the ultimate means by which a crust with a changed load may achieve isostatic equilibrium. In other words, it governs the behaviour of the upper layers of the Earth to such a degree that the determination of its physical and chemical properties can be safely said to be one of the genuinely fundamental problems in the Earth sciences.

And for obvious reasons it is a problem both difficult

and complex—characteristics which provide considerable opportunity for disagreement. On the other hand, there are some things that can be said with little fear of contradiction. Presumably few people would now object to, for example, the general definition of the asthenosphere as a layer with no enduring resistance to a shearing stress and thus with a finite strength small enough to be neglected (Le Pichon *et al.*, *Plate Tectonics*, Elsevier, 1973). It is interesting to note, however, that modern as this difinition may sound, the possibility of flow inherent in it had actually been implied by the acceptance of the principle of isostasy more than a century ago; and it was the problem of isostasy which later led Barrel (*J. Geol.*, **22**, 441 and 655; 1914) to incorporate the flow property into a layer model involving a 'lithosphere' and an 'asthenosphere'. In other words, by a curious coincidence the concept of an asthenospheric layer came about independently at almost exactly the time that Wegener was proposing continental drift, the phenomenon that would later require the characteristics of the asthenosphere to provide a convincing mechanism.

But it was to be a long time before the asthenosphere would be properly defined in non-isostatic terms, even though the process began with Gutenberg's early suggestion (*Z. Geophys.*, **2**, 24; 1926) that the general monotonic increase in seismic velocity with depth in the Earth may not be applicable to the uppermost mantle. Much later, in the light of many more and more modern seismic investigations, Gutenberg (*Physics of the Earth's Interior*, Academic Press, 1959) was able to propose the existence of an upper mantle low velocity zone having a minimum P wave velocity at a depth of about 100 km and a minimum S wave velocity within the depth range 100-200 km, the maximum decrease in velocity being a few percent for each type of wave but slightly greater for S waves than P waves. It now seems doubtful whether the P wave channel is universal, but the more pronounced S wave low velocity zone has been confirmed. It probably begins at a depth of 70-80 km beneath typical ocean basins and in the range 110-130 km beneath continental shields, in each case ending at a depth of about 250 km (Press, *J. geophys. Res.* **75**, 6575; 1970). The viscosity in the vicinity of this zone is also apparently low, both Gordon (*Geophys. J.*, **14**, 33; 1967) and McKenzie (*Geophys. J.*, **14**, 297; 1967) having estimated a value of about 10^{21} poise which thereafter rises very rapidly with depth. In summary, then, the asthenosphere seems to be a relatively thin upper mantle layer which varies somewhat in depth and thickness according to the tectonic environment above, in which seismic velocity is anomalously low (and seismic attenuation high), and in which viscosity is also particularly low.

But how does this peculiar combination of properties arise? According to Ringwood (in *The Earth's Crust and Upper Mantle*, American Geophysical Union, 1969), there are several ways in which zoning in the upper mantle might take place. One (mineralogical zoning) is based on the ability of material with his hypothetical 'pyrolite' (1 part basalt, 3 parts dunite) composition to crystallise into four distinct mineral assemblages with different physical properties. Which of these will form at any given time and place will be determined largely by the intersection of geotherms with the assemblages' stability fields; in other words, the assemblage forming will be the one stable at the relevant temperature and pressure. And since temperature and pressure vary with depth in the mantle, so will physical properties. A second possibility is chemical zoning in which "the extensive fractional melting and upward segregation of low-melting components that is inferred to have occurred beneath highly evolved con-

Recognition of *lac* initiator

PROTEIN synthesis in *Escherichia coli* begins after the ribosome and transfer RNA_f^{Met} have bound to a region of the messenger RNA containing the initiator triplet codon. An intriguing question is how the machinery recognises and selects the correct AUG (or GUG) codon out of others that may be fortuitously or otherwise present. Maybe a specific sequence of bases is recognised or maybe there is some aspect of the secondary structure with a recognisable shape.

An approach to this problem is to make use of the fact that initiation complex is stable in the absence of other charged tRNA species so that protein synthesis is prevented from taking place. It has been found that a region of the messenger RNA is protected from attack by endonucleases by the bound ribosome and it is tempting to believe that these protected regions contain the features that are recognised as well as the initiator codon.

The base sequence of several such ribosomal binding sites have already been published for various cistrons of various phages and a possible initiator triplet has invariably been found. In some of these sequences the protected sequence looks as if it could form a region of helical secondary structure with the codon in the loop, and it has been mooted that it is some aspect of this structure that is recognised by the *E. coli* ribosome.

This attractive notion seems to be confounded by work by Maizels (see page 647 of this issue of *Nature*). She had already reported the sequence of the first 63 bases of the lactose operon messenger RNA transcribed from the CAP-independent UV5 promoter mutant. The first AUG triplet occurs at positions 39-41 and is followed by 21 bases that code for the first seven amino acids of β-galactosidase. This suggested that translation might initiate at position 39 and that ribosomes should protect the surrounding region.

Maizels has gone on in her new article to sequence the region of this messenger that is protected by the ribosome in the initiation complex. The results gratifyingly show that the region around position 39 is indeed protected, though the precise length of the protected region depends on the activity and nature of the degrading endonuclease. It next turns out that no obvious secondary structure can be constructed with the codon in the loop.

The next step was to inspect the various ribosome protected regions obtained from the *lac* messenger and from the phages to see if common sequences are present. From this several generalisations emerge. First, there is always a nonsense triplet within 22 bases of the initiator codon, on the 5′ side; second, seven of the eleven sequences including the *lac* messenger show the quadruplet AGGA on the 5′ side; third, many of the sequences, again including *lac*, show a sequence PuPuUUUXPu (Pu is a purine and X is usually a purine). The significance of these findings is, however, further confused by the fact that these common subsequences do not occur in the same position relative to the initiator codon or even in the same order.

The question remains as to whether these results provide a tantalising glimpse at the requirements for initiator recognition or whether they are only fortuitous; for the possibility is still open that the binding of the ribosome to the protected region is not the primary event and that the recognition involves some other part of the messenger or even that the recognition process involves some other messenger specific factor.

E. G. RICHARDS

tinental regions results in zoning caused by layers which differ in chemical composition". In addition, regional variations in temperature–depth distributions will cause corresponding variations in elastic properties. In spite of these possibilities, however, any or all of which may play an actual part in the formation of the asthenosphere, the consensus is that they are not by themselves sufficient to account for, among other things, a decrease in S wave velocity from about 4.6 km s^{-1} immediately below the lithosphere to 4.3 km s^{-1} (or even 4.1 km s^{-1} in places) at a depth of about 100 km. This is why several workers, beginning with Anderson (*Sci. Am.*, **205**, 2; July 1962), have suggested that the low velocity zone implies a small degree of partial melting—a proposal which goes rather further than an earlier one to the effect that the low velocity part of the mantle is close to the melting point.

Since this suggestion was first made, the conditions under which a small but significant part of the mantle would remain in a state of partial melting have been examined in some detail. It soon became evident, for example, that the stability of a very small degree of partial melting would be difficult to maintain if the mantle were completely anhydrous. As Green (D.H.) and Ringwood have pointed out (*Beitr. Miner. Petrogr.*, **15**, 103; 1967), at pressures of 20-30 kb the melting temperature interval between the solidus and liquidus of olivine tholeiite and picrite is less than 100° C, and within this range dry pyrolite would probably become 30% molten. Even temperature fluctuations as small as 20° C would produce about 5% partial melting. In other words, for a dry mantle the degree of partial melting is very susceptible to temperature changes, making for an instability with which magma bodies would form and surface volcanism would occur on a greater scale than is actually observed. Or to put the matter another way, the assumption that partial melting occurs in the mantle because the geotherm rises above the dry solidus cannot be sustained because the geotherm would have to be finely controlled and constrained close to the solidus to prevent the production of too much liquid and the resulting instability.

So some stabilisation mechanism must be found; and one which has achieved a high degree of creditability in recent years is the presence of water. Numerous experimental studies have shown that even a very small proportion of water (say, 0.1%) will lower the melting temperatures of material thought to comprise the upper mantle by several hundred degrees, thereby increasing the interval between the solidus and the liquidus. The chemistry and mineralogy is quite involved, but the net physical effect of this increase is greater stability against temperature changes. After the occurence of a very small degree (say 1%) of partial melting—often termed incipient melting to distinguish it from the 5-30% partial melting required to form magmas—a comparatively large change in temperature will cause only a very small change in the degree of melting. And the fact that the amount of liquid produced varies little throughout a wide range of temperatures is widely thought to explain how a low velocity zone with little variation in properties can exist beneath a wide range of tectonic environments.

Details apart, however, what is clear from all this is that a great deal of thought and experimental work has gone into supporting the apparently simple proposition that the asthenosphere is a manifestation of incipient melting. This does not necessarily mean that the conclusion is right, of course—but at the same time, it does mean that anybody coming to the opposite view faces a difficult task in convincing others. In their article on page 617 of this issue of *Nature*, Green (H.W.) and Gueguen come to the opposite view. Whether their argument will be thought to carry the necessary conviction remains to be seen.

In the meantime, it is interesting to note the mild irony involved in the fact that Green and Gueguen come to their conclusion from a study of kimberlite pipes. The fact that kimberlite pipes (which occur in India, the United States and Siberia but are best known over about two million square kilometres of southern Africa) contain diamonds implies that the kimberlite magma originated at asthenospheric depths (pressures), or, as Green and Gueguen themselves argue, at lower asthenospheric depths. And the fact that they contain large numbers of xenoliths (genetically unrelated inclusions) acquired on the upward journey, not only of crustal rocks but also of material not known to occur in the local crust, implies that the 'unknown' xenoliths comprise a random sample of upper mantle rock types cut by the pipes. So the xenoliths are a valuable source of information on the composition of upper mantle materials (at least beneath stable continental regions)—materials whose studied behaviour led to the conclusion that the asthenosphere must be partially melted. But now Green and Gueguen have used the very same xenoliths (among other things) in quite a different way to show that the asthenosphere cannot be partially melted.

PETER J. SMITH

Magnetic superfluid

THE observation by Bozler, Bernier, Gully, Richardson and Lee of Cornell University (*Phys. Rev. Lett.*, **32**, 875; 1974) of a longitudinal nuclear magnetic resonance at the frequency which had been predicted by Leggett of the University of Sussex (*ibid.*, **31**, 352; 1973) suggests that theory is again starting to catch up with experiment in attempting to reach an understanding of superfluid ^{3}He.

For more than a decade theory had been well ahead in that a superfluid transition had been predicted, but had not been discovered experimentally. This prediction stemmed from an extension of the theory which Bardeen, Cooper and Schrieffer had devised (the BCS theory) to account for superfluidity of the electron gas in a superconducting metal, to describe assemblies of neutral particles, and liquid ^{3}He in particular. The basis of the BCS theory is the idea that, in the presence of an attractive interaction between themselves, the electrons can reduce their total energy by forming pairs with opposite spin and momentum. To break apart one of these co-called Cooper pairs requires a certain minimum amount of energy, so that any dissipative process which is unable to provide this minimum energy cannot occur, and flow of the electrons without dissipation of energy is therefore possible provided that a critical velocity is not exceeded. In the case of a superconductor the attractive interaction between the electrons, which is able to overcome their mutual Coulomb repulsion, arises through slight distortions in the lattice of positive ions in the region of each electron.

Like electrons, ^{3}He atoms also carry a net spin angular momentum of $\frac{1}{2}h$ and similarly, being fermions (unlike ^{4}He atoms), it is rigorously forbidden for more than one of them to be in the same state. Liquid ^{3}He is, of course, in other ways very different from a superconductor. In particular, there is no lattice and the atoms of which it is composed are electrically neutral. It was realised, however, that an effective attractive interaction might exist between two ^{3}He atoms if their mutual angular momentum was nonzero, so that a new form of Cooper pair might thus be created. This orbital angular momentum had to be quantised as lh, with l an integer, and a variety of suggestions was made concerning both the likely value of l and also the relative orientations of the two spins and the orbital motion of the pair which would give the lowest energy, and thus represent the most probable state of the assembly. Because such an assembly is composed of neutral atoms, the predicted state was referred to as superfluid rather than superconducting.

The observation in 1972 by Osheroff, a postgraduate

student at Cornell, that liquid ^{3}He under pressure undergoes two separate phase transitions as it is cooled below 3 mK seemed to indicate that the long predicted superfluid state had been discovered. Subsequent experiments at Cornell, La Jolla and Helsinki seem to have confirmed that both new phases of the liquid are indeed superfluids.

When a magnetic field is applied to normal liquid ^{3}He, above the superfluid transition temperatures, each nuclear spin has a choice of occupying one of two possible states separated by an energy $h\omega_L$, depending on whether it is parallel or anti-parallel to the field. The absorption of energy which occurs when electromagnetic radiation of frequency ω_L is applied at right angles to the magnetic field, is referred to as nuclear magnetic resonance (NMR), and is a consequence of transitions being induced between the two energy levels. It was found that as liquid ^{3}He cooled below the higher temperature phase transition into the so called A phase, the NMR frequency rose rapidly; and on reaching the second transition and entering the lower temperature B phase, the frequency suddenly reverted to the original value which is posessed in normal liquid ^{3}He. From these phenomena it was inferred that Cooper pairs in the A phase were formed with their spins parallel, producing a resultant spin angular momentum with consequent magnetic effects; but that those in the B phase were probably antiparallel, with no resultant spin and therefore no shift in the NMR frequency. It seems probable that in both the superfluid phases the orbital action of all the pairs is highly correlated so that there is a resultant orbital angular momentum describing the liquid as a whole, which might therefore be regarded as an orbital ferromagnet, and many of whose properties would clearly vary depending on the direction in which they were measured. An anisotropic superfluid of this sort is an object previously unknown in nature.

Much theoretical discussion has centred on the way in which the spins and orbital angular momenta are correlated with each other, but it now seems fairly well agreed that in the A phase the Cooper pairs each have a resultant orbital angular momentum of magnitude $1h$, and that the spin and orbital moments for each pair lie at an angle to each other. Leggett, in his 1973 paper, considered various states of this type and predicted the magnetic properties which might be observed for each. In particular, he showed that for one possible state, the so called axial state, there should be a longitudinal NMR absorbtion, in addition to the usual transverse one, whose frequency $\omega_0(T)$ was independent of magnetic field and which was connected by the simple relation $\omega^2=\omega_L^2+\omega_0^2(T)$ to the shift with temperature of the transverse NMR signal of frequency ω.

Members of the Cornell group have now carried out the experiment suggested by Leggett. They have been able to determine $\omega_0(T)$, first, by observing directly the predicted longitudinal resonance and, second, by measuring the shift with temperature of the transverse resonance. Values of $\omega_0(T)$ derived in these two different ways were indeed found to be in good agreement with each other, except very close to the upper transition temperature. Many features of the data require further investigation, notably the change with temperature of the linewidth of the longitudinal resonance, but the Cornell experiment does appear to have demonstrated unambiguously that the A phase of superfluid ^{3}He is in the axial state described by Leggett.

From a Correspondent.

Hexosaminidase and Tay-Sachs disease

from a Correspondent

ONE of the two main electrophoretically distinguishable N-acetyl hexosaminidases (hexosaminidase A) is deficient in tissues of patients with Tay-Sachs disease, a fatal inherited disorder of infants in which the complex lipid, ganglioside GM_2 accumulates excessively in the brain. Hydrolysis of the terminal N-acetyl galactosamine from GM_2 is presumably normally catalysed by hexosaminidase A but not hexosaminidase B. Several important questions still remain to be answered, however, concerning the nature of the relation of the enzymes to each other and also the low activity reported (Sandhoff and Wässle, *Hoppe-Seyler's Z. physiol. Chem.*, **352,** 1119; 1971) of purified hexosaminidase A towards GM_2.

That a genetic relationship exists between A and B was indicated by the discovery that in some patients both the A and B enzymes are deficient (Sandhoff's disease). The enzymes also resemble each closely—in particular they have the same molecular size, and antisera raised to either enzyme will cross react with the other form. Two main hypotheses have been seriously considered, one being that A is formed from B by an enzymatic conversion which affects a non-protein portion of the molecule (the converting enzyme being deficient in Tay-Sachs disease) and the other that the enzymes are both multimeric being made up of similar and dissimilar subunits.

These problems have been discussed again recently in several papers and some interesting new evidence has been presented (Srivastava *et al.*, *J. biol. Chem.*, **249,** 2043; 1974; Srivastava *et al. ibid.*, 2049; Srivastava and Beutler, *ibid.*, 2054; Lalley *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71,** 1569; 1974; Li *et al.*, *J. biol. Chem.*, **248,** 7512; 1973). Srivastava, Beutler and colleagues have purified the N-acetyl hexosaminidases of human placenta to homogeneity. They claim that the amino acid compositions of the two enzymes are different. The molecules were found to be multimeric by SDS (sodium dodecyl sulphate) electrophoresis (and if they are right they are hexamers). Immunological studies have demonstrated that a specific anti-A antibody can be formed in addition to the non-specific cross reacting anti A/anti B. Patients with Tay-Sachs disease possessed no A antigens whereas one of the two 'Sandhoff' patients had antigenically detectable A and B proteins, and the other patient had the A protein only. The authors conclude that the A and B enzymes possess subunits in common (β) and that at least A must have specific subunits (α) determined by a separate locus such that A is $(\alpha\beta)_n$ and B is $(\beta_2)_n$ or alternatively $(\beta\gamma)_n$ (γ representing a polypeptide subunit determined by a third locus). The defect in Tay-Sachs disease would represent a mutation causing the diminished production of α chains, whereas that in Sandhoff's disease would be a mutation of the β chain (the two patients described probably having different β mutations).

Suggestive evidence for this type of model came from the immunological findings, but the main support came from the amino acid analysis so it will be of particular interest to see whether the amino acid differences (which are certainly not great) can be substantiated.

Evidence which is consistent with the two locus model of Srivastava *et al.* (and also with the conversion model) is presented by Lalley *et al.* from their human–mouse cell hybrid results. As the human chromosomes are shed from hybrid cells the enzymes determined by genes on those chromosomes are also lost. On careful examination of 60 hybrid clones they observed the loss of A and B together, A without B, but never B without A, and concluded that the expression of A requires the presence of the gene coding for B as well as the gene for A expression, and that the two loci are on different chromosomes. They also provide a probable explanation for the conflicting results of another group (van Someren and van Henegouwen, *Humangenetik*, **18,** 171; 1973).

Synthetic substrates have largely been used to characterise the hexosaminidases. Srivastava *et al.* and also Li *et al.* report that much of the original activity

of their tissue extracts towards the presumed *in vivo* substrate GM_2 was lost on purification of hexosaminidase A. Li and colleagues, however, provide exciting new evidence that hexosaminidase A (but not B) activity towards GM_2 can be stimulated by a non-dialysable, non-enzymatic, heat-stable liver 'factor'. How does this finding fit in with the subunit model? Is the factor ever an integral part of the hexosaminidase A molecule? Further purification and characterisation of this component will clearly be informative.

East and West ribosomes

from a Correspondent

A SYMPOSIUM on ribosomes organised at Schloss Reinhardsbrunn from May 13–16 by the Biochemical Society of the German Democratic Republic provided a rare opportunity for scientists from East and West to discuss their work together at first hand. Many of the reports came from the two big ribosome groups in Berlin, with eukaryotes to the East of the Wall and prokaryotes to the West, and the time of the meeting was evenly divided between the two classes of ribosome.

To start with the prokaryotes, H. G. Wittmann summarised the work being done in West Berlin on mutant proteins from both subparticles of *Escherichia coli* and B. Wittmann-Liebold (W. Berlin) described amino acid sequences of many of these proteins; a clear pattern of amino acid changes in mutants of several proteins has emerged. The emphasis in *E. coli* has moved to the 50S particle, and R. Brimacombe (W. Berlin) reported two 50S ribonucleoprotein fragments, one containing proteins known to associate with 5S RNA. From the work of V. Erdmann (W. Berlin), it now clear that 5S RNA is capable of interacting with the TψCG loop of transfer RNA, and D. Richter (W. Berlin) showed further that oligonucleotide TψCG inhibits ribosomal functions associated with the A (aminoacyl tRNA) site, but does not affect the P (peptidyl tRNA) site. I. Rychlik (Prague) described a very specific inhibitor (TPCK) which prevents the formation of a ternary complex between tRNA, GTP and the elongation factor T_u. In other experiments on 50S sites, K. Nierhaus (W. Berlin) rebound single 50S proteins to lithium chloride cores, to identify the proteins involved in peptidyl transferase activity and chloramphenicol binding. A similar approach was used by G. Howard (Basel Institute for Immunology) to look for proteins involved in binding of elongation factor G and the antibiotic thiostreptone.

Affinity labelling is a technique which is being used with great success to pinpoint functional centres on the ribosome. O. Pongs (West Berlin) reported experiments with analogues of chloramphenicol and puromycin, and W. Möller (Leiden) an analogue of GTP. E. Küchler (Vienna) used affinity label analogues of both tRNA and mRNA designed to attach to protein, and also a tRNA analogue which reacted with the 3′ half of 23S RNA. A. Girshovitch (Poustchino) and D. Knorre (Novosibirsk) both preferred to use tRNA analogues which could attach to protein or RNA, and found that the RNA was preferentially attacked.

Coming now to the eukaryotic results, the affinity label method has also been used successfully here by J. Stahl (E. Berlin) to identify two proteins at the 60S A site with a puromycin analogue. H. Welfle (E. Berlin) and P. Westermann (E. Berlin) reported experiments on iodination of rat liver ribosomes. Those of Westermann were particularly interesting, as he was able to show which proteins are specifically protected from iodination by bound mRNA, tRNA and factors. W. Möller (Leiden) found that two acidic proteins from yeast 60S ribosomes could be interchanged with proteins L7/L12 from *E. coli*, with retention of EF activity. The surface of eukaryotic ribosomes is rich in pyrimidines, according to M. Saarma (Tartu) who suggested that these could interact with poly(A) as the first step in mRNA binding.

On the strictly functional side, H. Bielka (E. Berlin) showed that ribosomes from different tissues are very similar in activity, but that the corresponding cytosolic fractions are very variable. The ever-increasing complexity of mammalian initiation was summarised by T. Staehelin (Basel Institute for Immunology); the number of initiation factors now stands at six, and ATP is also involved. H. Bloemendahl (University of Nijmegen) described his calf eye-lens cell-free system, which seems a useful alternative to the reticulocyte system, and a good summary of current knowledge of ribosome-membrane interaction came from G. Blobel (New York). G. Shapira (Paris) talked about his new RNA species which stimulates protein synthesis; it is about half the size of tRNA, and seems not to be interchangeable between different systems.

Finally, the electron microscopists V. Vasiliev (Poustchino), N. Kiselev (Moscow) and G. Lutsch (E. Berlin) were in reasonable agreement that the smaller subparticle of both pro-and eukaryotic ribosomes is divided into two distinct regions of unequal size, but failed to agree on the shape of the large subparticle of eukaryotes.

Protein receptors of juvenile hormone

from our Insect Physiology Correspondent

IT has long been assumed that the lipid soluble juvenile hormone must be transported in the haemolymph in protective association with a hydrophilic carrier which was likely to be a protein. Emmerich and Hartmann (*J. insect Physiol.*, **19**, 1663; 1973) have found that in the haemolymph of adult female *Locusta* there are six distinct lipoprotein fractions and one of these (molecular weight 220,000) binds the juvenile hormone of cecropia. It closely resembles the lipoprotein which binds juvenile hormone in *Hyalophora cecropia* itself (Whitmore and Gilbert (*J. insect Physiol.*, **18**, 1153; 1972)). It seemed a reasonable suggestion that this might in fact be a specific carrier lipoprotein which could have a very important role in the action of the juvenile hormone in preventing its unspecific incorporation into the lipophilic phases of the cells and its rapid breakdown to inactive carboxylic and hydrated products.

But Kramer *et al.*, with Law (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 493; 1974), have now recognised the existence of a somewhat different specific juvenile hormone binding protein in the haemolymph of the hawkmoth caterpillar *Manduca*. The authors point out that the C_{18} juvenile hormone, the chief form in the silkmoth *Hyalophora cecropia*, has considerable solubility in water, yielding a monomeric solution exceeding 10^{-5}M. The binding protein has a molecular weight around 34,000; it has a much higher affinity for the hormone and its analogues than for the products of hydrolysis. Kramer *et al.* found that there is sufficient of this specific protein carrier in the haemolymph and it binds hormone at a sufficiently low dissociation constant, for it to take up virtually all hormone present in normal physiological conditions. They find, indeed, that the juvenile hormone is taken up by lipoprotein only when the binding protein has been saturated.

Besides transport in the haemolymph, the question arises as to whether, in analogy with the action of oestrogens in vertebrates, the juvenile hormone binds to a specific receptor in its target organs. It is well known that the insect epidermis is the chief site of action of the juvenile hormone. Schmialek *et. al.* (*Z. Naturforsch.*, **28C**, 173 and 453; 1973) have obtained new evidence of a receptor protein in the epidermal cells of the *Tenebrio* pupa. Using the juvenile hormone analogue 10, 11-epoxy-6, 7-*trans*-2, 3-*trans*-farnesylpropenylether as the active substance, they found that the target cells will accumulate this against

a concentration gradient and will degrade it far more slowly than non-target organs. The receptor has a molecular weight of 360,000 and seems to be a unique compound. It has a very high affinity for the active substance, a very low affinity for the breakdown products. It contains RNA in association with protein.

Filling the gap with the pin cherry

from Peter D. Moore
Plant Ecology Correspondent

IT is now fairly well established that during the course of succession leading to deciduous forest in temperate climates, the peak in plant species diversity occurs at about 100–200 years (Loucks, *Am. Zool.*, **10**, 17; 1970). Following this the full attainment of dominance by deep shade-casting trees and the establishment of a shade tolerant understorey community lead to the elimination of many light demanding plant species more typical of the seral stages. Natural climax forest, however, has an uneven population age structure and is subject to random perturbations resulting in the opening of the canopy, for example as a consequence of lightning strikes and windblows. A mature, stable forest in equilibrium with its environment possesses fragments of seral vegetation within its clearings, and its species diversity is therefore greater than one might suppose, if an adequate sample area is studied (see Hope Jones, *Br. Birds*, **65**, 291; 1972).

Marks (*Ecol. Monogr.*, **44**, 73; 1974) has made specific studies of these self-healing attributes of climax ecosystems. In particular he has concentrated on the modifications in life cycle, and dormancy and seed dispersal mechanisms associated with those species which recolonise areas laid bare by natural perturbations. In ecosystem terms, a gap represents a suboptimal utilisation of the available resources—for example, light, moisture, nutrients—and the species which are to exploit such a situation must either arrive rapidly by seed, or their dormant seeds already present in the soil must germinate and grow.

In New Hampshire, an important species which fills this role is the pin cherry (*Prunus pensylvanica*). It is not found in mature forest communities, but is restricted to clearings in which the forest has suffered disturbance. If the soil of a disturbed area contains abundant pin cherry seeds in a viable state, then a dense stand with a closed canopy develops within about 3 yr. In this case the climax species, sugar maple (*Acer saccharum*) and beech (*Fagus grandifolia*) grow beneath the canopy and assume dominance after about 25–35 yr. If the original soil density of pin cherry seeds is low, then birch and aspen become established at an early stage by seed coming from outside the system, and the final dominance of sugar maple and beech may be delayed until 80–90 yr have passed.

This buried seed strategy which results in the maintenance of pin cherry populations, demands a fairly high density of viable seeds surviving in the soil of the mature phase of the forest. Marks found densities varying between 300,000 and 500,000 viable seeds per hectare at two such mature forest sites in which pin cherry was rare or absent. Original fruit production by dense pin cherry stands was estimated to be between 2 and 3×10^6 fruits ha^{-1}. Germination is triggered by forest disturbance; in laboratory tests removal of endocarps stimulated germination. The longevity of seeds in soil is difficult to examine experimentally, but was estimated to be about 50 yr. The clearance of forest which had been in mature phase for 80–100 yr resulted in little pin cherry regeneration.

Once established, pin cherry grows and accumulates nutrients more rapidly than the final climax dominants, which accounts for its success during the early stages of forest recovery. As far as ecosystem regrowth is concerned, the nutrient demand of pin cherry results in the conservation of nutrient capital which might otherwise have been lost by leaching.

Stability in climax ecosystems is usually defined in terms of the system's capacity to recover from disruption. Evidently this stability depends ultimately on species, like the pin cherry, which may be totally absent from stands of mature climax dominants, or represented only by seeds lying dormant in the soil.

Polyadenylation in the cytoplasm

from Benjamin Lewin
Molecular Genetics Correspondent

THE purpose of nuclear polyadenylation remains obscure and an addition to this puzzle is now made by the observation of Slater and Slater (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 1103–1107; 1974) that cytoplasmic polyadenylation takes place in sea urchin eggs. It is by now well established that in mammalian cells a sequence of about 200 adenine residues is added to the 3′ end of an heterogeneous nuclear (hn) RNA molecule before cleavage of this region. The poly(A)-containing sequence is then transported to the cytoplasm to serve as messenger RNA. Addition of poly(A) takes place only in the nucleus of mammalian cells; since inhibiting the addition of poly(A) prevents processing of mRNA, it seems reasonable to suppose that poly(A) is implicated in some essential way in the maturation process.

But poly(A) is also present in viral messengers found only in the cytoplasm and in mitochondrial messengers. Speculation that it may therefore also have a direct role in translation seems to have been refuted by research in several laboratories, which has shown that mRNA lacking poly(A) can be translated as efficiently as mRNA containing it. But force to the argument that nevertheless poly(A) must be implicated in some way in the cytoplasmic function of mRNA is now lent by the results that Slater and Slater report.

Fertilisation of sea urchin eggs causes a sudden burst of synthesis of poly(A); the recent experiments of Slater *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 406–411; 1973) and Wilt (*ibid.*, 2345–2349) have suggested that this might represent the addition of poly(A) to previously existing messengers, a situation which contrasts with the addition of poly(A) only to newly synthesised nuclear transcripts in mammalian cells. Slater and Slater now report experiments to test this model. By incubating fertilised eggs with both ^{3}H-adenosine and ^{14}C-uridine, they distinguished newly synthesised mRNA (which possesses the ^{14}C label) from mRNA synthesised before fertilisation (which lacks this label). All RNA possessing poly(A) should contain a tritium label; and if this mRNA was synthesised previously the ^{3}H-adenosine should be located exclusively in the poly(A) segment, whereas if these molecules are newly synthesised some labelled adenosine should be present also in the other parts of the messenger.

The ^{3}H and ^{14}C labels show a different sedimentation distribution, which implies that they must enter different mRNA species; that is, the poly(A) is not added simply to newly synthesised messengers. The ^{3}H-adenosine which enters the mRNA fraction is found very largely in the poly(A) fraction which can be reclaimed after digestion with ribonuclease; this suggests that only the poly(A) part of the molecule is newly synthesised.

This implies that some mechanism must exist in the cytoplasm for polyadenylating messenger RNA, a conclusion which raises many important questions about the possible role of this polyadenylation. One obvious question is whether the messengers to which poly(A) is added after fertilisation possessed any poly(A) to start with or whether they completely lacked it. If messengers synthesised before fertilisation lack poly(A), then they must be produced by a mechanism in part different from that prevailing when

poly(A) is added at the nuclear stage. Similar questions to those asked about the nuclear addition of poly(A) can be directed to the cytoplasmic addition—are only certain messengers selected for polyadenylation, what happens to messengers which do not receive poly(A), does polyadenylation have some role in controlling translation? No function for poly(A) in the cytoplasm is yet apparent, but with teleological arguments becoming ever more popular in molecular and cell biology, many will be tempted to conclude that some important function of the poly(A) remains to be discovered.

Eliminating Doppler broadening

from a Correspondent

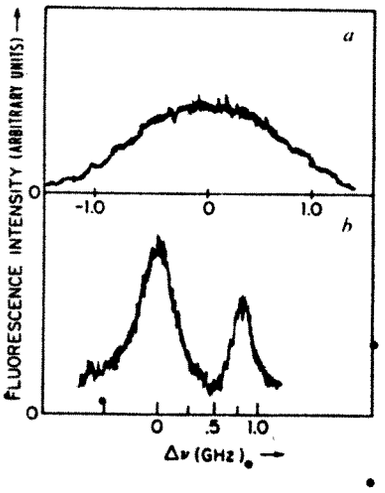
THE spectra of atoms (and molecules) are replete with information some of which is unfortunately inaccessible because the fine structure of spectral lines is often unresolved. Consequently, spectroscopists strive constantly to improve their resolution. The limitations imposed on spectral resolution are not, however, always instrumental but can be inherent in the atomic assembly. For example, atoms of a gas present a whole range of velocities to the direction of propagation of a light source because of their thermal motions. Now if ν_0 is the frequency of radiation required to raise an atom from its (sharp) lower state to a (sharp) upper energy state when it is stationary relative to the source, then an atom receding from the source 'sees' a number of waves per second (that is a frequency) less than ν_0. The receding atom must, of course, absorb radiation which it perceives to have frequency ν_0 and which relative to the stationary source must therefore exceed ν_0. That the Maxwell-Boltzmann distribution of atomic velocities in the source direction ensures a distribution of absorption frequencies even though each atom has sharp energy levels—the so-called Doppler broadening.

Doppler broadening can be overcome if one selects only atoms within a narrow band of velocities relative to the source so that they all absorb at the same frequency. Several velocity selection techniques are used, including atomic beams and laser saturation spectroscopy (see *Nature*, **235**, 127; 1972). A further, elegant method of dealing with Doppler broadening is now described independently by two groups (Biraben, Cagnac and Grynberg, *Phys. Rev. Lett.*, **23**, 643; 1974; Levenson and Bloembergen, *ibid.*, 645).

The essence of the technique used by these authors is very simple. Both groups investigate the normally forbidden 5S←3S electronic transition of the sodium atom by means of two-photon absorption. This transition is, in fact, a closely-spaced doublet (because of coupling of electronic and nuclear spin angular momentum) with separation 784 MHz but its resolution is precluded in the presence of the aforementioned Doppler broadening which confers a half-width of about 1,000 MHz on each component.

Low pressure sodium vapour is subjected to monochromatic radiation from a tunable laser at a frequency equal to one half that of the 5S←3S transition but corresponding to no other known energy state separation within the atom. Those few sodium atoms of thermal velocity such that the 5S←3S level separation is Doppler-shifted into resonance with twice the laser frequency can absorb simultaneously (but with low probability) two photons from the radiation and so raise atoms to the 5S state. As the laser is tuned, a weak Doppler broadened absorption profile (detected by counting photons emitted from the 5S state) is generated as shown in the figure (*a*). When, however, a mirror is positioned so as to reflect the radiation back through the sodium vapour and establish within it a radiation standing wave composed of two oppositely travelling waves, a given atom can absorb two photons by an additional mechanism—one from each of the counter-propagating beams.

It is clear therefore that for any atom the Doppler shift associated with absorption of a photon from one beam is equal and opposite to that associated with absorption from the other beam. The net result is that each and every atom, regardless of its velocity relative to the radiation source, absorbs two photons from the standing wave but only when the laser frequency is exactly one half the 5S←3S level separation. When the laser is tuned through this frequency the doublet character of the transition is resolved (see figure (*b*)), the Doppler broadening having been neatly eliminated.



One expects the weak Doppler broadened signal already mentioned as arising from absorption of two photons from one of the component travelling waves to provide a background pedestal to the observed doublet of figure (*b*), but this has been eliminated by an additional sophistication of the experiment. The two oppositely travelling beams are arranged to be circularly polarised in opposite senses. A rigorous selection rule governing transitions between atomic electron states precludes the 5S←3S transition if both photons are absorbed from a single beam of radiation circularly polarised in only one sense but not if one photon is absorbed from each of two beams of opposite circular polarisation. The weak Doppler broadened pedestal is therefore excluded while the sharp doublet remains.

Little evidence for Palaeozoic arthropod and plant interaction

from Barry Cox
Vertebrate Palaeontology Correspondent

ON April 30, the Palaeobotanical Group of the Linnean Society of London organised a symposium on the interrelationships of Palaeozoic terrestrial arthropods and plants, which took place at the society's rooms in Burlington House. Several contributions set the Palaeozoic scene. M. A. Calver (University of Leeds) discussed the depositional environment of the British Coal Measures. He showed the gradually decreasing frequency of the marine incursions into this low-lying area of coastal swamps, and briefly reviewed the two conflicting theories of the deposition of coal. R. H. Wagner (University of Sheffield) described the distribution of the Upper Carboniferous flora of the world. He contrasted the rich equatorial flora of Euramerica with the poorer and more conservative Gondwana flora, and showed that there were some indications of different swampland, dryland and upland floras.

Turning to the vertebrates, A. L. Panchen (University of Newcastle upon Tyne) described the ecology and distribution of Carboniferous tetrapods, and suggested that the tetrapods might have evolved in Euramerica. Though herbivorous reptiles probably evolved in the Upper Carboniferous, their remains are rare until the Lower Permian.

The general evolutionary background of the arthropod radiation was described by S. M. Manton (Queen Mary College, London). W. D. I. Rolfe (University of Glasgow) dealt with the

non-insectan arthropods of the Palaeozoic. The most spectacular of these were the 6-foot long arthropleurids of the Carboniferous of Euramerica, which seem to have lived within the leaf litter, splitting it apart with their flattened heads and bodies—and their gut contents suggest that they fed on lycopod material. The Palaeozoic mites seem to be related to living forms which feed on fungi.

The biology of Upper Palaeozoic insects was discussed by R. J. Wootton (University of Exeter). Insects were almost unknown before the Upper Carboniferous and, even then, knowledge of them is almost exclusively limited to the Euramerica coal swamp fauna. The modern herbivorous groups of insect were almost unknown in the Palaeozoic and holometaboly, which enables totally different adaptations of larva and adult, did not appear until the Permian. Such forms as *Stenodictya* may have been a sap sucker or have picked up spores from sporangia.

Finally, two contributors dealt specifically with the theme of this symposium. W. G. Chaloner (Birkbeck College, London) produced some possible (if contentious) examples of possible plant-animal interaction. These included some plants from the Rhynie Chert which appear to have been damaged during life, and the strong coats of some Devonian spores which may have been adjusted to resist chewing or digestion. P. D. W. Barnard (University of Reading) categorised the ways in which animals could have used plants as food, and the types of evidence of this activity that might have survived. The instances that he had been able to gather together, however, were few. Plumstead has described leaves of *Glossopteris* with ragged edges which could well have been caused by browsing arthropods. Holes in the seeds of *Samaropsis* have been described by Sharon, and Barnard himself has described holes in the nut-like megaspore of *Trigonocarpus*; both of these could well have been due to attack by insects. Finally, some coprolites contain spores, and a specimen of the reptile *Protorosaurus* contains conifer seeds in its abdominal region.

Despite Barnard's best efforts, one's prevailing impression of the symposium was of a production of Hamlet in which the Prince made only fleeting appearances, and indeed there seems little chance of any great variety of unequivocal evidence of the interaction between Palaeozoic plants and arthropods. The meeting also served to demonstrate what a high proportion of knowledge of Carboniferous life is derived from one single (and possibly unusual) environment—the swamplands of Euramerica.

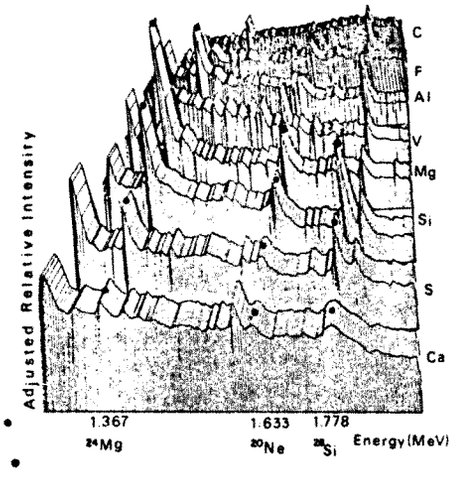
Why do pions eject 'alpha particles' from nuclei?

from Peter E. Hodgson
Nuclear Theory Correspondent

SEVERAL recent investigations have shown that when slow pions or kaons are captured by nuclei, the particles emitted promptly are very often such that the residual nucleus is an excited state of the target less one, two or more α particles or the equivalent numbers of neutrons and protons. The most plausible explanation is that somehow the pions or kaons eject α particles but this has not yet been established because the residual nucleus is identified only by the γ rays it emits, and the particles emitted previously are not detected.

A further important result has been obtained by V. G. Lind and colleagues working in the United States (*Phys. Rev. Lett.*, **32**, 479; 1974), who looked at the results of interactions of fast negative pions (220 MeV) with a range of light nuclei from carbon to vanadium. It is very difficult to detect directly the particles emitted from these intersections so, as in the previous work, the prompt γ rays were studied instead. These are emitted immediately after the particles resulting from the initial interaction. Some of the γ-ray spectra are shown in the figure, and it is notable that there are several peaks common to the spectra from different nuclei.

The energies of these γ rays identify the nucleus from which they were emitted, and it is then found that in the case of even–even targets the most likely residual nuclei correspond to removing one, two or several α particles (or the equivalent numbers of nucleons) from the target. In the case of the odd-A nuclei the γ rays correspond to the removal of a triton or an α particle or a triton plus an α particle from the target nucleus. The cross sections for these processes are comparable to the inelastic scattering cross sections. It is found that the proportion of multiple emissions of α particles compared with single emissions increases with nuclear size.



Some of the prompt γ-ray spectra emitted after interaction of 220 MeV negative pions with a range of nuclei. The lines marked with a solid dot appear in many spectra and correspond to the multiple removal of α particles.

The experimental technique does not distinguish between the emission of an α particle and the emission of two neutrons and two protons, since only the residual nucleus is identified. The relative likelihood of the two processes, and indeed of all possible processes and hence residual nuclei, can be calculated from statistical theory. It is found that this gives results completely different from those found experimentally so that multiple 'α' emission cannot be explained by the statistical theory.

These results have now been confirmed by studies of the interactions of both positive and negative pions with ^{27}Al and ^{28}Si by Ashery and colleagues (*Phys. Rev. Lett.*, **32**, 943; 1974) from Tel Aviv. The negative pions had an energy of 70 MeV and the positive pions energies of 25, 70 and 100 MeV.

Using essentially the same method as the United States group, they also found that there is a large cross section for removing an 'α particle' and leaving the nucleus in its first excited state. This occurs even below 25 MeV, which is the threshold for the emission of two protons and two neutrons, and this strongly supports the hypothesis of cluster emission.

It is found that at 70 MeV the cross section for 'α' emission is very similar to that for the elastic scattering of pions by free α particles, thus suggesting that the pions are simply knocking out pre-existing α particles from the target nuclei. Substantial cross sections are also found for reactions corresponding to the emission of deuteron clusters.

These experiments on the interaction of fast pions with nuclei thus give results similar to those found in studies of the interaction of slow pions or kaons. There seems to be some process that takes place in the initial stages of the interaction that leads to the preferential emission of α particles. This in turn suggests that there are α particles in the nuclear surface, a hypothesis for which there is already much evidence.

It is important to continue this work, and in particular to try to detect and identify directly the particles emitted in the early stages of the interaction. This is a difficult experimental problem but if it can be solved it will shed new light on the problem of α particles on the nuclear surface.

Origin of kimberlite pipes by diapiric upwelling in the upper mantle

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A diapiric mechanism is presented to explain geochemical data on the thermal structure of the upper mantle. It is emphasised that any model to fit the data cannot be a steady state model, and it is concluded that the data are inconsistent with the popular interpretation of the seismic low velocity zone as a partially melted region.

THE 'pyroxene geotherm' technique[1-3] utilises the experimentally determined partitioning of calcium[4] and aluminium[2] between coexisting clino and orthopyroxenes in order to determine the conditions of origin of garnet and spinel peridotites. Application of the technique to xenoliths from kimberlite pipes shows that: (1) the xenoliths are fragments of the upper mantle accidentally included in the kimberlite during its passage to the surface; (2) the xenoliths from each pipe describe a 'palaeogeotherm' which abruptly steepens at depths between 140 and 200 km (Fig. 1); (3) the point of inflection in each geotherm corresponds to chemical[5] and textural[6-8] changes. The shallower, 'normal' portion of the geotherm is characterised by coarsely crystalline harzburgites which have no significant preferred orientation and are strongly depleted in basaltic constituents; the steepened, deeper portion is represented by highly deformed, extensively recrystallised lherzolites capable of producing normal basalts by partial melting[9]. It has been pointed out[1,8,10] that the shape of the palaeogeotherms is unlikely to reflect a steady state situation and it has been proposed that the steepening results from shear heating which occurred in the seismic high attenuation, low velocity zone (HALVZ) during the breakup of Gondwanaland. We agree that the assumptions necessary for any steady state model are unreasonable but we question that the shear-heating model quantitatively satisfies the data. This article presents an alternative diapiric model, postulating upwelling from within or below the HALVZ.

The model

We assume that a small density inversion of unknown origin initiates upwelling at a depth of more than 200 km. The upwelling will be self perpetuating if the temperature gradient exceeds that for adiabatic expansion, a well known property of modern models of the thermal structure of the upper mantle[11]. The upwelling therefore becomes a diapir which very soon reaches a quasi-steady limit velocity. It is driven principally by the buoyancy force which results from the temperature difference between the interior of the diapir and the surrounding, undisturbed mantle. If the velocity of ascent is rapid compared with the rate of heat loss, the diapir may reach the solidus and partially melt. We assume that such melting occurs just below the point of the deepest xenoliths and that the kimberlitic magma is produced. The magma brings up fragments both from the top of the diapir, and from above it.

For quantitative comparison of this model with the geochemical data, the geothermal gradient along the axis of the diapir can be calculated (Fig. 2). The heat flow equation must therefore be solved. Neglecting heat sources[12]:

$$\rho c_p \left(\partial T/\partial t + v_i \nabla_i T\right) = K\nabla^2 T + (\eta/2)\left(\partial v_i/\partial x_k + \partial v_k/\partial x_i\right)^2 \quad (1)$$

where ρ is the density, c_p the heat capacity at constant pressure, T is temperature, t is time, v_i is the velocity of the moving rocks, K is the thermal conductivity and η the viscosity. The second, third and fourth terms are the contributions of convection, conduction, and viscous heating respectively. The well known difficulty in obtaining an analytical solution to equation (1) leads to a comparison of the contributions of the various terms thus simplifying the problem but remaining within the accuracy of the data. The relative importance of the convection and viscous heating terms is of paramount importance to the form of our solution. We assume that the spatial variation of velocity within the diapir can be satisfactorily approximated by a single average velocity $< v_3 >$. We also assume that the average temperature gradient $< \frac{\partial T}{\partial x_3} >$ is of the order of 1° C km^{-1}; $c_p = 10^7$ erg $^\circ$ C^{-1} g^{-1}; $\rho = 3$ g cm^{-3}, $\eta = 10^{21}$ poise and that $< \frac{\partial v_i}{\partial x_i} > = \frac{< v_3 >}{R}$ where R, the radius of the diapir, is 50 km. For this comparison, we calculate the average velocity by equating the driving force to the viscous drag on the diapir considered as a whole, and using an average temperature

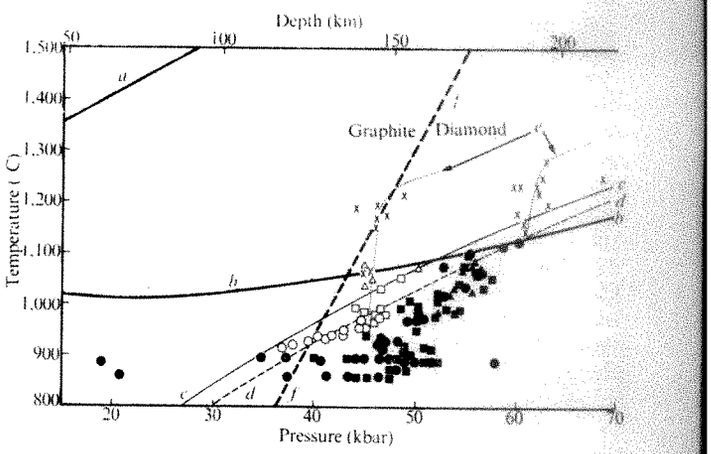


Fig. 1 Thermal and textural structure of the upper mantle beneath southern Africa at the time of emplacement of Cretaceous kimberlite pipes. Data and phase boundaries after Macgregor[3]: *a*, dry peridotite solidus; *b*, vapour saturated solidus (H_2O:CO_2 = 1:3); *c*, C and R shield geotherm; *d*, model geotherm (undisturbed); *e*, model geotherm (perturbed); *f*, graphite–diamond phase boundary. Open symbols, South West Africa region; closed symbols, Kimberley region; circles, coarse grained, undeformed xenoliths with coarse granular textures; squares, coarse grained, undeformed xenoliths with coarse tabular textures; triangles, plastically deformed xenoliths with porphyroclastic textures; crosses, plastically deformed xenoliths with mosaic textures. Shield geotherm of Clark and Ringwood[11] (*c*) given for comparison

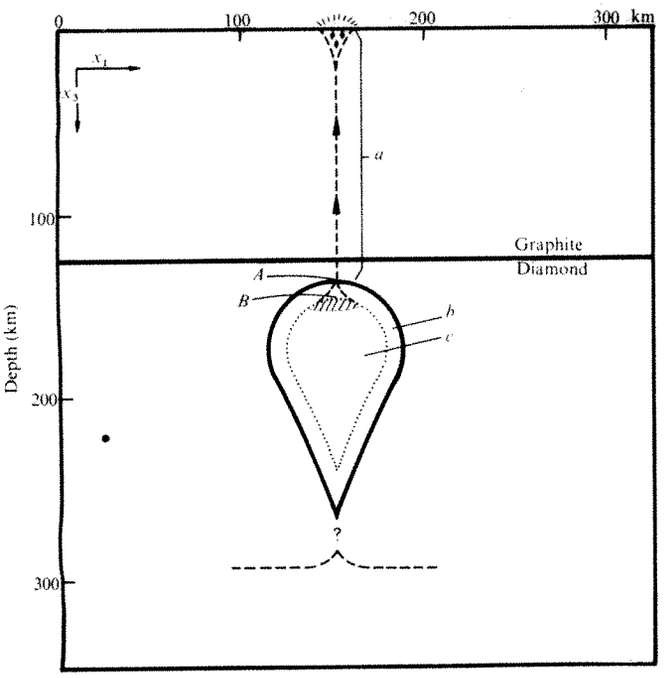


Fig. 2 Idealised drawing of a rising diapir at the time of melting and kimberlite formation. Region *a*, undisturbed lithosphere; region *b*, outer 'rind' of diapir affected by conductive cooling during upwelling; region *c*, interior of diapir cooled only by adiabatic expansion. Points *A* and *B* mark transitions from one region to another. Shaded area diagrammatically indicates melting at the top of region *c*.

difference of 100° C between diapir and surrounding rocks. The result, which is almost independent of the shape of the diapir[12], is $< v_3 > = 1$ cm yr^{-1}. Then:

$$\frac{\rho c_p < v_3 > < \partial T/\partial x_3 >}{(\eta/2)(< \partial v_3/\partial x_1 >)^2} \simeq 500 \qquad (2)$$

Even for these conservative assumptions, the convection term is dominant by more than two orders of magnitude. The likelihood that η is less than 10^{21} poise further reduces the importance of viscous heating, and therefore the shear heating term cannot have a controlling effect on our model.

A one-dimensional model can be used if the diapir is sufficiently large for heat loss at the sides not to affect appreciably the central axis. For the times relevant to the model, the heat diffusion distance[12] is less than 20 km, so that any diapir large enough not to be affected by shear heating (that is, $R \geqslant 25$ km) can also be treated as a one-dimensional problem; the side effects are unimportant in a first-order approximation. Finally, we assume that heat lost through the top of the diapir is carried away by the mantle flowing around the advancing mass. We thus obtain the model shown in Fig. 2. The calculated axial geotherm will have three parts: (*a*) an undisturbed geotherm above the diapir; (*b*) the top of the diapir affected by diffusive loss of heat; (*c*) the tail of the diapir, characterised by the adiabatic gradient. The simplified model matches parts *a* and *c* by a temperature perturbation (*b*) on the model geotherm, which is chosen as a good fit to the geochemical data between 100 and 200 km depth and fitted to the shield geotherm of Clark and Ringwood[11] at lesser and greater depths (Figs 1 and 3).

The perturbation obeys the heat flow equation:

$$\partial T/\partial t = (K/\rho c_p)(\partial^2 T/\partial x_3^2) \qquad (3)$$

The boundary condition at the top of the diapir is $T_0(t) = v_3 (\partial T/\partial x_3)_0 t$ where t is the time elapsed since the formation of the diapir, $K = 4 \times 10^4$ erg cm^{-1} s^{-1} ° C^{-1} (10^{-3} calorie cm^{-1} s^{-1} ° C^{-1}) and the other values are as already given. The boundary condition equates the temperature at the top of the diapir with that just above it in the undisturbed mantle; it is the expression of the heat flowing out at the top of the diapir. Between 250 km and 400 km depth, $(\partial T/\partial x_3)$ is relatively constant and for the calculations we have adopted 1.7 ° C km^{-1}. In this simplified form the problem has the analytical solution[13]:

$$T(x_3,t) = 4 < v_3 > (\partial T/\partial x_3)_0 \, t \, i^2 \operatorname{erf} [x_3 (Kt/\rho c_p)^{-\frac{1}{2}}] \qquad (4)$$

The calculations have been made for the shallowest and deepest data sets of Macgregor[3], representing the regions of South West Africa (Louwrencia pipe) and Kimberley (grouped data representing the Wesselton, Roberts Victor, Bulfontein and Jagersfontein pipes) respectively. We have chosen to model the undisturbed mantle with a single, representative geotherm because of the uncertainties in the data, the mixed origin of the Kimberley data, and the small effect separate geotherms would have on our results. The chosen geotherm also fits satisfactorily with the data from the Lesotho region[1,3]. The data allow the independent calculation of the time intervals of upwelling, the diapir velocities and then the depths of origin (Table 1, Fig. 1). The calculated depths of origin of the diapirs are surprisingly similar and perhaps represent the depth of the lowest portion of the HALVZ.

Discussion

The diapir model fits the geochemical data well and the adjustable parameters in the model are all chosen to be very reasonable values. Similarly, the calculated times and velocities are reasonable. The production of the kimberlite magma containing xenoliths is a natural consequence of the model, for once initiated, the diapir will rise until it reaches the surface without melting (becoming an alpine peridotite[14]) or it will partially melt and result in the production of kimberlite pipes. We assume that melting occurs at temperatures just above the hottest temperatures indicated by the analysed xenoliths. These temperatures always fall between the dry and water-saturated peridotite solidi[15,16], and the presence of small quantities of water is thus mandatory for the model. The amount cannot be precisely estimated because it is critically dependent on

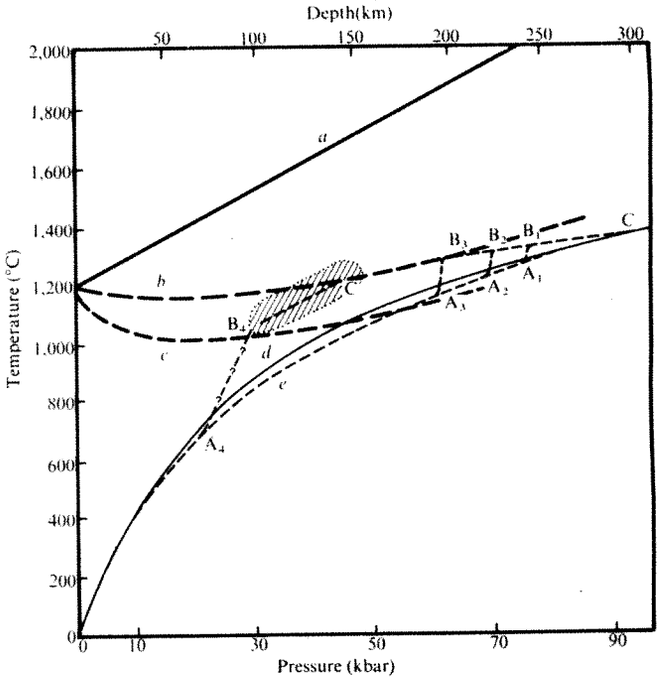


Fig 3 Thermal evolution of the rising diapir in the Kimberley region. *a*, Dry peridotite solidus; *b*, model solidus; *c*, wet solidus (H_2O:CO_2 = 1:3); *d*, C and R shield geotherm; *e*, model geotherm (undisturbed). Point *C* represents origin of the diapir on the undisturbed geotherm. Points A_i and B_i represent successive positions of points *A* and *B* of Fig. 2; *B* moves down the adiabat and *A* down the geotherm. Melting occurs at B_3. Shaded region encompasses data of 'second geotherm' of Macgregor[3]. A_4–B_4–*C'* represents possible earlier diapir responsible for these data.

Table 1 Duration of upwelling, velocity of ascent, and depth of origin of diapirs

Region	Mean velocity	Duration of upwelling	Original depth
South West Africa	5 cm yr^{-1}	3 Myr	290 km
Kimberley	3 cm yr^{-1}	3 Myr	280 km

collection and analysis of xenoliths which equilibrated on the solidus as well as on the accuracy of the pyroxene geotherm. Macgregor[3] points out that 'primary phlogopite' is present in region *a* (Fig. 2) of our model. Texturally this 'primary phlogopite' usually seems to be a replacement phase rather than part of the original paragenesis (Green, H. W. II, and Boullier, A. M., not yet published), but its coarse grain size and apparent stability with respect to the other phases of the rocks strongly indicates an origin which predates incorporation into the kimberlite magma. Thus the rocks do contain small quantities of water.

We have not yet considered the chemical and textural differences present in the xenoliths. The coarse grained harzburgites correspond to region *a* (Fig. 2) and the highly deformed lherzolites exist in region *b* (Fig. 1). We expect such an abrupt textural and chemical transition because the undepleted diapir flows plastically on its margins as it rises through the previously depleted, annealed upper mantle. The top of the diapir would be expected to be the only place where undepleted mantle could be sampled because only this region has lost enough heat to miss the solidus and stay intact.

Macgregor[3] has described a second group of xenoliths in the Kimberley region (Fig. 3). This atypical group plots at distinctly higher temperatures than the first, 'normal', group which is remarkably similar from pipe to pipe and from region to region. If there are two geotherms in the same region then there cannot be a steady-state situation. The second geotherm could represent the 'tail' of an earlier diapir which continued to rise at a reduced velocity after it had been partially melted. Alternatively, the second geotherm could represent an earlier diapir which rose more slowly and consequently never reached the melting point (for example, a velocity of ~ 0.1 cm yr^{-1} would produce the observed slope with our model). In either case the first diapir must precede the second diapir, which brings the fragments to the surface, by only a few tens of millions of years otherwise the internal temperature of the first diapir would be reduced to the normal, unperturbed geotherm. An interesting difference between these two possible explanations is that for the former possibility the older diapir 'tail' could be mineralogically and texturally indistinguishable from normal lithosphere of region *a* (Fig. 2) but in the latter case the first diapir should be chemically undepleted even though it might be texturally similar to normal lithosphere. Unfortunately, the data[3] are not separated in such a way as to allow these possibilities to be tested.

The discovery[3] of the 'second geotherm' in the Kimberley region emphasises the need for a time-dependent explanation for the geo chemical data, but it is not the only observation which supports this class of model. To make a steady state model fit the inflection in the palaeogeotherms a large and discontinuous change in thermal conductivity is required[1,3]. Variation in the depth of the inflection point and its correlation with the xenolith textural types indicate that the effect would have to be produced by the preferred orientation in the deformed rocks or by some inherent effect of deformation on thermal conductivity. Because of the rather weak preferred orientations in these rocks (A. M. Boullier, personal communication) an anisotropy of K of more than a factor of 50 would be necessary. Similarly, any effect of crystal defects on the thermal conductivity would seem to be small because such an effect depends on high concentrations of defects[17], whereas the high temperature deformation undergone by these rocks produces recrystallised grains with extremely low defect densities (Green, H. W. II, and Boullier, A. M., not yet published).

In an earlier model[1,5,8] shear heating in the low velocity zone is proposed to explain the steepened portion of the geotherms. Our calculations show that rates of strain exceeding 10^{-11} s^{-1} would be required to fit the data quantitatively, even assuming that there is no loss of heat by conduction. We consider such rates to be geologically unreasonable and therefore believe that the heat is better provided by a convective mechanism. Our diapiric model is the extreme case in which all of the heat is produced by convection. Other models can be constructed in which both upwelling and shear heating are involved[18], but they are more complicated and therefore more difficult to apply quantitatively.

Two additional consequences of the diapiric model are of considerable interest for understanding upper mantle processes. First, if the model is to be valid, the HALVZ cannot be partially melted; either another fluid[19] or a completely different mechanism[20] must be sought to explain the seismic data. (Interestingly, the same conclusion seems to be required for any model which equates the deformed xenoliths with the HALVZ because these rocks are unmodified and contain no distributed melt phase (Green, H. W., and Boullier, A. M., not yet published).) Second, if xenolith-bearing basaltic eruptions in tectonic and oceanic areas are also preceded by diapiric upwellings, as seems probable to us, then the flow mechanisms evident in the xenoliths reflect the mechanisms of diapirism which may not be the same as those of flow in the asthenosphere. Thus, the conclusions[21,22] that flow in the upper mantle is dominated by movement of dislocations which are climb-controlled does not necessarily apply to asthenospheric flow. These arguments do not, however, contravene those based on post-glacial uplift data and power-law creep in experiments[23].

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1 Boyd, F. R., *Geochim. cosmochim. Acta*, **37**, 2533 (1973).
2 Macgregor, I. D., *Am. Miner.* (in the press).
3 Macgregor, I. D., *Physics and Chemistry of the Earth, Kimberlite conference volume* (in the press).
4 Davis, B. T. C., and Boyd, F. R., *J. geophys. Res.*, **71**, 3567 (1966).
5 Nixon, P. H., and Boyd, I. D., in *Lesotho Kimberlites* (edit. by Nixon, P. H.), 48 (Lesotho National Development Corporation, Maseru, 1973).
6 Boullier, A. M., and Nicolas, A., in *Lesotho Kimberlites* (edit. by Nixon, P. H.), 57 (Lesotho National Development Corporation, Maseru, 1973).
7 Boullier, A. M., and Nicolas, A., *Physics and Chemistry of the Earth, Kimberlite conference volume* (in the press).
8 Nixon, P. H., Boyd, F. R., and Boullier, A. M., in *Lesotho Kimberlites* (edit. by Nixon, P. H.), 312 (Lesotho National Development Corporation, Maseru, 1973).
9 Kushiro, I., in *Lesotho Kimberlites* (edit. by Nixon, P. H.), 294 (Lesotho National Development Corporation, Maseru, 1973).
10 Boyd, I. D., and Nixon, P. H., in *Lesotho Kimberlites* (edit. by Nixon, P. H.), 254 (Lesotho National Development Corporation, 1973).
11 Clark, S. P., and Ringwood, A. E., *Rev. Geophys.*, **2**, 35 (1964).
12 Landau, L., and Lifchitz, E. M., *Mécanique des fluides* (edit. by Mir, M.), (1971).
13 Carslaw, H. S., and Jaeger, J. C., *Conduction of heat in solids*, (Oxford, 1959).
14 Loomis, T. P., *Bull. geol. Soc. Am.*, **83**, 2475 (1972).
15 Ito, K., and Kennedy, G. C., *Am. J. Sci.*, **265**, 519 (1967).
16 Boettcher, A. L., Mysen, B. O., and Modreski, P. J., *International conference on Kimberlites, extended abstracts*, 35 (1973).
17 Klemens, P. G., *Solid State Physics* (edit. by Seitz, F., and Turnbull, D.), **7**, 1 (1958).
18 Anderson, O. L., and Perkins, P. C., *J. geophys. Res.*, **79**, 2136 (1974).
19 Green, H. W. II, *Nature phys. Sci.*, **238**, 2 (1972).

[20] Gueguen, Y., and Mercier, J. M., *Phys. Earth planet. Interiors.*, **7**, 39 (1973).
[21] Green, H. W. II, and Radcliffe, S. V., *Earth planet. Sci. Lett.*, **15**, 239 (1972).
[22] Green, H. W. II, and Radcliffe, S. V., *in Flow and Fracture of Rocks, Geophysical Monograph*, **16**, 139 (Am. geophys. Union, Washington, 1972).
[23] Post, R. L., jun., and Griggs, D. T., *Science*, **181**, 1242 (1973).

Neural antigens of morphologically differentiated neuroblastoma cells

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Murine neuroblastoma cells in serum-free medium undergo correlative morphological and serological differentiation. The antigens specific to the differentiated cells are found in large quantities in mouse brain.

MURINE neuroblastoma cells can be induced to differentiate morphologically so as to resemble mature neurones if they are maintained in medium without serum[1]. This morphological differentiation is often not accompanied by biochemical differentiation, as studies of neurotransmitter biosynthetic enzymes[2], glycosphingolipid composition[3], tryptic glycopeptides[4], mouse alloantigens[5] and acrylamide gel patterns of solubilised membranes[6] have shown. Similarly, studies of the development of the ability to generate an action potential and of sensitivity to electrical and chemical stimulation, have produced no consensus on any correlation with morphological differentiation[7–10].

We have described a set of cell surface antigens on morphologically differentiated cells derived from clone N18 neuroblastoma[11]. They are also present in large quantities in particulate preparations of mouse brain, but not in spleen, liver or kidney. Their appearance is the only reported specific change in the cell membranes that correlates with the morphological differentiation of cultured neuroblastoma cells.

We report here that: (1) serological differentiation of N18 neuroblastoma cells correlates with the extension of processes in serum-free medium and (2) direct binding experiments with ^{125}I-labelled immunoglobulin confirm and partially quantify the presence of neurone-specific antigens found only on morphologically differentiated neuroblastoma cells.

Antisera specific to differentiated clone N18 murine neuroblastoma cells were produced by immunisation and adsorption as before[11]. The increased binding of antibodies (at saturating concentrations) to N18 cells maintained for 5 d in serum-free medium (N18-SF) compared with the binding to N18 cells grown in suspension culture on bacteriological plates[6] (N18-sus) implies that new antigens were present on the morphologically differentiated N18-SF cells (Fig. 1). Binding of ^{125}I-immunoglobulin to morphologically differentiated neuroblastoma cells, undifferentiated cells and mouse tissue correlated well with their antigenic activities measured by indirect immunofluorescence and complement fixation[11].

To examine the relationship between process formation and the presence of new antigens on neuroblastoma cells in serum-free medium we used the C1300 neuroblastoma clone 10D (ref. 11), which does not extend processes in serum-free medium. The serological reactivity of 10D cells grown with serum was indistinguishable from that of N18-sus cells measured by complement fixation, immunofluorescence or saturation binding (ref. 11 and Table 1). In addition, 10D cells maintained in serum-free medium for 5 d (10D-SF) neither extended processes nor bound more immunoglobulin than did N18-sus or 10D cells grown in complete medium (Table 1). The presence of new antigens on N18-SF cells was thus related to the formation of processes, and not to an effect of maintenance in serum-free medium.

Morphology and antigenicity studies

We next examined the serological reactivity and morphology of N18 cells maintained without serum for less than 5 d in an attempt to relate antigenicity and morphology. Morphological development increased steadily for 5 d. After 6 d the cultures tended to degenerate. Complement fixing ability (Fig. 2) of N18 cells in serum-free medium decreased initially for 2 d, probably because the cells were derived from near-confluent monolayers, and then increased for 3 d. Confluent cells were to some degree morphologically differentiated, thus a period in nonconfluent conditions may have been necessary for the antigens associated with this differentiation to be removed from the cell surface. Near-confluent cultures grown in complete medium had reactivity similar in both complement fixation and saturation binding assays to that of cells cultured for 3 d without serum. There was thus little serological differentiation during the first 2 d in serum-free medium, followed by a steady increase in antigenic reactivity.

Table 1 Antibody binding to neuroblastoma cells

Antigen	Immunoglobulin bound at saturation (ng)	% of N18-SF Value
N18-SF	48	100
N18-sus	37	78
10D	36	75
10D-SF	35	72

Values are the average from three experiments.

In general the primary morphological change that correlates with increasing immunological reactivity is the formation of steadily lengthening processes on the cells. Morphological development seems to precede antigenic development. The multispecificity of our antiserum is undoubtedly an important source of difficulty in attempting to correlate

discrete antigenic and morphological events.

We next examined the relationship of the new antigens to the antigens of various mouse organs. Our antisera were produced to N18-SF cells, and so the reactive antigens present on the other cells and tissues tested should have been subsets of those found on N18-SF. No relationship between (for example) the antigens present on brain and liver can however, be inferred from Fig. 1. The liver antigens, in whole or in part, could be a subset of the brain antigens, or the two sets of antigens could be independent. To determine the relationship(s) between them, we performed binding experiments using ^{125}I-immunoglobulin adsorbed with particulate material (PM) from various mouse organs. Liver was chosen as a non-neural source of antigen because it binds relatively large amounts of the immunoglobulin (compared with other non-neural tissues) and has low levels of innervation.

Adsorption of immunoglobulin with particulate preparations from liver removed antibodies that bind not only to liver but also to all the other organs and cells tested (Table 2). Antibodies that bind to spleen and heart were removed when the immunoglobulin was adsorbed with liver PM. Therefore the reactive antigens found on these tissues were probably identical to those of liver. In a reciprocal experiment spleen adsorbed all binding activity to heart and liver (data not shown). A small amount of the antibodies that bind to kidney were not removed by adsorption with liver PM. It is not clear whether these antibodies reacted with the neural antigens which were present as a consequence of the innervation of kidney, or with some other antigen not shared by the liver. Binding of liver-PM-adsorbed immunoglobulin to brain, N18-sus and N18-SF cells was reduced by approximately equal amounts. The antigens found on liver seemed to be present on all the cells and organs tested and are probably common to all mouse cells. Immunisation of a xenogeneic animal, the rabbit, would be expected to raise antibodies to species-specific antigens.

As adsorption of the immunoglobulin with brain PM removed all antibodies which bind to other organs (Table 2), brain must contain all the antigens found on the other organs, but not all those present on the cultured neuroblastoma cells. The absolute binding measured in ng to N18-SF cells of brain-adsorbed antibody was reduced more than the binding of such antibody to N18-sus. This reduction suggests the presence of neural antigens found on N18-SF but not on N18-sus. The difference in binding between N18-SF and N18-sus [Δ(SF-sus)] could be reduced 67% by ad-

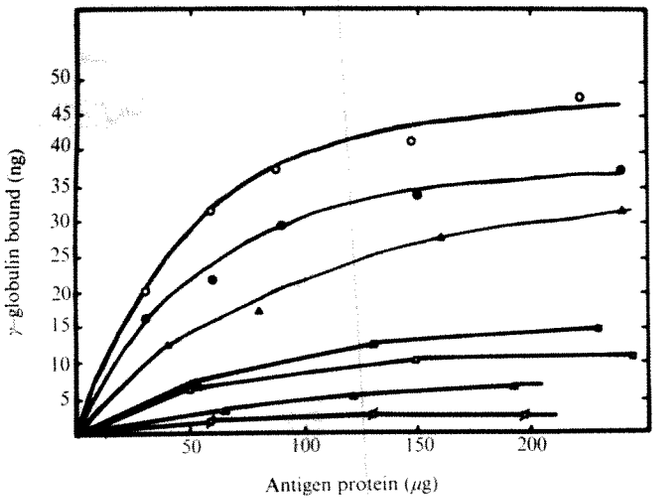


Fig. 1 Binding of ^{125}I-labelled immunoglobulin to mouse organs and cultured neuroblastoma cells. Antisera production and cell culture have been described. All experiments utilised the bleeding from rabbit 147 on the 20th day of the immunisation protocol[11]. Unless otherwise noted, all operations were performed at 0°–8°C. γ-Globulin was isolated by the method of Fahey and Horbettt[14] and labelled by a large scale modification of the method of Greenwood *et al.*[15] using carrier-free ^{125}I (New England Nuclear Corp.). Labelled immunogloblin was separated from unbound iodine on Sephadex G25, diluted with an equal volume of 5 mg ml^{-1} bovine albumin in isotris buffer[16], and stored in aliquots at −20° until use. The specific activity was 0.7 Ci g^{-1}. Cells and organs were prepared as before[11]. Brain samples did not include the olfactory bulb. Spleen samples included only the expressed cells and not the capsule. Whole liver and kidney were used. Heart samples were taken from the caudo-lateral left ventricle wall. There were no substantial differences in saturation binding with tissues from A/J, B_6AF/J, C3H, C57B, HeJ or Swiss Webster mice. Labelled immunoglobulin (260 μg) was shaken for 5 h in a mock adsorption procedure. After filtration, 5 μg was added to each antigen sample. All tissues are from a 60-d-old Swiss Webster mouse. The ^{125}I-immunoglobulin and antigen PM were combined in isotris buffer plus 10% normal rabbit serum (final volume of 1.5 ml) incubated 12-16 h, then centrifuged for 23 min at 45,000*g*. The supernatant was discarded. The pellet was resuspended by vortexing for 15 s in 1.2 ml isotris containing 2 mg ml^{-1} bovine albumin (BA). After a second centrifugation for 26 min at 45,000*g*, the pellet was resuspended in 1.2 ml isotris containing 2.0 mg ml^{-1} BA. The suspensions were then filtered (at room temperature) over a Whatman GF-A glass fibre paper which had been presoaked overnight in 2.5 mg ml^{-1} BA in isotris. The tube was washed twice with 2 ml isotris, and the washes filtered. The filters were rinsed twice with 2 ml absolute ethanol and dried for at least 30 min at 75°C before counting. For each curve the ng of antibody bound by the equivalent amount of HeLa cells is subtracted to obtain the specific binding. This background in the linear portion of the binding curve is 10-15% of the values obtained using N18-SF as the antigen. That the γ-globulin which sticks to HeLa cells is bound nonspecifically is demonstrated by the observations that (a) this binding is a linear nonsaturating function of added HeLa cell protein, (b) exhaustive adsorption of antiserum with an antigen reduces the binding of antiserum to that antigen down to but not below the levels obtained with HeLa cells, and (c) adding excess unlabelled antiserum reduces binding of labelled antiserum to N18-SF down to but not below the level obtained with the same amount of HeLa cell protein. HeLa cells do not react with this antiserum in either complement fixation or immunofluorescence assays. ○, N18-SF; ●, N18-sus; △, brain; ■, kidney; □, liver; ▲, spleen; ⊡, heart.

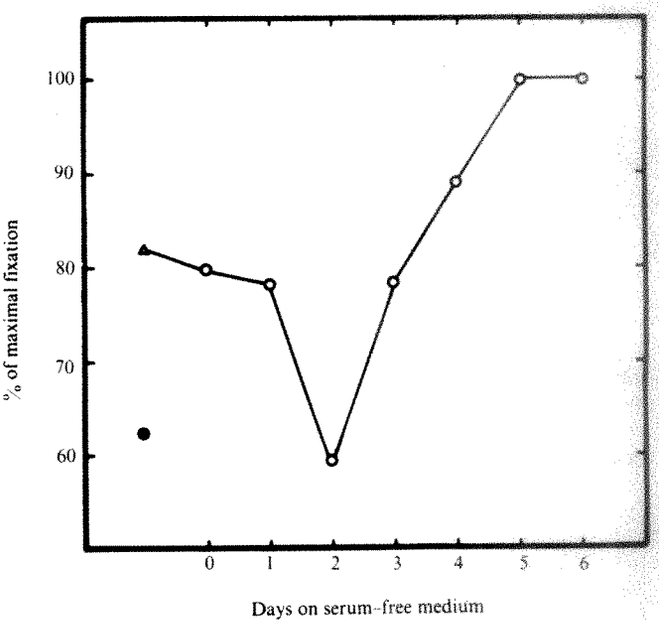


Fig. 2 Complement fixation of N18 cells maintained for varying periods on serum-free medium. Data are expressed as percentages of the maximal fixation obtained with N18 cells maintained 5 d on serum-free medium. Confluent or late log phase cultures were treated with Viokase and plated as before[11] to obtain cultures subsequently maintained in serum-free medium. △, Late log or confluent cultures; ●, N18-sus; ○, N18.

Table 2 Organ adsorption of antibody binding activity

Antigen used for test	% Antibody binding activity adsorbed by: Brain	Liver
N18-SF (differentiated)	75 (32.0)	38 (16.0)
N18-sus	79 (27.2)	51 (17.5)
Brain	88 (22.0)	56 (15.0)
Kidney	95 (14.0)	75 (10.5)
Liver	93 (10.0)	>95 (10.8)
Spleen	>95 (6.2)	92 (5.8)
Heart	>95 (2.5)	>95 (2.5)
Δ(SF-sus)	67 (6.8)	< 5 (0.1)

Two aliquots of 130 μg of ^{125}I-immunoglobulin were adsorbed with 5 mg of liver and brain respectively. This is the amount of antigen necessary to adsorb 90% of the antibodies which bind to brain and about 1.5 times the amount necessary to adsorb 90% of the antibodies which bind to liver. A volume containing 5 μg of adsorbed antibody was added to each antigen sample (150 μg). The mixtures were incubated for 12 h at 8°C. All cells and organs were the same preparations as those described in Fig. 1. Numbers in brackets are ng of antibody binding activity removed.

sorption with brain PM but less than 5% by adsorption with liver PM (Table 2, last line). N18-SF is the only antigen source able to adsorb totally the binding immunoglobulins in the antiserum.

These and other adsorption experiments established the following relationships among the sets of antigens reactive with this antiserum. (1) The set of antigens present on a tissue includes as a subset all reactive antigens of tissues with a lesser total reactivity. For example, the set of reactive antigens present on liver PM includes as subsets those reactive antigens present on spleen and heart PM, while the set of reactive antigens present on brain includes all those of kidney, liver, spleen and heart. (2) Approximately two-thirds of the antigens present only on the morphologically differentiated form of N18-cells are found only on neural tissue. Among antibodies that bind to brain are some that bind to morphologically differentiated but not

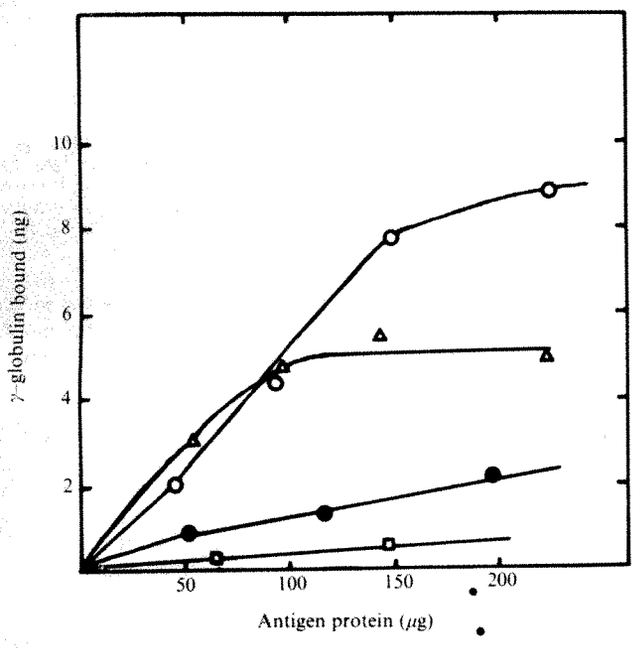


Fig. 3 Binding of ^{125}I-immunoglobulin adsorbed with N18-sus. ^{125}I-immunoglobulin (120 μg) was adsorbed with 3.3 mg of N18-sus PM for 16 h. A volume containing 5 μg was added to each antigen sample and the mixture was incubated 14 h at 8°C. Organs were from a 45-d-old C57BL mouse. Symbols as in Fig. 1.

to undifferentiated neuroblastoma cells.

We attempted to confirm the neuronal specificity of Δ(SF-sus) by further methods. Adsorption of the immunoglobulin with N18-sus removes all antibodies that bind to all cells and tissues tested, except N18-SF and brain (Fig. 3). For such an adsorbed immunoglobulin preparation, Δ(SF-sus) was 6.0 ng, while Δ (brain-sus) was 3.4 ng or 57% of Δ(SF-sus). This value is close to the 67% of Δ(SF-sus) already given (Table 2) as specific to neural tissue. Each of these calculations gives a minimal value because in each

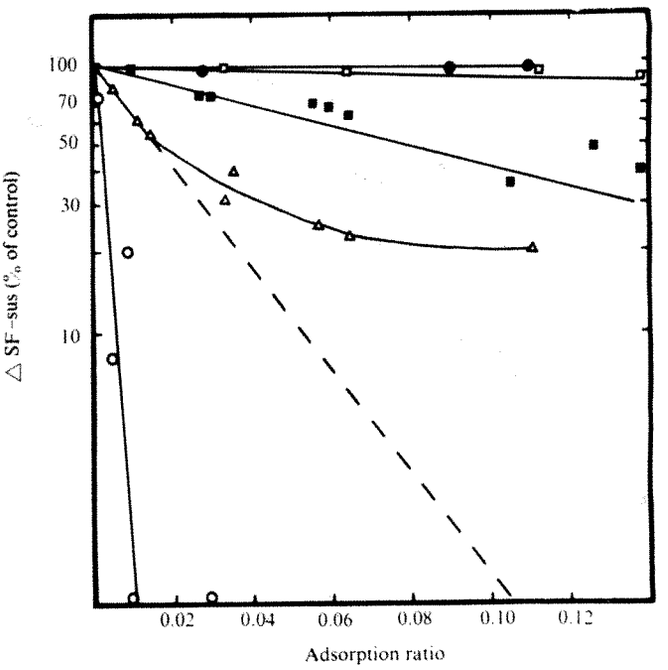


Fig. 4 Exhaustive adsorptions with PM from mouse organs. The reduction in Δ(SF-sus) is plotted as a function of the amount of PM used to adsorb. Spleen cells could not be used in these exhaustive adsorption experiments, due to their affinity for rabbit γ-globulin. This binding is probably due to the presence of receptors for the F_c portion of immunoglobulin molecules[12]. The adsorption ratio is defined as the mg of organ PM per μg ^{125}I-immunoglobulin used in the overnight adsorption. By comparison this ratio in the adsorptions of Table 2 is 0.038. The adsorption ratios at which 90% of the binding activity to each antigen is removed by adsorption with that same antigen are liver, 0.026; brain, 0.038; N18-SF, 0.018. Values for muscle and kidney were not determined. The lines drawn are linear regression fits to the data points in all cases except brain. The dashed line is an extension of the line of best fit to the first three brain adsorption points. The remainder of the solid curve for brain is a free-hand fit of the points. ○, N18-SF; △, brain; ■, kidney; □, liver; ●, skeletal muscle.

case the adsorption of antibody molecules was incomplete. Any unadsorbed binding material was assumed to not be specific to neural tissue.

If adsorption and subsequent direct binding assays were performed with particulate preparations from monolayer 10D-neuroblastoma cells rather than N18-sus cells, the ability of brain to adsorb the remaining (N18-SF specific) immunoglobulin preferentially was unchanged (data not shown). Thus, brain reacted preferentially with an antiserum made specific for the morphologically differentiated state of neuroblastoma cells, whether N18-sus or 10D monolayer PM was used for adsorption. In summary, antigens found only on the differentiated form of N18 neuroblastoma cells were also present on brain but not other organs, when assayed by both direct binding and adsorption.

To determine whether antigens present only on the

morphologically differentiated form of N18 neuroblastoma cells are present in lesser concentrations on particulate material from organs other than brain, we performed binding studies with immunoglobulin exhaustively adsorbed with skeletal muscle, liver and kidney as well as brain PM. The adsorbed immunoglobulins were tested against both N18-SF and N18-sus to determine whether antibodies reactive with antigens unique to the differentiated neuroblastoma cells had been removed.

Liver and skeletal muscle PM did not adsorb significant amounts of the Δ(SF-sus)-specific immunoglobulins (Fig. 4). Adsorptions with brain preparations produced a multiphasic curve. The ratio of the slope of the adsorption curve with N18-SF to that of the initial slope of the brain adsorption curve (dashed line) is 12 : 1, indicating that the specific activity of Δ(SF-sus) antigens was twelve times greater on N18-SF cells than on brain. The slope of the brain adsorption curve seems to be reduced at about 50% reduction of Δ(SF-sus). Therefore the remaining Δ(SF-sus) antigens adsorbed by brain were present in brain with specific activities less than one-twelfth of the activity of these antigens in N18-SF cells. In total 80% of the Δ(SF-sus) antigens was adsorbed by brain PM in exhaustive procedures.

If the linear regression line is extrapolated, the specific activity of the Δ(SF-sus) antigens in kidney is one-seventh that of brain (1/84th the specific activity of these antigens in N18-SF). However, the kidney adsorption curve appears to saturate at ratios of 0.10 to 0.14. (Larger adsorption ratios were difficult to examine because of nonspecific affects of large amounts of PM.) Tissues other than brain might be expected to contain small amounts of neural antigens due to their intrinsic innervation. If the kidney adsorption curve does saturate at 50%, then (i) approximately 30% of the Δ(SF-sus) antigens cannot be detected by this exhaustive adsorption method in any organ but brain; (ii) half the Δ(SF-sus) antigens are enriched in brain by a factor of seven to one hundred or more when compared with other organs, and (iii) the remaining 20% cannot be detected in any of the organs tested, including brain. Similar calculations, based on complement fixation[11] and saturation binding analysis, suggest that the set of antigens unique to morphologically differentiated N18 cells is present in at least 100 times the specific activity on such cells as on morphologically undifferentiated neuroblastoma cells (N18-sus, 10D or 10D-SF).

Table 3 Antigen classes of murine neuroblastoma cells

Proposed antigen class on:	Presence of antigen on: HeLa	Liver Spleen and so on	Brain	N18-sus	N18-SF
Undifferentiated cells					
I Common Mouse	−	+	+	+	+
II Neural	−	−	+	+	+
III Cell-Specific	−	−	−	+	+
Differentiated cells only					
IV Neural-specific	−	−	+	−	+
V Cell-specific	−	−	−	−	+

−, Will not adsorb; +, will adsorb.

Proposed antigen classes

The xenogeneic antiserum we used revealed a spectrum of particulate antigens on N18 neuroblastoma cells. Morphologically undifferentiated cells have antigens which seem to be shared by all mouse organs and histocompatibility types. They are the mouse common antigens (type I in Table 3). Antibodies to these antigens can be removed from the antiserum by adsorption with PM from either brain, liver or spleen.

Antibodies to the second class of antigens present on morphologically undifferentiated cells can be removed only by adsorption with particulate preparations from brain, and not by any other organs tested. By this criterion and also by direct binding studies, these are neural antigens (type II in Table 3).

Antibodies to the third class of antigens present on morphologically undifferentiated N18 cells are not adsorbed by any of the organs tested. These constitute type III and may be the result of cellular adaption to culture. They may be present only on the tissue (sympathetic nervous system) or cell of origin of the tumour. They might even be related to the malignant transformation of these cells and (or) to their associated A type virus particles[15]. These antigens are called cell-specific in Table 3.

Adsorption experiments demonstrated that morphologically differentiated N18 cells possess all these classes of antigens. In addition, such cells also have antigens related specifically to their morphological differentiation. Approximately half to two-thirds of the antibodies unique to morphologically differentiated cells are adsorbed only by PM from brain (type IV in Table 3). The remainder of the antibodies to antigens unique to morphologically differentiated cells are similar to the third class of antigens described here in that they are not adsorbed by any mouse organ or cell (including 10D-SF) except N18-SF (type V). We hope subcellular fractionation techniques and (or) tolerance inducing methods will facilitate the production of more specific antisera that may be used for detailed study of individual antigenic correlates of morphological differentiation of the murine neuroblastoma in culture.

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1 Seeds, N. W., Gilman, A. G., Amano, T., and Nirenberg, M. W., *Proc. natn. Acad. Sci. U.S.A.*, **66**, 160-167 (1970).
2 Richelson, E., *Nature new Biol.*, **42**, 175-177 (1973).
3 Yogeeswaran, G., Murray, R. K., Pearson, M. L., Sanwal, B. D., McMorris, F. A., and Ruddle, F. H., *J. biol. Chem.*, **248**, 1231-1239 (1973).
4 Glick, M. C., Kimki, Y., and Littauer, U. Z., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1682-1687 (1973).
5 Schachner, M., *Nature new Biol.*, **243**, 117-119 (1973).
6 Schubert, D., Humphreys, S., DeVitry, F., and Jacob, F., *Devl Biol.*, **25**, 514–546 (1971).
7 Harris, A. J., and Dennis, M. J., *Science*, **167**, 1253-1255 (1970).
8 Nelson, P. G., Ruffner, W., and Nirenberg, M. W., *Proc. natn. Acad. Sci. U.S.A.*, **64**, 1004-1010 (1969).
9 Peacock, S., Minna, J., Nelson, P. G., and Nirenberg, M. W., *Expl Cell Res.*, **73**, 367–377 (1972).
10 Schubert, D., Harris, A. J., Heinemann, S., Kidokoro, Y., Patrick, J., and Steinback, J. H., in *Tissue culture of the nervous system* (edit. by Sato, G.), (Plenum, New York and London, 1973).
11 Akeson, R., and Herschman, H. R., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 187–191 (1974).
12 Cline, M. J., Sprent, J., Warner, N. L., and Harris, A. W., *J. Immun.*, **108**, 1126-1128 (1972).
13 Kuff, E. L., Lueders, K. K., Ozer, H. L., Wivel, N. A., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 218–222 (1972).
14 Fahey, J. L., and Horbett, A. P., *J. biol. Chem.*, **234**, 2645-2651 (1959).
15 Greenwood, F. C., Hunter, W. H., and Glover, J. S., *Biochem. J.*, **89**, 114-123 (1963).
16 Levine, L., in *Handbook of experimental immunology* (edit. by Weir, D. M.), 707 (Blackwell, Oxford, 1967).

Age of early Acheulian industries from the Peninj Group, Tanzania

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Geophysical data strongly indicate that stone assemblages, which include bifaces and cleavers, from East Africa, are at least a million years old. Since these artefacts seem to be much older than any of their known counterparts in Europe, the question of the date of man's expansion from the tropics into cool, temperate regions is raised for discussion.

ONE of the most long lasting and widespread classificatory entities in human prehistory is the set of stone industries collectively known as Acheulian[1-3]. These industries are characterised, among other things, by the prominence of a series of tools variously called 'bifaces' or 'hand axes'. Representative assemblages are widely distributed; they occur in most regions of Africa, in southern and atlantic Europe, in the Levant and across into peninsular India. Isolated finds referable to the Acheulian have been reported from northern and central Europe, and from continental Eurasia, but north-east of the Alps, Caucasus, Zagros and Himalayan chains of mountains this kind of industry is conspicuously absent or at best rare and atypical.

With regard to age, it has long been known that the Acheulian industrial pattern was in practice through much of that rather vague segment of geological time classified as the 'Middle Pleistocene', though some of the last Acheulian assemblages to be made, belong in the early part of the 'Upper Pleistocene'. The upper and lower boundaries of the 'Middle Pleistocene' are not well fixed stratigraphically and evaluations of their age in years have been highly uncertain and variable from region to region. Consequently estimates of the time span represented by the Acheulian have also been varied and uncertain. In particular the relative age of early representatives of the industry from different regions has been in doubt.

We are reporting here new geophysical evidence from East Africa which provides strong indications of the chronometric age of early Acheulian assemblages in that subcontinent. The evidence raises very important questions with regard to inter-regional correlations and with regard to the timetable for changing patterns in the biogeography of early man. Although we raise these issues, their resolution must lie in part in the application of geophysical techniques to artefact-bearing sections other than those in East Africa.

The new data come primarily from a group of sedimentary and volcanic formations in Northern Tanzania, namely that of the Peninj, west of Lake Natron. The evidence consists of potassium-argon dates and palaeomagnetic determinations based on samples interstratified with artefact-bearing horizons. Although we are continuing to elaborate and check the evidence, the main features of the results seem sufficiently well established to warrant summary publication.

The Peninj Group

In an area about 50 miles north east of Olduvai, on the west side of Lake Natron a long sequence of sediments and volcanics has yielded a hominid fossil, mammal fossils, early Acheulian assemblages and geophysical evidence for dating. The regional sequence has previously been described[4,5] but is summarised here in Fig. 1 and Table 1. A series of K-Ar age determinations has been made by one of us (G.C.) and is also shown in Table 1.

Assemblages of early Acheulian artefacts were obtained by excavation from two localities in the northern sector of the 40 km strip over which the sedimentary sequence has been studied. The archaeological horizons were stratified within the Humbu Formation and in each case the artefacts closely overlie a major basaltic tuff unit.

One of the units dated by K-Ar, the 'Wa-Mbugu basalt' is an olivine basalt flow bedded within basaltic tuffs of the Humbu Formation some 15–20 km to the south of the sites. Another dated flow, the 'intra Moinik basalt', is stratified within the base of the overlying Moinik Formation in outcrops about 8 km to the north. Unless the stratigraphic boundary between the Humbu Formation and the Moinik Formation is markedly time-transgressive from north to south, these two units bracket the age of the Acheulian occurrences. Field relations and the nature of the contrast between the two units make time transgression seem unlikely. In any event, the two flows clearly are close in age to the age of the Acheulian industry. An australopithecine mandible was recovered from beneath the basaltic tuffs and is definitely older than both flows[6,7].

Potassium-argon age determinations

Table 1 shows the results of K-Ar and palaeomagnetic determinations made on various volcanic rocks from the West Natron succession. These K-Ar measurements were made by the standard techniques in use in the Berkeley K-Ar laboratory[8]. The palaeomagnetic results were obtained by T. Reilley working in Nairobi on samples collected by Isaac and Musset[9]. Although there is a fair degree of concordance between stratigraphy and K-Ar ages, the results do not show complete internal consistency

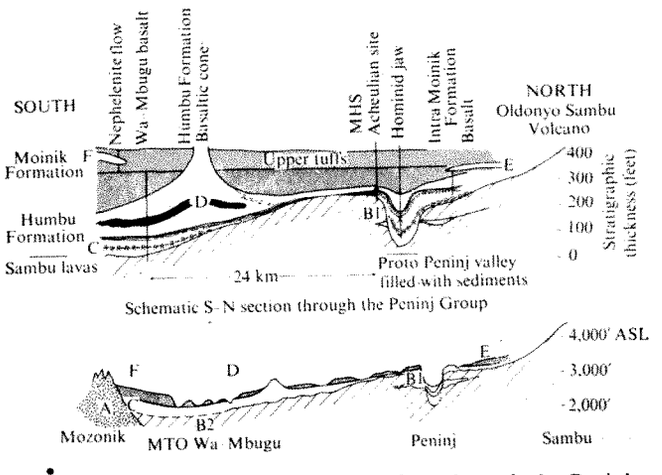


Fig. 1 Diagrammatic north-south sections through the Peninj Group in the West Natron area. The upper section shows the disposition of stratigraphic units without regard to topography. The lower section indicates topographic relations. Letters refer to lava flows listed in Table 1. (For a map see ref. 4, Fig. 1.)

Table 1 Potassium-argon and magnetic polarity determinations made on the succession of volcanic rocks in the West Natron area

Stratigraphic unit and rock type	Run number	K (wt %)	Radiogenic Ar[1]	Radiogenic Ar (%)	Apparent age × 10^6 (Myr)	Polarity	Degree of alteration
Moinik Formation							
(F) Nephelinite	KA 1814f	Grossly inconsistent with stratigraphy				R	
intra Moinik basalt	KA 2410	1.02	0.210	14.4	1.33 ± 0.05	?	5% zeolite
(E)	KA 2589	1.01	0.249	10.6	1.38 ± 0.09	?	
Humbu Formation							
(D) Wa-Mbugu olivine basalt (1964 sample)	KA 1754	0.729	0.200	26.0	1.55 ± 0.03	N	3–5% analcime
	KA 1754R	0.729	0.294	10.7	2.27 ± 0.06	N	
Wa-Mbugu olivine basalt (1969 samples)	KA 2382	0.812	0.181	5.7	0.96 ± 0.10	N	10–15% zeolite
	KA 2382R	0.678	0.220	2.1	1.21 ± 0.36	N	10–15% zeolite
	KA 2578	0.765	0.132	2.7	0.97 ± 0.25	N	10–15% zeolite
	KA 2646	0.750	0.161	6.9	1.21 ± 0.10	N	10–15% zeolite
(C) Hajaro basalt	Too altered for dating purposes					R	
Sambu lavas							
(B_1) Basalt from Peninj Gorge	KA 1755	0.949	0.321	15.4	1.90 ± 0.07	N	~2% zeolite
	KA 1755R	0.949	0.298	18.6	1.77 ± 0.04	N	
(B_2) Basalt from Malambo Gorge	KA 2570	0.579	0.361	4.9	3.50 ± 0.46	R	
Mozonik Volcanics							
(A) Biotite lava	KA 1757	6.86	3.88	18.4	3.18 ± 0.08	?R	
Results grossly inconsistent with stratigraphy:							
(F) Moinik Formation nephelinite	KA 1814	5.84	4.82	40.3	4.60 ± 0.07	R	
	KA 2250	6.27	2.99	49.4	2.68 ± 0.06	R	

and some interpretation is necessary. Palaeomagnetic data for several of the lava flows provide valuable control in assessing the reliability of the apparent ages.

R. L. Hay has carried out petrographic and X-ray diffraction examinations of several of the samples in order to assess the degree of alteration. Of the samples used in runs KA 2382, 2578 and 2646 in a letter, he reports as follows: "A little of the zeolite is isotropic (= analcime ?) but most is weakly birefringent. A diffractogram indicates a substantial amount of phillipsite which is presumably the birefringent zeolite". Hay's estimates of the overall percentage of zeolites are shown in Table 1. There are indications that the most heavily zeolitised lavas may be giving anomalously young ages.

Interpretation of the individual measurements must now be discussed. The oldest unit dated is the Mozonik Volcanics for which biotite flakes from tuffs give an age of 3.2 Myr (KA 1757). A lava flow elsewhere on Mount Mozonik showed reversed polarity and presumably part of the volcano's active life belonged in the Gilbert Reversed Epoch.

The Sambu lavas consist of a thickness of more than 1,000 feet of basalt flows apparently originating from Oldoniyo Sambu (Fig. 1). These lapped around the Mozonik volcanic centre. The flows have normal polarity except for thin localised flows at the very top of the succession. During an interval between a lower set and an upper set of flows, a major canyon was cut and filled with sediments. Under chronological scheme A in Fig. 2 the dates of 3.5 ± 0.46 Myr would be accepted and the dates of 1.9 and 1.77 Myr (KA 1755 and 1755R) for one flow in the upper set would be regarded as erroneous and the whole sequence assigned to the Gauss Normal Epoch. Under scheme B, the younger K-Ar results would be accepted as being consistent with an Olduvai event age. The first interpretation seems to be in better accord with the large scale of the formation.

The Wa-Mbugu basalt is bedded within the Humbu Formation at a level correlated with basaltic tuffs that overlie the australopithecine mandible and underlie the Acheulian industry. This single localised flow consistently showed normal polarity. A scatter of K-Ar apparent age values has been obtained, which seem to fall into two groups. A sample which showed only relatively slight alteration has given ages with a mean of 1.91 Myr (1754 and 1754R) whereas two samples with 10–15% of zeolite yield apparent ages between 0.96 and 1.21 Myr (2382, 2382R, 2578 and 2646). If the older dates are accepted an Olduvai Event age is implied, if the younger ones are accepted—a Jaramillo Event age.

Two runs on a sample of the intra Moinik Formation basalt flow have produced consistent results with a mean of 1.35 Myr (2410, 2589). Unfortunately the polarity of this unit is unknown since all samples collected turned out to have been struck by lightning.

In the southernmost exposures a nephelinite flow is stratified within the upper tuff complex of the Moinik Formation, well above the equivalent level of the artefacts. This flow has reversed polarity (Site MZN113 of Reilley[9]) but attempts at K-Ar dating have produced anomalous results—2.6 and 4.6 × 10^6 Myr. Nephelinites, in our experience and in that of other laboratories, frequently give unreliable results and attempts to date this rock have been abandoned. The polarity evidence is, however, of great importance in evaluating the other determinations. Since the nephelinite with reversed polarity is stratified above the Wa-Mbuga Basalt in the same sections, the normal polarity of the basalt cannot pertain to the Brunhes Normal Epoch but must belong to the Jaramillo or Olduvai Events, as implied also by the K-Ar results.

Figure 2 shows alternative chronologies for the Peninj Group. Alternative A involves acceptance of the date of 1.35 Myr for the intra Moinik basalt and the older set of ages for the Wa-Mbugu Basalt. Under this scheme the 1.77–1.9 Myr age for a Sambu lava flow must be rejected as stratigraphically inconsistent. Alternative B depends on rejection of the intra Moinik basalt date of 1.35 Myr and acceptance of the younger scatter of dates for the zeolitised Wa-Mbuga Basalt samples. We regard alternative A as more likely to prove correct.

While this paper was being revised, McIntyre, Mitchell and Dawson[10] reported K-Ar dates for volcanics from the area immediately to the south of the Natron basin. They conclude that major movements occurred along the Manyara-Natron

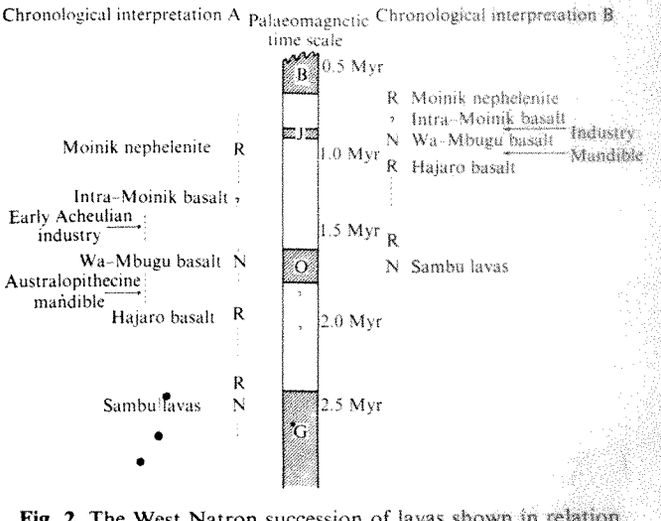


Fig. 2 The West Natron succession of lavas shown in relation to two alternative patterns of correlation of the available potassium-argon and palaeo-polarity data. Alternative A is favoured—see text.

Table 2 K-Ar or palaeomagnetic age determinations for Acheulian sites

Industry	Site	Apparent age × 10^6 (Myr)	Comments and Source
Late Acheulian	Kapthurin	<0.02	Ref. 24
Acheulian	Torre in Pietra	≤ 0.429	Mean of 7, ref. 8
Acheulian	Olorgesailie	0.425	Ref. 25
Lower Acheulian and Developed Oldowan	'Ubeidiya	≥ 0.64	Ref. 21
		≥ 0.68	
Acheulian	Kariandusi	0.928	Ref. 9
		0.946	
		1.1	
Lower Acheulian	Olduvai EFHR	~1.4	(palaeomagnetic) Ref. 16

This list is restricted to instances where the sample source is closely associated with the formation in which the sites are stratified. Several of these dates are problematic. For a general review see ref. 23.

fault between 1.15 and 1.2 Myr ago. This episode almost certainly corresponds to the fault dislocations which terminated the accumulation of the Humbu and Moinik Formations along the western flanks of the Natron basin. The new information lends strong support to chronological interpretation A.

Taken as a whole the geophysical data seem to be unambiguous in their indication of an age of the order of 1.0 to 1.5 Myr for the early Acheulian sites in the Humbu Formation. Table 2 lists other Acheulian occurrences, dated by K-Ar or polarity stratigraphy. The extreme paucity of data is apparent and it is for this reason that we have reported our results in spite of their lack of precision.

In addition to the K-Ar age determination there are a number of uranium decay series measurements on carbonates associated with Acheulian industries, for example those of the Riviera and the Casablanca region[11], the Afar[12] and Isimila in Tanzania[13]. Where these methods have given rise to finite age estimates they are less than 300,000 yr. In most instances the associated industry is a 'Middle' or 'Late' Acheulian, so that the uranium series dates do not at present contribute to the problem of estimating the age of the early Acheulian. Some geochemists have important reservations about regarding Th-U measurements on many materials, as 'dates'[14].

Implications

Whichever alternative chronology is adopted from Fig. 2, the age estimates for the Humbu Formation industries are very much greater than the values commonly entertained for the earliest Acheulian in the Mediterranean basin or Europe where estimates based on stratigraphic considerations commonly range between 300,000 and 500,000 yr. And yet the combined evidence of the various East African sections gives a very strong indication that at least some stone tool kits of Acheulian type began to be made in East Africa more than a million years ago. The Olduvai evidence reported by M. D. Leakey[15] and by Brock, Hay and Brown[16] is unambiguous in its demonstration that the earliest Acheulian assemblages are of Matuyama Reversed Epoch age (that is $\geq$ 0.7 Myr), and the K-Ar dates and stratigraphy of the Olduvai sections like the Natron section implies an age of between 1.0 and 1.6 Myr.

Until recently it seemed possible that the apparent discrepancy between East Africa and Europe was caused primarily by a tendency of European stratigraphers to undervalue the duration of the 'Middle Pleistocene', but recent palaeomagnetic work has resulted in the determination of the position of the Brunhes-Matuyama boundary in two important European Pleistocene sections—namely, those of the Netherlands and of Poland. Expressed in biostratigraphic and climate-stratigraphic terms, the boundary has been found by Mont-Frans to lie in the lower portion of strata classified as 'Cromerian' in the Netherlands[17] and Kukla has located it in the early Biharian of Poland[18]. The oldest stone tool assemblages which contain hand axes in Europe, however, are normally assigned to the 'Elster' (or Mindel) stratigraphic complex which succeeds the 'Cromerian'.

Evidence for hominid occupancy of Europe before Elster–Mindel times is very scanty with the allegedly late Villafranchian site of the Grotte du Vallonet near Nice constituting one of a very few important possible documents[19]. Another possible pre-Mindel occurrence is that of Abbeville, where hand axes were collected by D'Ault du Mesnil in 1875–96 (ref. 20). These may have been of late Cromerian age, but full stratigraphic information is not now available.

The 'Ubeidiya Formation contains what seem to be the oldest geophysically-dated artefacts in Eurasia. Lava flows believed to be stratigraphically above Acheulian and Developed Oldowan occurrences, have been dated at 0.64 and 0.68 Myr (ref. 21).

Palaeomagnetic stratigraphy has not yet been reported for such important Mediterranean basin sequences as those of Latamne (Syria), Ternifine (Algeria) or Sidi Abder Rahman (Morocco). The Indian sites are similarly unknown geochronological quantities, though new work in Java may soon throw light on the age of the apparently 'non-Acheulian' *Homo erectus* population there[22].

If, as seems likely, further work substantiates the present indications of an antiquity of a million years or more for the earliest East African Acheulian assemblages, and, if the apparent ages of less than half a million years are sustained for Europe, then understanding of the timing of developments in human cultural geography will have to be profoundly modified. It will be necessary to consider the possibility that hominids were essentially restricted to tropical and warm temperate zones for a much larger fraction of their evolutionary history than has hitherto been envisaged. For these questions to be resolved it is urgent that there be an intensification of efforts to secure chronometric determinations and palaeomagnetic data from Middle Pleistocene, artefact-bearing sedimentary sequences in areas outside East Africa.

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[1] Oakley, K. P., *Frameworks for Dating Fossil Man* (second ed.) (Aldine, Chicago, 1966).
[2] Leakey, L. S. B., *Adam's Ancestors* (Methuen, London, 1934).
[3] Bordes, B., *The Old Stone Age* (McGraw Hill, New York, Toronto, 1968).
[4] Isaac, G. Ll., *Quaternaria*, **7**, 101 (1965).
[5] Isaac, G. Ll., in *Background to Evolution in Africa* (edit. by Bishop, W. W., and Clark, J. D.) (University of Chicago Press, Chicago and London, 1967).
[6] Leakey, L. S. B., and Leakey, M. D., *Nature*, **202**, 3 (1964).
[7] Tobias, P. V., *Anthropologie*, **3**, 43 (1965).
[8] Evernden, J. F., and Curtis, G. H., *Curr. Anthrop.*, **6**, 343 (1965).
[9] Reilley, T., thesis, Trinity College, Dublin (1968).
[10] McIntyre, R. M., Mitchell, J. A., and Dawson, J. B., *Nature*, **247**, 354 (1974).
[11] Stearns, E. C., and Thurber, D. L., *Quaternaria*, **7**, 29 (1965).
[12] Roubet, C., *L'Anthropologie*, **73**, 503 (1969).
[13] Howell, F. C., Cole, G. M., Kleindienst, M. R., Szabo, B. J., and Oakley, K. P., *Nature*, **237**, 51 (1972).
[14] Broecker, W. S., and Bender, J. L., in *Calibration of Hominoid Evolution* (edit. by Bishop, W. W., and Miller, J. A.) (Scottish Academic Press, Edinburgh, 1972).
[15] Leakey, M. D., *Nature*, **232**, 380 (1971).
[16] Brock, A., Hay, R. L., and Brown, F. H., *Abstracts with Programs* (Geological Society of America, 1972).

[17] Montfrans, H. M. van, *Earth Planet. Sci. Lett.*, **11**, 226 (1971).
[18] Kukla, J., *Geol. För. Stockh. Förh.*, **92**, 148 (1970).
[19] Lumley, H. de, *C. r. hebd. Séanc. Acad. Sci., Paris*, **256**, 4261 (1963).
[20] Howell, F. C., in *Recent Studies in Paleoanthropology* (edit. by Clark, J. D., and Howell, F. C.), *Spec. Publs Amer. Anthrop.*, **68**, (1966).
[21] Horrowitz, A., Siedner, G., and Bar-Yosef, O., *Nature*, **242**, 186 (1973).
[22] Jacob, T., *Antiquity*, **182**, 148 (1972).
[23] Isaac, G. Ll., in *Calibration of Hominoid Evolution* (edit. by Bishop, W. W., and Miller, J. A.) (Scottish Academic Press, Edinburgh, 1972).
[24] Bishop, W. W., in *The Late Cenozoic Glacial Ages* (edit. by Turekian, K.) (Yale University Press, New Haven, 1971).
[25] Miller, J. A., in *Background to Evolution in Africa* (edit. by Bishop, W. W., and Clark, J. D.) (University of Chicago Press, Chicago, 1967).

Mechanism of action of the toxic lectins abrin and ricin

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The mechanism of action of the plant toxins abrin and ricin is described. The toxins inhibit protein synthesis probably by inactivating the 60S ribosomal subunits, interfering with chain elongation. Only eukaryotic ribosomes are attacked by the toxins.

PROTEINS toxic to animal cells are widely distributed but in only a few cases is their mechanism of action at the cellular and molecular level known in detail. Recently, considerable progress has been made in work on the toxic proteins abrin and ricin, whose mechanism of action is now rather well understood. The facts learned about the binding of abrin and ricin to cell receptors and their subsequent uptake into cells is of considerable general interest, since recent evidence suggests that similar mechanisms may apply in the case of other toxic proteins and possibly also in the case of some protein hormones and growth factors.

Abrin and ricin, which are present in the seeds of *Abrus precatorius L.* (Leguminosae) and *Ricinus communis* L. (Euphorbeaceae) respectively, have been known since the end of the last century (for review see ref. 1). Previously it was thought that the toxic action of these proteins in animals is due to the strong haemagglutinating properties of abrin and ricin preparations. More recent work has shown, however, that the agglutinating activity is associated with closely related, nontoxic agglutinins present in the same seeds[2-7] and that the highly purified toxins possess little or no agglutinating ability. Abrin and ricin have been reported to be more toxic to malignant than to normal cells and attempts to treat cancer in animals and man with abrin and ricin have been reported[8].

Structure

Abrin and ricin are glycoproteins with molecular weights of 60,000 to 65,000 (refs 2, 4, 7, 9–11) (Fig. 1). The whole toxins move as single bands in polyacrylamide gels in the presence of sodium dodecyl sulphate, but on treatment with 2-mercaptoethanol, they are split into two peptide chains[5,6,9-11], moving at rates corresponding to molecular weights between 30,000 and 35,000 (Table 1). Thus, the toxins consist of an A and B chain, joined by SS bonds (Fig. 1). We have separated the two peptide chains in their native form by ion exchange chromatography[5,6]. The isolated peptides thus obtained are not contaminated to any significant extent with the other chain. If a mixture of the isolated A and B chains is dialysed to remove 2-mercaptoethanol, the disulphide bridges between the two chains are reformed (S.O., A. M. Pappenheimer, and A. A. Harper, in preparation). The reconstituted proteins are almost as toxic as the native proteins (Table 2), demonstrating that the peptide chains do not lose their activity during the separation. Hybrid proteins consisting of the A chain of abrin and the B chain of ricin and *vice versa*, were also prepared (S. O., A. M. Pappenheimer, and A. A. Harper, in preparation). Such hybrids have toxicities close to those of the native toxins (Table 2).

Both toxins consist of a neutral and an acidic peptide chain (Table 1). In the case of abrin, the A chain is acidic, whereas in ricin the B chain has the low *p*I value. In both toxins the

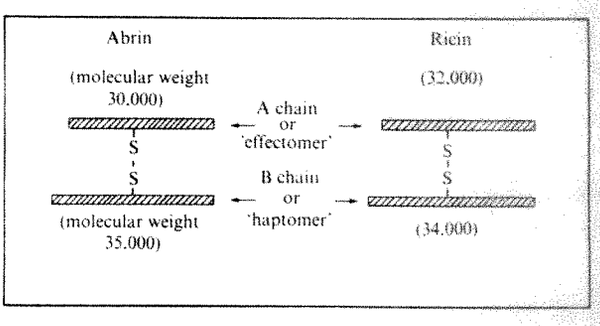


Fig. 1 Schematic structure of abrin and ricin.

B chains contain most of the carbohydrates (Table 1).

The structure and properties of abrin and ricin are surprisingly similar although they come from the seeds of two unrelated plants. Although the toxins are immunologically different (refs 5, 6 and 12 and S. O., K. R., A. P., and T. Christensen, in preparation) and show differences in their amino-acid composition (in preparation) the many similarities suggest that the toxins descend from a common ancestor molecule.

Effect *in vivo*

Abrin and ricin are extremely toxic to animals and man. After intraperitoneal administration the LD_{50} doses in mice were 40 ng and 100 ng for pure abrin and ricin, respectively[5,6]. These very small doses lead to death in the course of a few days, whereas larger doses have a more rapid effect. But animals never die earlier than 8–10 h after intraperitoneal administration of toxin. In animals intoxicated with large doses, haemorrhages in the viscera and serous cavities are found, whereas in animals treated with smaller doses multifocal necrosis develops in the parenchymous organs and in the germinal centres of the lymphatic organs[13]. Previous work revealed little or no change in oxygen consumption and energy metabolism, and the serum concentration of various metabolites studied remained unchanged in the early stage of intoxication[14].

Table 1 Physical properties of abrin, ricin and their constituent peptide chains

Toxin	Molecular weight*	*p*I†	Carbohydrate content‡
Abrin	65,000	6.1	—
Abrin A chain	30,000	4.6	0
Abrin B chain	35,000	7.2	7.4
Ricin	65,000	7.1	—
Ricin A chain	32,000	7.5	2.4
Ricin B chain	34,000	4.8	6.5

*Measured by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate[5].
†Measured by isoelectrofocusing (S. O., K. R., A. P., and T. Christensen, in preparation).
‡In % (w/w). Measured by gas chromatography (S. O., K. R., A. P., and T. Christensen, in preparation).

The selective inhibition of protein synthesis by abrin and ricin was first shown by Lin *et al.*[14,15] and our data in Fig. 2 confirm their experiments. It is shown that when 1-ml samples of Ehrlich ascites cells were treated with 0.1 μg of the toxins, the incorporation of ^{14}C-leucine into acid-precipitable material decreased after a lag time of about 30 min and stopped after 2 h (Fig. 2*a*). The incorporation of labelled thymidine was also inhibited by this concentration of the toxins (Fig. 2*b*), although after a longer lag time. On the other hand, the incorporation of ^{14}C-uridine was only slightly inhibited by 0.1 μg ml^{-1} of the toxins (Fig. 2*c*).

Different functions of the two peptide chains

The toxic effect *in vivo* seems to depend on the intact structure of the toxins[16]. Thus, we found that the isolated peptide chains had only ~ 1% of the inhibiting effect on protein synthesis in cells compared with that of the intact toxins (data not shown). A similar relationship was found when the toxic activity was tested in living animals (Table 2). A mixture of the two chains (reduced toxins) had the same low effect as isolated A or B chains. As pointed out above, reconstituted toxins or hybrid proteins show about the same toxicity as native abrin and ricin. (S. O., A. M. Pappenheimer, and A. Harper, in preparation).

Table 2 Biological properties of abrin, ricin and their constituent peptide chains

	Toxicity	Inhibition of cell-free protein synthesis*	Indirect haemagglutination†
	LD_{50} (μg)‡	Amount giving 90% inhibition (i μg ml^{-1}§	Amount giving visible agglutination (i μg ml^{-1})
Abrin	0.04	1.00	0.05
Abrin A chain	> 10.00	0.01	1.00
Abrin B chain	> 10.00	5.00	0.05
Abrin (reduced)¶	> 10.00	0.02	—
Abrin (reconstituted)‖	0.15	—	—
Ricin	0.10	1.00	0.04
Ricin A chain	> 10.00	0.01	5.00
Ricin B chain	> 10.00	3.00	0.04
Ricin (reduced)¶	5.00	0.03	—
Ricin (reconstituted)‖	0.20	—	—
Hybrid (abrin A chain/ricin B chain)‖	0.15	—	—
Hybrid (ricin A chain/abrin B chain)‖	0.30	—	—

* The cell-free system (0.5 ml) was an unfractionated rabbit reticulocyte lysate to which was added salt solution, an energy donating system, ^{14}C-leucine and the 19 other (unlabelled) amino acids. The system is essentially that of Lingrel as described elsewhere[21].
† Washed human erythrocytes (blood group B$^-$) were mixed with toxins or their peptide chains in 1 ml of 10 mM Tris-HCl (*p*H 7.5), 0.14 M NaCl for 60 min at 20° C. Then 5 μl of antiabrin or antiricin was added and the samples were observed under the microscope after 9 h. Conditions as described elsewhere[5,6].
‡ The amount giving 50% death when injected intraperitoneally into mice as described elsewhere[5,6].
§ Measured after 60 min incubation at 30° C.
¶ Pretreated with 1% 2-mercaptoethanol overnight at 4° C.
‖ Equimolar amounts of A chain and B chain were mixed and dialysed to remove 2-mercaptoethanol and reoxidise the disulphide bridge as described elsewhere (S. O., A. M. Pappenheimer, and A. Harper, in preparation).

The results indicate that only molecular species consisting of A and B chains joined by disulphide bridges have toxic effects on intact cells and living animals.

When the effect on protein synthesis of abrin, ricin and their constituent peptide chains was studied in a cell-free system from rabbit reticulocytes, we obtained results which in several respects were opposite to those obtained with intact cells[5,6,16]. Thus, reduced toxins, consisting of a mixture of A and B chains were much more potent than the intact toxins (Table 2). Experiments with the isolated chains showed that the inhibitory effect on protein synthesis is associated with the A chain of the toxins, whereas the isolated B chains were comparatively inactive. The results suggest that when the A chain is bound with a disulphide bridge to the B chain, it is prevented from inhibiting protein synthesis in a cell-free system. Possibly, the moderate inhibition observed when intact toxin is added (Table 2) may be accounted for by the fact that the cell-free protein

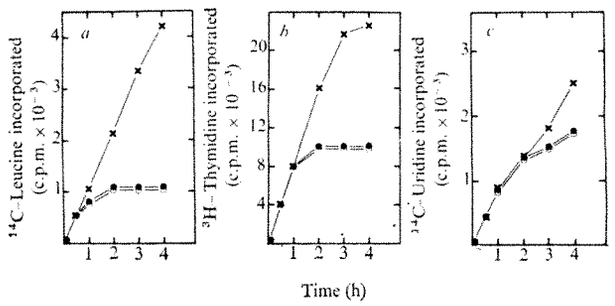


Fig. 2 Effect of abrin and ricin on the synthesis of protein (*a*), DNA (*b*), and RNA (*c*) in Ehrlich ascites cells *in vitro*. Abrin and ricin were extracted and purified to homogeneity as described elsewhere[5,6]. To 1-ml samples of Ehrlich ascites cells, washed and suspended in incubation medium as described elsewhere[19], were added 0.5 μCi ^{14}C-leucine (specific activity 331 Ci mol^{-1}), 10 μCi methyl-^{3}H-thymidine (specific activity 24.5 Ci mol^{-1}) or 0.5 μCi ^{14}C-uridine (specific activity 505 Ci mol^{-1}) as indicated. The samples were incubated at 37° C, aliquots were removed after different times and the acid-precipitable radioactivity was measured as described elsewhere[19] with the exception that in the case of (*c*), the aliquots were poured directly into cold 5% trichloroacetic acid rather than pretreated with 0.1 N NaOH, ×, Control; ○, 0.1 μg abrin; ●, 0.1 μg ricin.

synthesising system contains a low concentration of 2-mercaptoethanol capable of liberating small amounts of A chain.

Since a complete toxin consisting of both A and B chains is required for activity on whole cells, it is clear that the B chain must have a specific function. Our recent experiments indicate that the B chain is necessary for binding the toxins to the cell surface[5,6]. In indirect haemagglutination experiments, where erythrocytes, pretreated with isolated B chain (or intact toxin), were exposed to an antiserum against the toxin (Table 2), the cells agglutinated, indicating that the antibody molecules form bridges between the cells with anchor points in the B chains or intact toxin molecules bound to the cell surface. Under similar conditions, pretreatment of the cells with A chain did not result in agglutination with antiserum, and we have been able to demonstrate this in a more direct way. Sepharose particles possessing covalently bound intact toxins or isolated B chains will bind erythrocytes, whereas particles with bound A chains will not (Fig. 3).

Binding to surface receptors

The B chains of the toxins probably bind to galactose-containing receptors on the cell surface. We found that the haemagglutination by antisera of erythrocytes pretreated with B chains could be inhibited in the presence of galactose or lactose[7]. Furthermore, in the presence of lactose no erythrocytes became bound to Sepharose particles containing covalently bound toxins (Fig. 3). Also, the ability of abrin and ricin to inhibit protein synthesis in Ehrlich ascites cells is strongly reduced

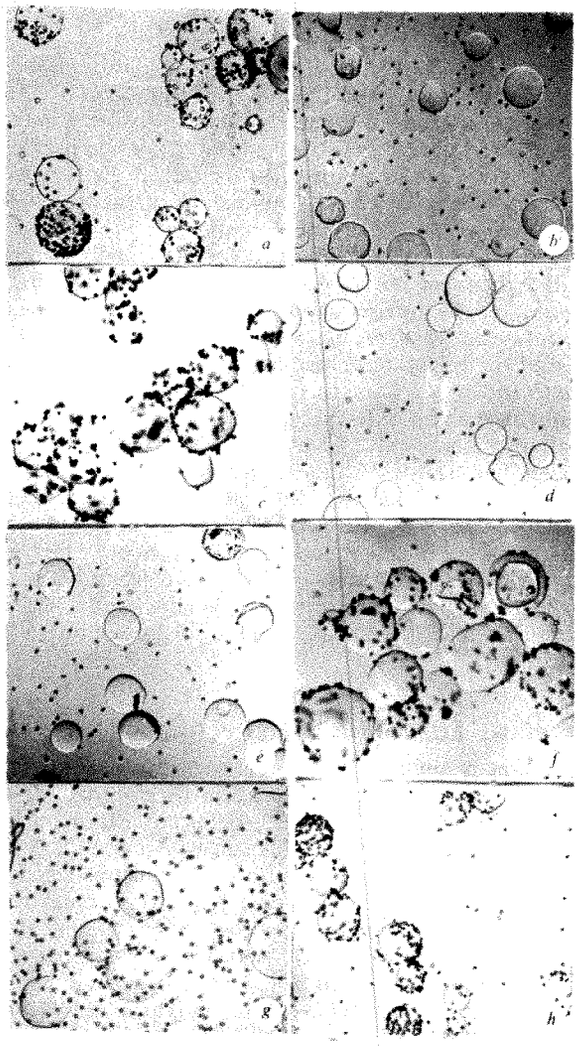


Fig. 3 Binding of erythrocytes to Sepharose particles with covalently bound toxins or B chains. Abrin and ricin or their constituent peptide chains were linked covalently to cyanogen bromide-activitated Sepharose particles, (Pharmacia, Uppsala, Sweden) 10 mM lactose was present during the binding procedure to prevent the toxins from becoming bound by their specific binding sites to galactose residues of Sepharose. Sepharose was washed several times at high and low *p*H and with 0.1 M lactose to remove unbound material. It was then washed extensively with 0.1 M sodium phosphate (*p*H 7.1) containing 0.14 M NaCl and subsequently it was resuspended in the same buffer. One drop was mixed with about 10^7 human erythrocytes and mixed carefully by gentle shaking. Where indicated, 10 mM lactose was added. The mixture was examined under the microscope. *a*, Abrin; *b*, abrin (lactose); *c*, ricin; *d*, ricin (lactose); *e*, abrin A chain; *f*, abrin B chain; *g*, ricin A chain; *h*, ricin B chain.

when lactose is present in the culture medium (Fig. 4). In the presence of 10 mM lactose about twice as much abrin was necessary to reduce the protein synthesis by 50% as when lactose was omitted, and in the case of ricin about 30 times the concentration of toxin was required to obtain the same toxic effect as in the absence of lactose. Similar data have been obtained with ricin by other authors[17]. Evidence that only the B chain is involved in the binding of the toxins to galactose was obtained in experiments where we applied isolated chains of the toxins to a column of Sepharose 4B (which contains galactose residues). In this case the A chain passed through the column, whereas the B chain was retained and could be eluted with galactose[6]. The ability of the toxins to bind to Sepharose columns has been utilised in several laboratories for the purpose of purification of the toxins[5,6,10,17].

The toxins seem to have one binding site for galactose-containing carbohydrates, whereas the nontoxic agglutinins present in the same seeds have two binding sites[7]. Thus we found in equilibrium dialysis experiments that each toxin molecule binds one molecule of lactose, whereas the agglutinins bind two lactose molecules per molecule. Abrin and ricin may therefore be considered as monovalent lectins. The binding constants were 8×10^3 l mol^{-1} (abrin) and 1.5×10^4 l mol^{-1} (ricin).

On the surface of different cells about 1×10^7 to 2×10^7 sites to which the toxins can bind seem to be present[17,18]. The presence of a higher number of sites on the surface of malignant cells than on their normal counterparts may in part explain why abrin and ricin seem to be more toxic to malignant than to normal cells[18].

Penetration of the toxins into cells

Since the B chains seem to bind the toxins to the cell surface we shall call this chain the 'haptomer' (Fig. 1). The A chain which carries the toxic activity, is denoted as the 'effectomer'. It is likely that after the toxins are bound to the cell surface by the haptomer, the A chain (or possibly the whole toxin) is transported into the cytoplasm to its site of action, the ribosomes (see below). It should be noted that whereas in a cell-free system protein synthesis is inhibited immediately after addition of the toxins[5], we always found a lag of at least 30 min (at 37° C) when cells in culture were treated with the toxins, even

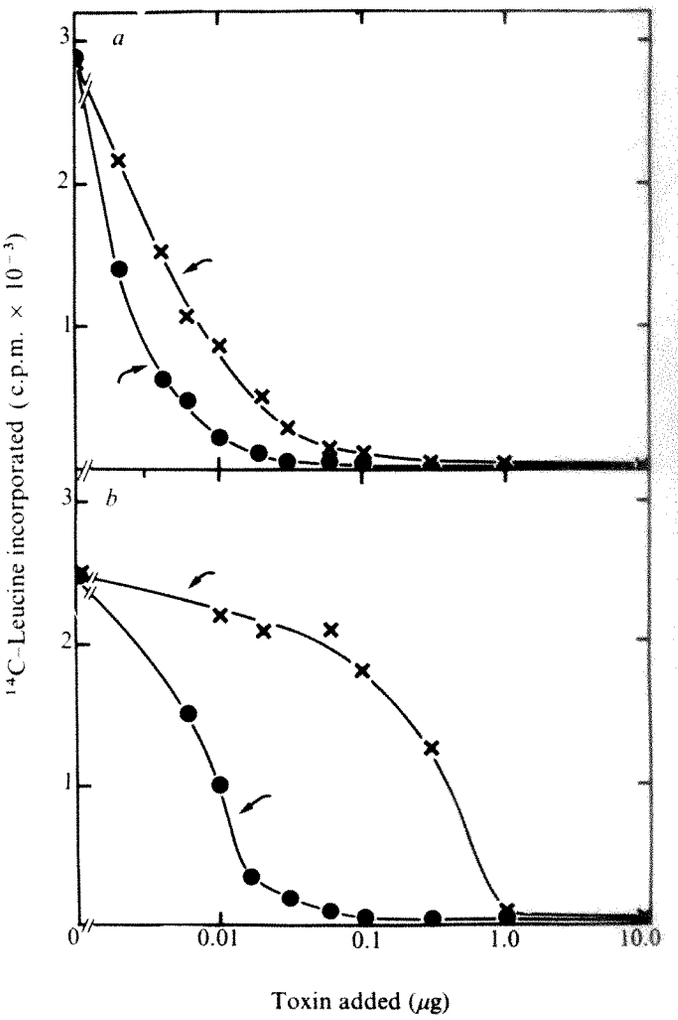


Fig. 4 Effect of lactose on the ability of abrin (*a*) and ricin (*b*) to inhibit protein synthesis in Erhlich ascites cells. To 1-ml samples of a solution of Ehrlich ascites cells were added increasing amounts of toxin as indicated in the absence (●), and presence (×) of 10 mM lactose. The samples were incubated as in Fig. 2*a* and after 1 h, 0.5 μCi of ^{14}C-leucine was added to each tube. The samples were further incubated for one more hour. Then aliquots were removed from each sample and the acid-precipitable radioactivity was measured.

Table 3 Protective effect of antitoxins added to Ehrlich ascites cells at different times after abrin and ricin

Time of toxin interaction with the cells before addition of antitoxin min	Inhibition of protein synthesis Abrin %*	Ricin %*
0	0	0
5	33	11
10	67	44
30	92	78
60	100	100

To 1 ml samples of a suspension of Ehrlich ascites cells prepared as in Fig. 2*a* was added 1.0 μg of abrin and ricin. After incubation at 20° C for the indicated periods of time, a neutralising amount of antiabrin and antiricin was added. The samples were incubated at 20° C for a total of 60 min, then the incubation was continued at 37° C for 2 h to enable the effect of the toxins to develop (see Fig. 2). ^{14}C-Leucine (0.5 μCi) was then added and the acid-precipitable radioactivity was measured as in Fig. 2*a*.

* Percentage reduction in radioactivity incorporated (control: 4,500 c.p.m.).

when the toxin concentration of the medium was as high as 100 μg ml^{-1} (ref. 19). This lag is greater at lower temperatures (being 6 h at 20° C). Since the binding of the toxins to the cell surface seems to be rapid, in seems that the transportation of the toxin (or the free A chain) into the cytoplasm accounts for most of the lag. Our finding (Table 3) that the toxic effect can be counteracted by antitoxins only during the first 30 min of the 6 h lag at 20° C, indicates that during the remaining 5.5h the toxins are not exposed on the outside of the cells.

They are possibly engulfed by endocytotic vesicles from which they are slowly liberated into the cytoplasm. It is generally believed that such vesicles merge with lysosomes. Our recent finding that the toxins are extremely resistant to proteolytic enzymes may be significant in this respect (S. O., K. R., and A. P., in preparation). The view that the cellular uptake of abrin and ricin involves endocytosis is supported by our finding that these toxins are unable to inhibit protein synthesis in reticulocytes which have little or no endocytotic activity[19], even though the toxins bind readily to the surface of these cells. It is interesting to note that many viruses are taken up by a similar mechanism in that they bind to specific receptors on the surface of susceptible cells (viropexis) and are then transported into the cytoplasm by endocytotic activity.

Mechanism of protein synthesis inhibition

The A chains of abrin and ricin have an immediate inhibitory effect on protein synthesis in a cell-free system from rabbit reticulocytes[5,6]. To see whether abrin and ricin act on the ribosomes or on some soluble factors, we fractionated the lysate into ribosomes and a postribosomal supernatant, which were incubated separately with and without A chains from abrin and ricin[20]. After incubation, the toxins were inactivated by addition of sufficient amounts of specific antitoxins. Subsequently, the ribosomes and the supernatants were recombined and a cell-free protein synthesising system was prepared. When the ribosomes had been pretreated with the toxins, virtually no incorporation of labelled amino acids into protein occurred, whereas pretreatment of the supernatant did not inhibit the amino acid incorporation. The fact that abrin and ricin inactivated isolated ribosomes indicate that a cofactor is not necessary. Our results indicate that only the eukaryotic type of ribosome is susceptible to the toxins, as protein synthesis in a cell-free system from *Escherichia coli*[20] was not reduced, whereas the incorporation of labelled amino acids into proteins in a cell-free system from rat liver[20] and wheat germ (our unpublished work) was strongly inhibited.

Not only protein synthesis directed by natural messengers, but also poly(U)-directed polymerisation of labelled phenylalanine, is inhibited[21]. The inhibition was strongest in the presence of a low concentration of Mg^{2+} ions (4 mM) and could be partly overcome by increasing the concentrati (our unpublished data).

In a cell-free system the toxins seem to inhibit the elongation of already initiated peptide ch sucrose gradient centrifugation we found that whe of new peptide chains was inhibited, the polysom preserved if the incubation was carried out in th the toxins and that the toxins prevented the runoff ordinarily observed under such conditions[21,22].

When the ability of isolated ribosomes to suppo tion of radioactive puromycin into acid-precipit was measured, no difference was found whether or ribosomes had been preincubated with the tox published results). Similar findings were made by *et al.*[23] and by L. Carasco (personal communi data obtained so far thus indicate that the toxins d formation of the peptide bond.

Recent findings of Montanaro *et al.*[23] indica inhibits the ribosome-linked GTP hydrolysis catal and Sperti *et al.*[24] have obtained evidence that the 6 subunit is the site of action of ricin. Data from our tory indicate that abrin also inactivates the 60S s likely therefore that the toxins act by modifying t somal subunits in some way, rendering them un with EF2.

Enzymatic activity of the effectomer

The minimum amount of abrin and ricin nece HeLa cells in culture was estimated to be less th molecules per cell (unpublished data). Since cells contain about 10^5 to 10^6 ribosomes and sinc that a substantial fraction of the ribosomes must b to kill the cells, we inferred that the toxins s catalytically[16]. This view is supported by our fin cell-free systems thousands of ribosomes were inact 30 min by one toxin molecule[5,6,16]. Our subsequent the inactivation of ribosomes by the toxins is a time ture-dependent process is also consistent with the toxins possess enzymatic activity[20]. The possibi A chains of the toxins activate an enzyme which the the ribosomes, is unlikely since the A chains inacti ribosomes in simple buffer solutions[20]. It is not kn the toxins are specific proteases or specific RNases eukaryotic ribosomes in a similar way as colicin E bacterial ribosomes (for review see ref. 1).

Implications

The finding that the toxic plant proteins abrin and of a haptomer moiety responsible for the binding to surface receptors of susceptible cells and an eff responsible for the specific toxic action, seem to be able interest. Thus, it has prevoiusly been shown tha toxin consists of an enzymatically active A chain wh protein synthesis by inactivating the elongation and a B chain which binds the toxin to specific surface of susceptible cells (for review see ref. 25 is now accumulating that cholera toxin[26], botul and tetanus toxin[28] may similarly consist of a hapto effectomer moiety. Also it seems possible that endocytosis after specific binding to the cell surf widespread in nature. It is an interesting possibilit and ricin pass the cell membrane by routes originall for the uptake of physiological proteins, like certai and growth factors.

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1 Olsnes, S., *Naturwissenschaften*, **59**, 497 (1972).

[2] Ishiguro, M., Takahashi, T., Funatsu, G., Hayashi, K., and Funatsu, M., *J. Biochem.*, **55**, 587 (1964).
[3] Khan, A. H., Gul, B., and Rahman, M.A., *J. Immun.*, **96**, 554 (1966).
[4] Waldschmidt-Leitz, E., and Keller, L., *Hoppe-Seyler's Z. physiol. Chem.*, **350**, 503 (1969).
[5] Olsnes, S., and Pihl, A., *Eur. J. Biochem.*, **35**, 179 (1973).
[6] Olsnes, S., and Pihl, A., *Biochemistry*, **12**, 3121 (1973).
[7] Olsnes, S., Saltvedt, E., and Pihl, A., *J. biol. Chem.* (in the press).
[8] Tung, T.-C., Hsu, C.-T., and Lin, J.-Y., *J. Formosan med. Ass.*, **70**, 569 (1971).
[9] Funatsu, M., Funatsu, G., Ishiguro, M., and Nanno, S., *Jap. J. med. Sci. Biol.*, **23**, 264 (1970).
[10] Nicolson, G. L., Blaustein, J., and Etzler, M. E., *Biochemistry*, **13**, 196 (1974).
[11] Wei, C. H., Hartman, F. C., Pfuderer, P., and Yang, W.-K., *J. biol. Chem.* (in the press).
[12] Ehrlich, P., *Collected papers* (edit. by Dale, H.), **2**, 21 (Pergamon, London, 1957).
[13] Waller, G. R., Ebner, K. E., Scroggs, R. A., Das Gupta, B. R., and Corcoran, J. B., *Proc. Soc. exp. Biol. Med.*, **121**, 685 (1966).
[14] Lin, J.-Y., Kao, W.-Y., Tserng, K.-Y., Chen, C.-C., and Tung, T.-Ch., *Cancer Res.*, **30**, 2431 (1970).
[15] Lin, J.-Y., Liu, K., Chen, C.-C., and Tung, T.-C., *Cancer Res.*, **31**, 921 (1971).
[16] Olsnes, S. and Pihl, A., *FEBS Lett.*, **21**, 48 (1972).
[17] Kaneko, I., Satoh, H., and Ukita, T., *Biochem. biophys. Res. Commun.*, **50**, 1087 (1973).
[18] Nicholson, G. L., and Lacorbiere, M., *Proc. natn. Acad. Sci. USA*, **70**, 1672 (1973).
[19] Refsnes, K., Olsnes, S., and Pihl, A., *J. biol. Chem.* (in the press).
[20] Olsnes, S., Heiberg, R., and Pihl, A., *Molec. Biol. Rep.*, **1**, 15 (1973).
[21] Olsnes, S., and Pihl, A., *FEBS Lett.*, **20**, 327 (1972).
[22] Olsnes, S., and Pihl, A., *Nature*, **238**, 459 (1972).
[23] Montanaro, L., Sperti, S., and Stirpe, F., *Biochem. J.*, **136**, 677 (1973).
[24] Sperti, S., Montanaro, L., Mattioli, A., and Stirpe, F., *Biochem. J.*, **136**, 813 (1973).
[25] Pappenheimer, A. M., jun., and Gill, D. M., *Science*, **182**, 353 (1973).
[26] Cuatrecasas, P., Parikh, I., and Hollenberg, M. D., *Biochemistry*, **12**, 4253 (1973).
[27] Sugiyama, H., Das Gupta, B. R., and Yang, K. H., *Proc. Soc. exp. Biol. Med.*, **143**, 589 (1973).
[28] Craven, C. J., and Dawson, D. J., *Biochim. biophys. Acta*, **317**, 277 (1973).

Role of myosin light chains in calcium regulation

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The evidence that rabbit DTNB light chains bind to 'desensitised' scallop myosin and restore calcium sensitivity, that is, they functionally replace the scallop EDTA light chain, may suggest that these light chains are involved in some type of calcium regulatory mechanism in rabbit myosin in vivo.

In skeletal muscle, contraction is regulated by the interaction of calcium ions with the specific protein troponin, which is attached to the actin filaments[1]. In molluscan muscles[2] and in some primitive invertebrates[3], on the other hand, contraction is regulated by the interaction of calcium with myosin. Molluscan myosin preparations bind calcium and their ATPase activity in the presence of pure actin is calcium dependent[2]. The calcium dependence of the actin-activated ATPase activity of scallop myosin requires the presence of a specific light chain, called the EDTA light chain because it is released from myosin if the divalent cation concentration of the medium is lowered by the addition of EDTA[4]. The release of the light chain results in a loss of calcium sensitivity, that is, the myosin is 'desensitised' and the calcium requirement for the actin activated ATPase activity is abolished. Recombination of the EDTA light chain with the densensitised myosin in the presence of divalent cations restores calcium sensitivity, showing that the light chain functions as a regulatory subunit.

Comparative studies on the calcium regulatory systems of a wide variety of species[3] have revealed that the muscles of some invertebrate species contain both myosin-linked and actin-linked calcium regulation. These results prompted an investigation of a possible role in calcium regulation of the myosin light chains in other species, such as vertebrates, whose muscles seem only to show actin-linked calcium regulation.

The number, size and chemical characteristics of myosin light chains from a variety of vertebrate species and different muscle types are rather diverse[5–13] and, as yet, there is no unambiguous demonstration of the role of the subunits in the functions of the myosin molecule. Rabbit skeletal myosin, the most extensively studied, contains three light chain components and these may be divided, on the basis of chemical studies[14] into two distinct classes—(1) the so-called 'alkali' light chains[15] with molecular weights of 21,000 and 17,000 which have extensive sequence homology[16] and have been implicated in regulation of the enzymatic activity of the myosin[17,18] and (2) the DTNB light chain with a molecular weight of about 19,000, which is selectively released from myosin by treatment with 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB)[7,15]. In view of the evidence[14] that this DTNB light chain is not required for the hydrolytic activity of the myosin, since its selective removal by treatment with DTNB does not significantly alter the ATPase activity of the residual myosin, a possible role for this light chain in calcium regulation was investigated.

DTNB light chain and rabbit myosin

Treatment of rabbit skeletal myosin with 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB)[15], which blocks about 80% of the myosin thiol groups, leads to the selective release of 50–60%, (about 1 mol) of the DTNB light chains. Treatment with EDTA alone[4] does not remove the DTNB light chain. The loss of 1 mol of DTNB light chain does not significantly alter the properties of the residual myosin; it has little effect on the ATPase activity or on the ability of the myosin to interact with actin, and a calcium-sensitive complex is formed when this myosin is mixed with actin containing the calcium regulatory proteins troponin and tropomyosin. Similarly, if subfragment 1 is prepared from DTNB-treated myosin by papain cleavage[19], no intact DTNB light chains are detectable by polyacrylamide gel electrophoresis in the presence of SDS (Fig. 1). Double reciprocal plots of the actin-activated ATPase of this subfragment[20] yields a value for V_{max} (12 μmol ATP min^{-1} mg^{-1}) similar to that obtained with subfragment 1 prepared from untreated myosin. This subfragment also interacts with actin containing tropomyosin and troponin to form a calcium-sensitive complex. The functional significance of the DTNB light chain is therefore unclear and its presence is apparently not required for the normal biochemical functions of the

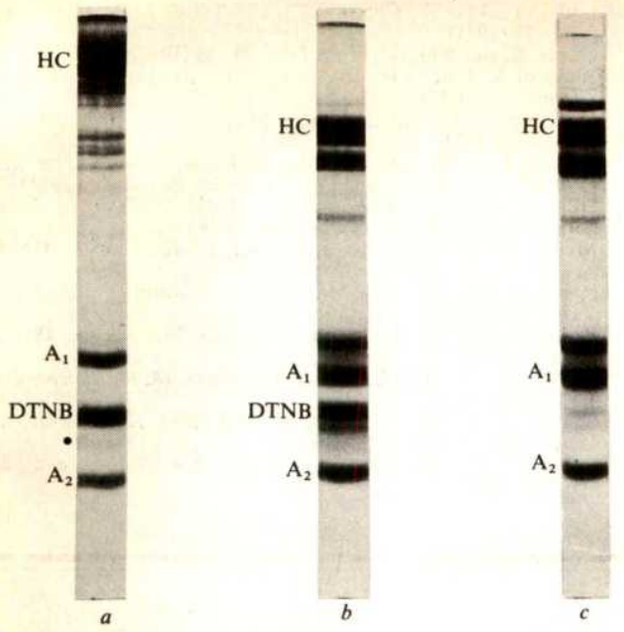


Fig. 1 10% SDS polyacrylamide gel electrophoresis[29] of: *a*, rabbit myosin; *b*, subfragment 1 prepared from untreated rabbit myosin; *c*, subfragment 1 prepared from DTNB-treated rabbit myosin. Buffer system used was 100 mM Tris bicine (*p*H 8.3), 0.1% SDS and the gels were stained with 0.5% Coomassie brilliant blue. HC, heavy chains of myosin and subfragment 1; A_1, 'alkali' 1 light chain; DTNB, DTNB light chain; A_2, 'alkali' 2 light chain.

myosin. It is difficult to accept that this light chain is a tightly bound contaminant of the myosin, especially in view of the stoichiometric amounts found in myosins from several species[10]. An alternative explanation is that the DTNB light chains in the intact muscle may have a function that has been lost during extraction and purification of the myosin; for example, it may, like the EDTA light chain in molluscan myosin, be involved in some type of calcium regulation. To explore this possibility, the interaction of the DTNB light chain with 'desensitised' molluscan myosin preparations was studied.

DTNB light chain and 'desensitised' molluscan myosin

Treatment of scallop myofibrils, actomyosin or myosin with 10 m M EDTA (ref. 4) leads to the release of 1 mol of the EDTA light chain and to a loss of calcium sensitivity, that is, the preparations are 'desensitised' and the calcium requirement for the actin-activated ATPase activity of the myosin is abolished. Rabbit DTNB light chains were added to preparations of desensitised scallop myofibrils, actomyosin and myosin and the mixtures were washed extensively to remove nonspecifically bound light chains. Polyacrylamide gel electrophoresis in the presence of SDS of these preparations (Fig. 2) shows that the DTNB light chain combined readily with these desensitised preparations. In the case of the desensitised myofibril and actomyosin preparations, the binding of the DTNB light chain always leads to a restoration of calcium sensitivity (Table 1) that is, the DTNB light chain can replace the scallop EDTA light chain. In the case of purified myosin preparations, however, the results are somewhat more complicated. Although the DTNB light chain always binds to the desensitised myosin preparations (Fig. 2), its ability to restore calcium sensitivity to these preparations is rather variable. The results in Table 2 illustrate that the DTNB light chain can in some cases, fully restore calcium sensitivity to the desensitised myosin. In certain other preparations, however, even though the DTNB light chain is bound, the myosin remains completely insensitive to calcium. These desensitised myosin preparations, however, become calcium sensitive when combined with the scallop EDTA light chain. This lack of calcium sensitivity when the DTNB light chain binds to certain desensitised myosin preparations must be due to rather subtle alterations in the structure of the myosin, and indicates that the DTNB light chain requires a rather more 'specific myosin configuration' than the EDTA light chain does, in order to bind in the correct position and restore calcium sensitivity. The reasons for the variability in resensitising myosin with the DTNB light chain are at present unclear. Since the desensitised myofibril and actomyosin preparations can always be resensitised by the addition of the DTNB light chain, the presence of actin in these preparations probably stabilises the structure of the desensitised myosin. We have no evidence to suggest that the DTNB light chain interacts with either the actin or tropomyosin in these preparations.

To measure directly the amount of DTNB light chain bound to the myosin in these desensitised preparations, the light chains were radioactively labelled by alkylating their thiol groups with ^{14}C-labelled iodoacetic acid. This treatment has no effect on the ability of the light chain to bind and to restore calcium sensitivity. Using these labelled DTNB light chains, it was found that calcium sensitivity was completely restored when about 1 mol of light chain is bound per myosin molecule, and the extent of restoration of calcium sensitivity correlates approximately with the amount of light chain bound (Tables 1 and 2). Only 1 mol of DTNB light chains is bound, even when a large excess of light chain is added. These values are consistent with our previous findings[21], that 1 mol of EDTA light chain is released when the myosin is desensitised by treatment with EDTA. These results and the fact that neither intact myofibrils nor myosin bind the DTNB light chain unless the EDTA light chain has been removed, further suggest that the DTNB light chain must bind at approximately the same position on the myosin heavy chains as the EDTA light chain.

Release of the EDTA light chain from scallop myofibril and myosin preparations decreases the amount of calcium bound by the myosin in these preparations by about 40%

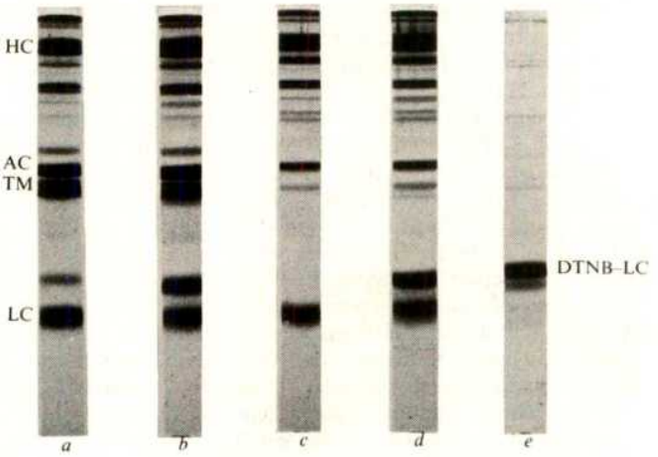


Fig. 2 10% SDS polyacrylamide gel electrophoresis demonstrating the binding of rabbit DTNB light chains to desensitised scallop myofibril and myosin preparations. *a*, Desensitised scallop myofibrils; *b*, desensitised myofibrils plus DTNB light chain; *c*, desensitised scallop myosin; *d*, desensitised scallop myosin plus DTNB light chain, *e*, DTNB light chain. The isolated rabbit DTNB light chain was added in large excess to the desensitised scallop preparations in 40 mM NaCl, 1 mM $MgCl_2$, 5 mM phosphate buffer (*p*H 7.0) and these preparations were washed extensively to remove the unbound light chains. HC, myosin heavy chains; AC, actin; TM, tropomyosin; LC, scallop light chains; DTNB-LC, rabbit DTNB light chain.

Table 1 Effect of the DTNB light chain on the ATPase activity of desensitised scallop myofibrils

^{14}C–DTNB light chain added (mol per mol myosin*)	^{14}C–DTNB light chain bound (mol per mol myosin*)	Actin-activated ATPase (μmol ATP mg^{-1} min^{-1} at 25° C) 0.1 mM EGTA	0.1 mM Ca^{2+}	Ratio EGTA/Ca^{2+}
Control	—	0.186	0.194	0.96
0.22	0.16	0.170	0.203	0.84
0.44	0.36	0.147	0.240	0.61
0.70	0.60	0.070	0.232	0.34
1.10	0.90	0.031	0.238	0.13
1.50	1.18	0.022	0.246	0.09
Native myofibrils	—	0.018	0.208	0.08

*Assuming that the myosin content of desensitised myofibrils is 65%, the molecular weight of the myosin is 425,000 and that of the DTNB light chain is 19,000.

The desensitised myofibrils were incubated for 10 min with increasing amounts of the ^{14}C-labelled DTNB light chain in 40 mM NaCl, 1 mM $MgCl_2$, 5 mM phosphate (*p*H 7.0) and washed four times with 10 volumes of the same solution. Aliquots were taken for ATPase measurements[4] and for liquid scintillation counting to determine the amount of light chain bound.

(ref. 4). Full calcium binding is regained when the EDTA light chain and the desensitised myosin are recombined (Table 3). When the DTNB light chain is combined with desensitised myofibril and myosin preparations, however, the amount of calcium bound is increased by only 10–20%. This rather lower figure raises the question, especially in view of the results in Table 1 and 2 which indicate that the DTNB light chain can fully restore calcium sensitivity to these preparations, whether there is a correlation between the restoration of calcium sensitivity and the amount of calcium bound. To answer this question, the ATPase activities and the amount of light chain bound by desensitised myofibrils were assayed after the calcium binding measurements. The results in Table 3 indicate that during our calcium binding procedure[4] there was a slight decrease in the amount of DTNB light chain bound (0.89 mol light chain bound per mol myosin) and a decrease in calcium sensitivity (compare Table 1). Therefore, when the DTNB light chain combined with desensitised myofibrils, the restoration of calcium sensitivity seems to parallel the increase in the amount of calcium bound by these preparations.

The DTNB light chain is tightly associated with scallop myosin, since it is not released by repeated reprecipitation or under mild dissociation conditions. The binding of light chain to myosin heavy chain also seems to be specific for the DTNB light chain, since the other types of light chains, that is, the rabbit 'alkali' light chains isolated from DTNB-treated myosin by treatment with guanidine hydrochloride[9] and purified by chromatography on DEAE-cellulose[14] do not bind and do not confer calcium sensitivity to these desensitised preparations. To confirm this observation, rabbit myosin was treated with 5 M guanidine hydrochloride[22] which dissociates the 'alkali' and DTNB light chains, and both types of light chains were added to a desensitised scallop myofibril preparation, which selectively binds solely the DTNB light chain and restores calcium sensitivity. Preliminary evidence indicates that this type of 'regulatory' light chain may be a common feature of all myosins, for example, cardiac, gizzard smooth, lobster and frog striated muscle myosins all contain a light chain component that behaves, with respect to desensitised scallop muscle preparations, like the rabbit DTNB light chain. (J. K.-J., E. M. Szentkiralyi and A. G. Szent-Györgyi, manuscript in preparation).

Scallop EDTA light chain and rabbit myosin

Since the rabbit DTNB light chain combined with desensitised scallop myosin, it was natural to enquire whether the scallop EDTA light chain crossreacts with DTNB-treated rabbit myosin. To answer this question, EDTA light chains were radioactively labelled by iodinating their tyrosine residues with ^{125}I (ref. 24) under very mild oxidising conditions. These ^{125}I-labelled light chains function normally with desensitised scallop myosin and bind to DTNB-treated rabbit myosin (about 0.6 mol light chain bound per mol of myosin) but they have no effect on the actin-activated ATPase activity of rabbit myosin when mixed with actin in the presence and absence of calcium, that is, the myosin remains calcium insensitive. Native rabbit myosin binds less than 0.1 mol labelled EDTA light chain per mol myosin, which suggests that the DTNB light chain and EDTA light chain binding sites on this myosin are probably the same or in the same region of the myosin heavy chains. Since the EDTA and DTNB light chains are interchangeable in their respective myosins one might have supposed that these light

Table 2 Desensitised scallop myosin and rabbit DTNB light chain

^{14}C–DTNB light chain added (mol per mol myosin*)	^{14}C–DTNB light chain bound (mol per mol myosin*)	Actin-activated ATPase (μmol ATP mg^{-1} min^{-1}) 0.1 mM EGTA	0.1 mM Ca^{2+}	Ratio EGTA/Ca^{2+}
Control	—	0.201	0.215	0.93
0.30	0.24	0.130	0.220	0.59
0.60	0.52	0.076	0.216	0.35
0.82	0.74	0.052	0.238	0.22
1.52	0.94	0.057	0.234	0.24
2.98	1.08	0.048	0.186	0.26
Native myosin	—	0.030	0.242	0.13

*Assuming that the molecular weight of scallop myosin is 425,000 and the DTNB light chain is 19,000.

Desensitised myosin was mixed with varying amounts of the light chain in 0.6 M NaCl, 1 mM $MgCl_2$ 10 mM phosphate (*p*H 7.0) and dialysed against 40 mM NaCl, 1 mM $MgCl_2$, 5 mM phosphate (*p*H 7.0). The precipitated myosin was washed extensively and then dissolved in 0.6 M NaCl (*p*H 7.0). For the ATPase measurements, the myosin was mixed with rabbit actin (2:1 w/w).

Table 3 Correlation between the restoration of calcium sensitivity and the calcium binding of scallop myofibrils

	Actin-activated ATPase (μmol ATP mg^{-1} min^{-1} at 25° C) 0.1 mM EGTA	0.1 mM Ca^{2+}	Ratio EGTA/Ca^{2+}	Calcium binding μmol Ca per g muscle at 10^{-6} M Ca^{2+}
Native myofibrils	0.020	0.281	0.07	2.05
Desensitised myofibrils	0.232	0.251	0.93	1.34
Desensitised myofibrils + scallop EDTA light chain	0.032	0.278	0.12	1.94
Desensitised myofibrils + DTNB light chain	0.093	0.262	0.35	1.62

Excess EDTA light chains and DTNB light chains were mixed with desensitised myofibrils as described in Table 1. The myofibrillar preparations were subjected to our calcium binding procedure[4], then assayed for ATPase activity[4] and the amount of bound DTNB light chain measured.

chains may have considerable chemical similarity. However, a comparison of their chemical structures as revealed by amino acid analyses and tryptic peptide maps[4,14] indicates no obvious sequence homology. The question of whether these different light chains have highly conserved regions, which may be the heavy chain binding sites, will therefore require more extensive knowledge of their amino acid sequences, which is now under investigation.

Implications

Our knowledge about the role of the DTNB light chain in rabbit myosin is extremely limited. The evidence that rabbit DTNB light chains bind to desensitised scallop myosin and restore calcium sensitivity, that is, they functionally replace the scallop EDTA light chain, is the first indication of a possible functional role of these light chains in rabbit myosin. It may be that these light chains are involved in calcium regulation in rabbit muscle or they may be remnants of a myosin-linked regulatory system that has lost its ability to respond to calcium, due to alterations in the heavy chains. To decide definitely whether vertebrate muscles have a myosin-linked calcium regulatory system similar to that found in molluscan muscles, one must establish directly that these myosins are able to respond to calcium. The available biochemical evidence, however, indicates that purified vertebrate myosins bind rather small amounts of calcium (at 10^{-6} M free calcium) in the presence of physiological levels of free magnesium, and show a calcium insensitive ATPase activity when mixed with pure actin[1,24]. This does not however, exclude the possibility that the myosin binds calcium *in vivo*, since it can be argued that during extraction and purification, this ability has been irreversibly lost. Some support for such a speculation may be provided by recent X-ray diffraction studies[25,26] on stretched frog muscles, which indicate but do not prove, that the myosin cross bridges may be able to respond to changes in the level of free calcium in the muscle. The evidence for and against some type of calcium control by the myosin in vertebrate muscles has been extensively discussed in a recent review of Weber and Murray[27], who conclude that the question of whether there is some type of control mechanism on the myosin in these muscles remains unresolved.

The evidence presented here indicates that the DTNB light chain and EDTA light chain seem to be interchangeable. Even if their overall functions are similar, that is, switching the myosin 'on' in response to the release of calcium during activation, we cannot exclude the possibility that the exact mechanisms involved maybe rather different. For example, in molluscan muscles, calcium released upon activation acts directly on the myosin[2], whereas in rabbit muscle, calcium may act indirectly on the myosin by activating a specific calcium requiring protein kinase[28], known to be able to phosphorylate selectively the DTNB light chain, and thus under *in vivo* conditions this light chain may be phosphorylated which may result in the myosin being switched 'on'[28].

To answer the questions of whether vertebrate myosins contain a regulatory control mechanism and what is the nature of such a mechanism, and to establish the role played by the DTNB light chains, therefore, requires further work specifically with the intact muscle.

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1 Ebashi, S., Endo, M., and Ohtsuki, I., *Q. Rev. Biophys.*, **2**, 351 (1969).
2 Kendrick-Jones, J., Lehman, W., and Szent-Györgyi, A. G., *J. molec. Biol.*, **54**, 313 (1970).
3 Lehman, W., Kendrick-Jones, J., and Szent-Györgyi, A. G., *Cold Spring Harbor Symp. quant. Biol.*, **37**, 319 (1972).
4 Szent-Györgyi, A. G., Szentkiralyi, E. M., and Kendrick-Jones, J., *J. molec. Biol.*, **74**, 179 (1973).
5 Locker, R. H., and Hagyard, C. J., *Archs Biochem. Biophys.*, **122**, 521 (1967).
6 Gaetjens, E., Bárány, K., Bailin, G., Oppenheimer, H., and Bárány, M., *Archs Biochem. Biophys.*, **123**, 82 (1968).
7 Gazith, J., Himmelfarb, S., and Harrington, W. F., *J. biol. Chem.*, **245**, 15 (1970).
8 Samaha, F. J., Guth, L., and Albers, R. W., *J. biol. Chem.*, **245**, 219 (1970).
9 Perrie, W. T., and Perry, S. V., *Biochem. J.*, **119**, 31 (1970).
10 Lowey, S., and Risby, D., *Nature*, **234**, 81 (1971).
11 Sarkar, S., Sreter, F. A., and Gergely, J., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 946 (1971).
12 Weeds, A. G., and Pope, B., *Nature*, **234**, 85 (1971).
13 Kendrick-Jones, J., *Phil. Trans. R. Soc. Lond.*, **B265**, 183 (1973).
14 Weeds, A. G., and Lowey, S., *J. molec. Biol.*, **61**, 701 (1971).
15 Weeds, A. G., *Nature*, **223**, 1362 (1969).
16 Weeds, A. G., and Frank, G., *Cold Spring Harbor Symp. quant. Biol.*, **37**, 9 (1972).
17 Stracher, A., *Biochem. biophys. Res. Commun.*, **35**, 519 (1969).
18 Dreizen, P., and Gershman, L. C., *Biochemistry*, **9**, 1688 (1970).
19 Lowey, S., Slayter, H. S., Weeds, A. G., and Baker, H., *J. molec. Biol.*, **42**, 1 (1969).
20 Eisenberg, E., Zobel, C. R., and Moos, C., *Biochemistry*, **7**, 3186 (1968).
21 Kendrick-Jones, J., Szentkiralyi, E. M., and Szent-Györgyi, A. G., *Cold Spring Harbor Symp. quant. Biol.*, **37**, 47 (1972).
22 Gershman, L. C., Stracher, A., and Dreizen, P., *J. biol. Chem.*, **244**, 2726 (1969).
23 Bray, D., and Brownlee, S. M., *Analyt. Biochem.*, **55**, 213 (1973).
24 Bremel, R. D., and Weber, A., *Nature*, **238**, 97 (1972).
25 Haselgrove, J. C., thesis, Univ. Cambridge (1970).
26 Huxley, H. E., *Cold Spring Harbor Symp. quant. Biol.*, **37**, 361 (1972).
27 Weber, A., and Murray, J. M., *Physiol. Rev.*, **53**, 612 (1973).
28 Perrie, W. T., Smillie, L. B., and Perry, S. V., *Biochem. J.*, **135**, 151 (1973).
29 Weber, K., and Osborn, M., *J. biol. Chem.*, **244**, 4406 (1969).

LETTERS TO NATURE

PHYSICAL SCIENCES

Structure of the visible Jovian clouds

DURING the 1972 apparition of Jupiter we studied hydrogen quadrupole line intensities at the centre of the planetary disk, by observing the S(1) lines of the H_2 (3–0) and (4–0) quadrupole bands which are respectively centred at 12268.8 and 15704.01 cm^{-1}. These observations show that the abundance of hydrogen detected at these frequencies is extremely variable because of rapid changes in the structure of the visible clouds, which are confirmed by photographs of the planet.

We observed with the Coudé spectrograph of the 61-cm (24-inch) telescope at the Table Mountain Observatory. Spectra were obtained at 1.6 Å mm^{-1} reciprocal dispersion in the (4–0) band and 2 Å mm^{-1} in the (3–0) band, and were recorded on photographic plates using a single stage S–25 image intensifier tube. The plates were traced with a digital microphotometer and the digital data were reduced to intensity tracings by a computer routine using conventional techniques of photometry. The photometric calibration was checked by measuring, on each tracing, the equivalent widths of several Fraunhofer lines selected to match approximately the intensities and frequencies of the Jovian H_2 lines. The measured intensities of the Fraunhofer lines were systematically low on the tracings and linear least-squares fits were used to correct the measured H_2 lines. The error estimates of one standard deviation (σ) computed from these correction processes are given with the individual measurements.

In Figs 1 and 2 we show the measured equivalent widths of the (3–0)S(1) and (4–0)S(1) hydrogen quadrupole lines as a function of the SYSTEM I longitude. The magnitude of the (4–0)S(1) equivalent widths (EW) varies from 4.2 ± 0.7 to 14.9 ± 2.4 mÅ. This is in contrast to the previously published observations[1,2] which had all centred at about 10 mÅ for the EW and seemed to be independent of both the observer and the terrestrial conditions. The (3–0)S(1) equivalent widths show a similar variation at 34.5 ± 11.3 to 56.9 ± 6 mÅ.

The measurements have been interpreted by constructing a realistic model of the Jovian atmosphere in which all the processes of radiative transfer have been accurately considered[1]. Hydrogen quadrupole lines exhibit the phenomenon of collision narrowing before the more usual pressure broadening occurs, and so it is important that the correct line shape is used in an analysis[3]. We have used the Galatry profile which accurately represents the quadrupole line shape. The nominal model of the Jovian atmosphere proposed by Divine[4], and an H/H_e ratio of 6:1 (ref. 5), which are both consistent with present knowledge, are used as a basis for this analysis.

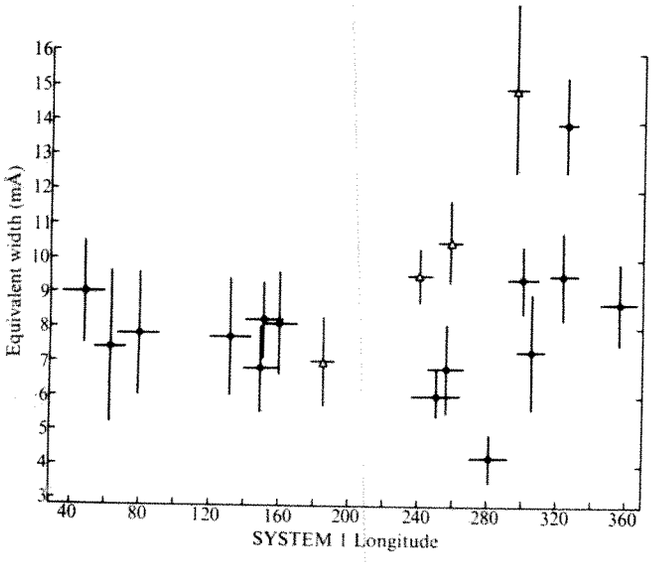


Fig. 1 The variation of the equivalent width of the (4–0)S(1) quadrupole line in the Jupiter spectrum with the SYSTEM I longitude. Solid circles, observations made June 14–30, 1972; triangles, August 17–19, 1972.

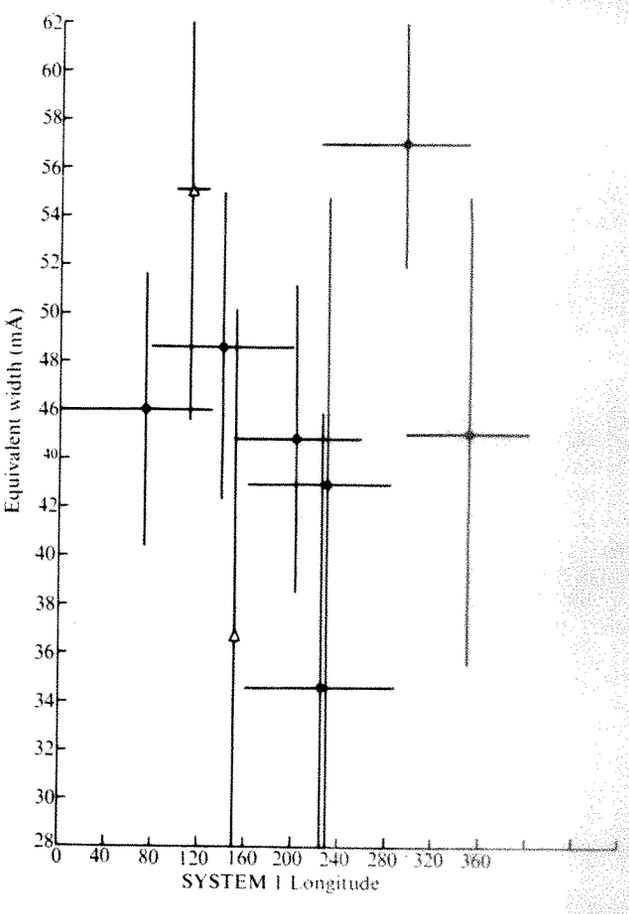


Fig. 2 As Fig. 1 but for the (3–0)S(1) line. Solid circles, June 6–28, 1972; triangles, August 16–18, 1972.

In the equatorial region of Jupiter a structure of two distinct cloud layers is consistent with all observations[5]. The upper cloud may be composed of ammonia crystals, but the composition of the lower layers is unknown[1]. The upper cloud layer is optically thin[1] and we found that it has a negligible effect on the equivalent widths which we observed at the centre of the disk. The lower cloud is optically thick and behaves like a reflecting surface. Consequently, the changes in the derived effective pressure at the level of formation of weak lines, such as the (4–0)S(1) line, will indicate the variability of the top of this dense, lower cloud.

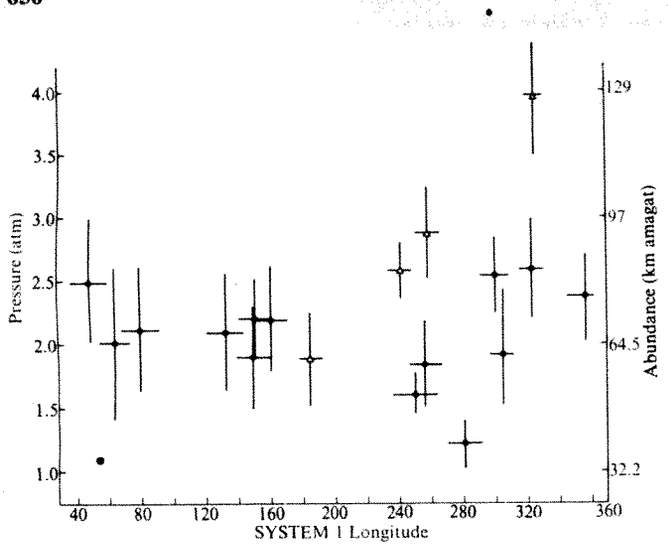


Fig. 3 The variation of the effective pressure at the level of the formation, and the relative abundance of hydrogen, as a function of the SYSTEM I longitude derived from observations of the (4–0)S(1) quadrupole line (Fig. 1).

From the (4–0)S(1) observations we have derived a possible variation of 1.22 ± 0.18 to 4.03 ± 0.93 atm in the effective pressure with a corresponding relative abundance of 39.4 ± 5.9 to 145 ± 30 km amagat (Fig. 3). (1 amagat is the unit of density or volume referring to a gas at 0° C and 1 atm pressure). The analysis of the (3–0)S(1) indicated similar variations of 1.045 ± 0.41 to 2.29 ± 0.51 atmospheres for the effective pressure with the relative abundance varying between 33.85 ± 3.15 and 74.5 ± 16.5 km amagat (Fig. 4). This is a stronger, saturated line which is less sensitive to changes of cloud structure, than the weak (4–0)S(1). These results indicate the futility of quoting a single value for either the effective pressure or the relative abundance derived from spectroscopic observations of a dynamically active planet such as Jupiter.

There are two significant features in the observations illustrated in Figs 1 and 2. First, for the SYSTEM I longitude in the range $160° \leqslant \lambda \leqslant 280°$ the observations carried out during June 1972 show a significant decrease in all of the measured line strengths. For the (4–0)S(1) line this requires the effective pressure at the level of line formation (and therefore at the position of the cloud top) to decrease from $\sim 2 \pm 0.5$ to $\sim 1.25 \pm 0.2$ atm and the hydrogen abundance varies between 68 ± 15–39 ± 13 km amagat. For the (3–0)S(1) line the pressure varies between $\sim 1.6 \pm 0.5 - \sim 1 \pm 0.4$ atm and the abundance

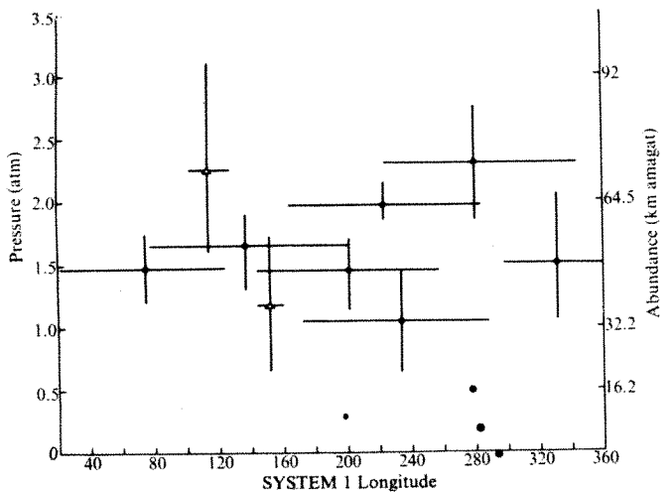


Fig. 4 As Fig. 3 but for the observations of the (3–0)S(1) quadrupole lines (Fig. 2).

varies between $\sim 54 \pm 16 - \sim 33 \pm 13$ km amagat. If these variations are interpreted in terms of changes in the position of the top of the dense, lower cloud, the effective pressure derived from the (4–0)S(1) observations requires this cloud to rise by ~ 25 km from the 2 atm level, during the observational period.

Photographs from the Lowell Observatory International Planetary Patrol, taken at times corresponding to our spectroscopic observations, show significant changes with SYSTEM I longitude of the detailed appearance of the broad equatorial zone. Keay *et al.*[7] have detected temperature variations greater than 40 K in the equatorial zone and have found a strong correlation between temperature and the colours of cloud features in the zones. This increases our confidence in both the detection of fluctuations in the apparent molecular hydrogen abundance and in the interpretations of variations, through a large range of temperature and pressure, in the position of the top of the lower cloud.

With this information we can estimate an upper limit to the vertical velocity of the atmosphere in the neighbourhood of the cloud tops during the observation period. We are assuming that our observations refer to some portion of the planet as it rotates. Hide[6] suggests an upper limit on the vertical velocity W (m s^{-1}) of a fluid element may be derived from the equation

$$W \leqslant U^2 d / f L^2 \qquad (1)$$

where f is the vertical component of rotation (W 10^{-4}s $^{-1}$). As our observations refer to the equational zone we used values of $L \sim 6 \times 10^6$ m for its width and $U \sim 100$ m s^{-1} for a typical horizontal velocity[5]. Inserting a value of $d \sim 25$ km for the height through which the cloud top has moved, equation (1) gives a reasonable estimate of the upward velocity necessary to account for this change in cloud structure:

$$\sim \leqslant 7.5 \text{ cm s}^{-1} \qquad (2)$$

Hide[6] has suggested a value of $\sim \leqslant 10$ cm s^{-1} by observing the changes in colour brought about by the supposed vertical mixing in the atmosphere.

The second significant feature of the observations occurred during August 1972 when an increase in line strength was measured for all lines over the SYSTEM I longitude range of 180–320°. For the (4–0)S(1) line this means that the effective pressure must increase (the level of the lower cloud must decrease) from 1.38 ± 0.77 to 4.43 ± 0.93 atm and the abundance must increase from 61.25 ± 11.75 to 145 ± 30 km amagat. The corresponding changes for the less sensitive (3–0)S(1) line are from approximately 1.18 ± 0.52 to 2.26 ± 0.89 atm and 38.25 ± 16.75 to 73.75 ± 29.25 km amagat. Assuming that we are referring to a single portion of the disk this variation requires the lower cloud to decrease in height by 25 km from ~ 1.38–4.43 atm level during the observational period of two Earth days. From equation (1), the downward velocity, W, will be $\leqslant 7.5$ cm s^{-1}.

This patrol of H_2 quadrupole lines has shown that the abundance of hydrogen and the level of formation of these quadrupole lines is extremely variable. The observations of Keay *et al.*[7] support our conclusions.

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[1] Hunt, G. E., *Mon. Not. R. astr. Soc.*, **161**, 347–363 (1973).
[2] Margolis, J. S., and Hunt, G. E., *Icarus*, **18**, 593–598 (1973).
[3] Hunt, G. E., and Margolis, J. S., *J. quant. Spectrosc. Radiat. Transfer*, **13**, 417–426 (1973).
[4] Divine, N., *The Planet Jupiter*, NASA SP-8069 (1971).

[5] Newburn, R., jun., and Gulkis, S., *Space Sci. Rev.*, **3**, 179–271 (1973).
[6] Hide, R., *Proc. R. Soc.*, **A336**, 63–84 (1974).
[7] Keay, K. S. L., Low, F. J., Rieke, G. H., and Minton, R. B., *Astrophys. J.*, **183**, 1063–1073 (1973).

Model for the binary X-ray source 2U1700-37

Six X-ray sources have been found which show a periodic variability in their X-ray flux which may indicate orbital motion of a binary system[1].

Two of these sources, Her X-1 and Cen X-3, have pulsed emission with periods respectively of 1.24 s and 4.8 s. From the variation of the pulsed emission period due to the Doppler effect during the orbital motion it is possible to deduce some parameters of the orbit and some physical properties of the system.

A possible model for these X-ray sources is the following: a binary system is made up of a neutron star (perhaps a black hole in the case of Cyg X-1), which is the X-ray source and a star which fills its Roche lobe. The X-ray luminosity is provided by the mass transfer from the star through the internal Lagrangian point and that is accreted by the neutron star.

In the case of Her X-1 and Cen X-3, if the neutron star is rotating and has a strong magnetic field, then the matter is accreted chiefly by the magnetic poles producing hot spots that emit X rays and consequently a pulsed emission. This has been studied in some detail by Davidson and Ostriker[2] and Gnedin and Sunyaev[3].

The evolutionary picture of such a system, with special emphasis on Cen X-3, has been given by Van den Heuvel[4], who has studied the evolution of a close binary system in which a large mass transfer occurs.

The matter transferred from the primary star to the neutron star (X-ray source) is likely to have angular momentum. Therefore a rotating disk will be formed, which gradually spirals inwards as turbulent viscosity transports its angular momentum outward. The spectrum of the radiation emitted by the disk was calculated by Pringle and Rees[5] and Shakura[6].

The model explains reasonably well the properties of Her X-1, Cen X-3, Cyg X-1 and 2U0900–40 (ref. 7) but it fails in the case of 2U1700–37 (see ref. 1).

Here I suggest that in the case of the source 2U1700–37, the primary star, an 07f type star (HD153919, refs 1, 7 and 8) is not filling its Roche lobe and that the matter which is accreted by the neutron star is due to a strong stellar wind from the primary star.

If the primary star fills the Roche lobe, we have a relation between ψ, which is the half angle in the orbital plane subtended by the critical lobe as seen from the X-ray source and the ratio M_s/M_x, where M_s is the mass of the primary star and M_x that of the X-ray source.

I first assume that the orbital inclination angle i is 90°. Then, $\psi/180°$ is the fraction of the period during which the X-ray source is eclipsed. From the data given by Jones *et al.*[1], $\psi \sim 58°$. I note that 2U1700–37 is the source which shows the greatest occultation time, almost one-third of the period.

If $\psi \sim 58°$ the mass ratio is $M_s/M_x \sim 240$.

On the other hand, recent measurements of radial velocity of HD153919 by Van den Heuvel[9] show a nearly sinusoidal variation with an amplitude of 23 km s^{-1}. Since the period is 3.41 d (ref. 1), the mass function is:

$$\frac{M_x \sin^3 i}{(1+M_s/M_x)^2} = 4.3\times10^{-3} M_\odot \qquad (1)$$

With the mass ratio calculated above and $i = 90°$, and using equation (1): $M_x \sim 250 M_\odot$ and $M_s \sim 6\times10^4 M_\odot$, which are very implausible values. The results are even worse when $i < 90°$. I conclude that the primary star does not fill the Roche lobe.

I now assume that the primary star has a strong stellar wind. The primary star is an 07f type and thus has enough ultraviolet radiation to ionise the wind well beyond the neutron star orbit and therefore the accreted material consists mainly of protons and electrons.

I suggest that the occultation of the X-ray source is not only due to the eclipse but also due to the Thompson scattering of the X rays in the extended envelope of the primary star. This seems to be supported by the gradual transition from the visibility to the occulted phase and by the variability on time scales of minutes[1] which can reflect irregularities in the wind, that is partially driven by the X-ray source (J. Aarons, unpublished).

A similar analysis was made by Pringle[10] in the case of Her X-1. In this case, since the primary star is likely to be a FO type, ionisation of the envelope is negligible. There is only a kind of 'Stromgren surface' produced by the soft X rays emitted by the neutron star.

To make some crude calculations of the stellar wind parameters, I assume a constant wind velocity V. In this case, matter conservation requires a wind density $n(r)$, which falls as the inverse square of the distance r from the star, namely,

$$n(r) = \frac{K}{r^2} \qquad (2)$$

where K is a constant to be calculated. I further assume that $i = 90°$.

Then, the optical depth in a given direction θ is

$$\tau(\theta) = \int_0^\infty n(x,\theta)\sigma_T \, dx \qquad (3)$$

where θ is the angle between the vectors from the X-ray source to the observer and from the X-ray source to the centre of the primary star. σ_T is the Thompson cross section (6.65×10^{-25} cm^2) and x is related to r by the equation

$$r^2 = a^2 + x^2 - 2ax\cos\theta \qquad (4)$$

where a is the distance between the X-ray source and the primary star.

From equations (2), (3) and (4)

$$\tau(\theta) = (K\sigma_T/a\sin\theta)(\pi - \theta) \qquad (5)$$

Since the occultation of the X-ray source starts at $\theta \sim 58°$ I assume that $\tau(58°) > 1$. I assume further that at phase 0.5, $\tau \lesssim 1$. Then, from equation (5)

$$1 \gtrsim \frac{K\sigma_T}{a} > 0.4 \qquad (6)$$

Now if $M_x \sim 0.8 M_\odot$ for the neutron star mass, from equation (1) $M_s \sim 12.6\ M_\odot$.

Then, from Kepler's third law

$$G(M_s + M_x)/a^3 = 4\pi^2/P^2 \qquad (7)$$

and with the values obtained above:

$$a \sim 1.6\times10^{12} \text{ cm}. \qquad (8)$$

Therefore, from equations (6) and (8)

$$2.4\times10^{36} \gtrsim K > 9.6\times10^{35} \text{ cm}^{-1} \qquad (9)$$

Since the limits above do not differ by more than a factor three I assume

$$K \sim 2\times10^{36} \text{ cm}^{-1} \qquad (10)$$

to obtain order of magnitude estimates.

From equation (2) the density of the wind at the neutron star orbit, $n(a)$ can be derived. Using the figures given by equations (8) and (10)

$$n(a) \sim 8\times10^{11} \text{ cm}^{-3} \qquad (11)$$

To obtain an X-ray luminosity L_x of about 5×10^{36} erg s^{-1} requires an accretion rate of about $7\times10^{-10} M_\odot$ yr^{-1}. This figure is obtained from the relation

$$L_x \approx (GM_x/R_x)(dM_x/dt) \qquad (12)$$

where I have assumed $R_x \approx 10^6$ cm.

Since the neutron star moves supersonically with respect the wind, the accretion rate is given by

$$dM_x/dt = (4\pi(GM_x)^2/V_R^3)\, n(a)\, m_H \qquad (13)$$

where V_R is the relative velocity between the neutron star and the wind and m_H is the proton mass. From equation (13) and the values calculated before

$$V_R \sim 1{,}640 \text{ km s}^{-1} \qquad (14)$$

Since

$$V_R^2 = V_0^2 + V^2 \quad (15)$$

where $V^2 \sim GM_s/a$ is the neutron star orbital velocity, the wind velocity V is

$$V \sim 1{,}610 \text{ km s}^{-1} \quad (16)$$

On the other hand, the rate of loss of mass dM_s/dt from the primary star due to the wind is

$$dM_s/dt = 4\pi KVm_H \quad (17)$$

and, with the parameters derived above,

$$dM_s/dt \sim 10^{-4} M_\odot \text{ yr}^{-1} \quad (18)$$

The calculated wind velocity and mass loss rate are in good agreement with observational measurements carried out by Morton[11] and Carruthers[12] from the spectra of hot stars, which give a certain credibility to the model.

The data seem to indicate the presence of a secondary minimum at phase 0.5. This can be explained, in principle, in the framework of my model, since the matter is accreted mainly after passing the tail shock[13], increasing locally the optical depth and reducing the X-ray intensity observations are made through the accretion cone near phase 0.5.

If the total angular momentum carried out by the wind is zero, then there should be an increase in the orbital period of the order of 5 s yr^{-1} because of the mass loss calculated above, a result which is very important from the observational point of view. (Aldo Treves called my attention to this point.)

Optical observations (*UBV* photometry) of this system have been made since March 1973 in São Jose dos Campos (São Paulo). The results, with a more detailed analysis of this system, will be presented soon.

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1 Jones, C., Forman, W., Tanambaum, H., Schreier, E., Gursky, H., Kellog, E., and Giacconi, R., *Astrophys. J. Lett.*, **181**, L43 (1973).
2 Davidson, K., and Ostriker, J. P., *Proc. IAU Symp.* 55 (Reidel, Dordrecht, 1973).
3 Gnedin, Y. N., and Sunyaev, R. A., *Astr. Astrophys.*, **25**, 233 (1973).
4 Van den Heuvel, E. P. J., and Heise, J., *Nature phys. Sci.*, **239**, 67 (1972).
5 Pringle, J. E., and Rees, M. J., *Astr. Astrophys.*, **21**, 211 (1972).
6 Shakura, N. I., *Soviet Astr.—AJ*, **16**, 756 (1973).
7 Jones, C., Forman, W., Tanambaum, H., Schreier, E., Gursky, H., Kellog, E., and Giacconi, R., *Bull. Am. Astr. Soc.*, **4**, 329 (1972).
8 Hearp, S. R., *Astrophys. J. Lett.*, **181**, L71 (1973).
9 Van den Heuvel, E. P. J., *IAU Circ. No. 2526* (1973).
10 Pringle, J. E., *Nature phys. Sci.*, **243**, 90 (1973).
11 Morton, D. C., *Astrophys. J.*, **147**, 1017 (1967).
12 Carruthers, G. R., *Astrophys. J.*, **151**, 269 (1968).
13 Bondi, H., and Hoyle, F., *Mon. Not. R. astr. Soc.*, **104**, 273 (1944).

Astronomical demonstration of an infrared upconverter

AN upconverter based on a laboratory system[1] developed at the Optical Sciences Center (OSC), University of Arizona, has been used to convert infrared photons from astronomical sources to higher frequency quanta that were detected with a pulse-counting photomultiplier photometer. These observations, made at the Coudé focus of the Steward Observatory 90-inch (229-cm) reflector on Kitt Peak, represent the first successful astronomical demonstration of infrared upconversion, although infrared laser heterodyne radiometry has recently been used to detect the Sun[2] and α Boo[3].

Upconversion is accomplished in an optically nonlinear medium, which in our upconverter is a lithium iodate crystal 5 cm long. Essentially 100% quantum conversion efficiency (QE) was achieved using a 0.6943-μm high peak power ruby laser pump developed at the OSC. This laser seems to be unique among published systems for its efficient generation of TEM_{00}-mode radiation. Details of its design can be found in ref. 4. A 1.064-μm Nd:YAG pumped version of the upconverter yielded a QE of 0.1% (ref. 5). The Nd-YAG pumped upconverter was selected for the astronomical test because it has an infrared spectral bandwidth greater by a factor of 700 and because the effective duty cycle of the Nd-YAG laser is 65 times greater than that of the ruby laser. Therefore, in spite of its lower QE, the Nd-YAG-pumped upconverter is more efficient by a factor of 45 for the detection of thermal astronomical sources.

The astronomical infrared signal at frequency ν_s is phase matched with the pump radiation at frequency ν_p by adjusting the angle of the crystal. The upconverted signal, $\nu_u = \nu_p + \nu_s$, is detected by a pulse counting photometer using a cooled gallium arsenide photomultiplier (RCA C31034). The astronomical source is acquired at the Coudé focus of the 90-inch reflector and directed into the upconverter entrance aperture. The infrared radiation is focused at the appropriate position within the nonlinear crystal. Guiding is accomplished by viewing the image of the source reflected by a dichroic beam splitter to a reticle eyepiece.

Because of the extreme sensitivity of phase matching to angular perturbations, the alignment of the telescope optical axis with the laser pump beam in the nonlinear crystal is extremely critical. We have found it necessary to devote considerable time to this task, and to install auxiliary HeNe alignment lasers to monitor the orientation of optical elements in the system.

The instrument simultaneously upconverts radiation between 3.2 μm and 5.0 μm. This spectral bandwidth of 1.8 μm is the largest ever reported for an upconverter operating in this spectral region[5]. The central wavelength of the operating bandwidth was selected by rotating the lithium iodate crystal about a perpendicular to its optical axis.

During the observations, the laser was pulsed at rates ranging from 0.5 to 4 s^{-1} with a stable pulse width of 2.0 ms and a typical pulse energy of 0.3 J. The Nd:YAG laser is capable of operating at 30 s^{-1}. But we usually operated at 0.5 s^{-1} to permit real-time statistical analysis of the data using an HP-45 portable calculator. Raw data also were printed on paper tape.

The observations were made on the nights of September 8 to September 14, 1973. We attempted to detect the Moon, Mars, α Aur, α Tau, α Ori, Venus, α CMa, ρ Per, NGC1068, α Her, and β Peg. Integrations taken on each object were followed by integrations on the adjacent blank sky. This less-than-optimum detection procedure was used because the telescope was not equipped with a wobbling secondary mirror. The statistical detections are relative to the adjacent sky. An ice dark slide and a glowbar resistor source were also observed. The glowbar data were used to monitor system stability. The ice dark slide data indicated that in practice the thermal background of the detector was definitely less than that due to the telescope and sky.

Multiple statistically significant detections of the Moon, α Tau, α Ori, Venus and α Her were accomplished. Typical data from individual observations with integration times of 0.1 and 0.2 s on September 10, 1973 are summarised in Table 1. These integration times were adequate to yield 2σ (standard deviation) detections of the first four listed sources. Combining data from multiple observations of this type, higher confidence results are obtained.

Table 1 Typical observational data

Object	Signal-to-noise ratio	Integration time* (s)
Moon	2.4	0.10
α Ori	2.2	0.20
α Tau	1.6	0.20
Venus	2.8	0.10
α CMa	0.4	0.20

*Laser pulse of duration 2.0 ms at 0.5 pulse s^{-1}.

Tests showed that a system quantum efficiency (SQE) of approximately 0.001% was attained. This value includes quantum efficiency losses in filters and in the photomultiplier tube. To increase SQE, improvement in the quality of lithium iodate crystals is required, because the currently available material suffers from an absorption which is 0.5 μm wide and centred at 4.3 μm. This absorption results in excess thermal noise from the upconverter when operated at room temperature. We used dielectric interference filters to remove stray flashlamp light from the laser pump beam. The present state of the art should allow better filters, with high transmissivity at 1.064 μm and very low transmissivity at wavelengths below 0.95 μm, to be made. With improvements in the crystal and filter quality, a theoretical SQE of approximately 1.0% could be attained by an upconverter of this type.

We plan to test the upconverter as a device for infrared spectroscopy. This is possible because the upconversion process preserves the frequency coding of the input infrared signal. It will be possible to detect the entire upconverted spectrum simultaneously with a photoemissive image tube because the infrared spectrum is shifted to the 0.83-μm region.

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[1] Gurski, T. R., *Appl. Phys. Lett.*, **23**, 273–275 (1973).
[2] Veth, C., de Grauuw, Th., Shelton, J. C., and van de Stadt, H., *Astr. Astrophys.*, **26**, 479–482 (1973).
[3] de Batz, B., Granes, P., Gay, J., and Journet, A., *Nature phys. Sci.*, **245**, 89–90 (1973).
[4] Gurski, T. R., *Appl. Optics*, **11**, 2105–2106 (1972).
[5] Gurski, T. R., thesis, Univ. Arizona (1974).

Is the zodiacal light intensity steady?

CONTINUED refinement of data on zodiacal light from OSO–5 (ref. 1) confirms our conclusion[2,3] that the absolute value of both the surface brightness and polarisation of the zodiacal cloud varied by less than 10% over the 4-yr period from January 1969 to January 1973. These results are in stark contrast with the recently published satellite measurements of Levasseur and Blamont[4].

The blue photometer, one of six, which recorded the bulk of the zodiacal light measurements comprised a broadband system with an effective wavelength of 4,180 Å, an 11° circular field of view and a fixed polaroid. The photometer viewed along the anti-sail spin axis of the satellite which rotated once every 2 s. The spin axis was held in a plane whose normal was directed at the Sun. Its movement within this plane varied from 0.1° to 1.0° d^{-1}.

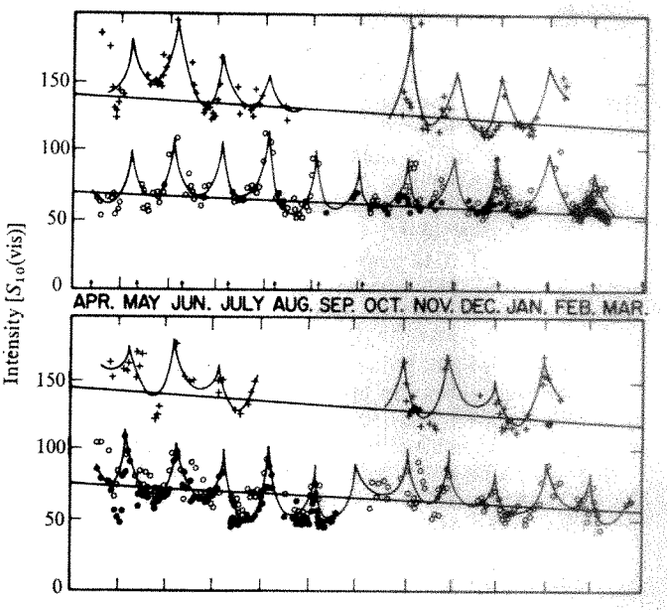


Fig. 1 Zodiacal light data of Levasseur and Blamont (Fig. 4 of ref. 4).Mean value of the intensity for eight directions: top graph $\epsilon<0$; +, ecliptic plane; ○, $+45°\beta$; ●, south pole. Bottom graph $\epsilon>0$; +, ecliptic plane; ○, $\pm45°\beta$; ●, north pole.

Measurements were then made at $\varepsilon=90°$ and varying ecliptic latitudes, usually with $\beta=\pm35°$. Readings were taken at the rate of three a second for 15 min of each 96-min orbit with a night-time look height of 400 km or higher. There were 15 orbits a day for most of four years. Usually, 1,880 readings or 5 min of data were reduced to determine the brightness and polarisation for that night. So detection of changes down to a time scale of hours was possible through the four year period with the following exceptions.

The previously published results[2,3] did not include readings when: (1) the Moon was within 50° of the field of view (one week out of four); (2) a first magnitude object was on the edge of the field of view (seldom); (3) the galactic latitude was less than 30° (four out of 12 New Moon periods). Given these exclusions an absolute calibration of 10% over four years was made. The relative calibration precision over any New Moon period was found to be 5% or less.

The effect on the Moon on telescope 3 was most evident during either first or third quarter depending on the orientation of the spin axis. At these times the Moon could be within 50° of the spin axis. The airglow telescopes in the opposite sail direction were severely hampered any time the Moon was above the satellite horizon. This was because protruding parts of the spacecraft at times allowed reflected moonlight to fall within 50° of the spin axis. (Nightglow measurements were not made with the Moon up.)

The six-parameter least-squares fit made in reducing a 5-min data interval proved of great value in detecting contamination light scattering into the optics from the Moon, moonlight reflected from the Earth, and bright stars and planets near the field. In such cases a considerable variation of intensity at the spin rate as well as at the polaroid frequency, which was twice the spin rate, was seen. Without such a check it would have been more difficult to avoid periodic variations with a lunar period in the data.

So the effects of the Moon may be appreciable any time it is above satellite horizon. The time of maximum effect depends on the particular geometry involved. We have

placed a periodic variation typical of reflections, with lunar frequency, over the data of Levasseur and Blamont (Fig. 1). No attempt at an exact fit to the data has been made, and much of the Full Moon data had already been removed. Nevertheless the chief peaks in the data of Levasseur and Blamont fall between first quarter and Full Moon, except for the initial peak which comes within a few days of launch. Further the New Moon data are quite free of the fluctuations otherwise seen, and a straight line drawn through their New Moon data has a consistent slope in all four sets of data indicating photometer degradation of some $20 s_{10}$[vis] per year in excess of the calibrations that were used to reduce the data.

Data with galactic latitude less than $\pm 30°$ have also now been studied. For data closer to the galactic plane, the absolute calibration errors increased from 10% to 30% due to increased problems of star background and diffuse galactic light subtraction. The relative calibration precision over any New Moon period increased from 5% to 10%. Within these limits again no variations were found in either the brightness or polarisation of the zodiacal light.

A relative variation of 10% or less was observed during the periods April 15 to 29, 1971, and November 2 to 16, 1971. These results are in direct conflict with the satellite measurements of Levasseur and Blamont[4] who have recently reported enhancements of 50% on April 21, 1971, with a half enhancement lasting 4 d, and a 100% enhancement on November 7, 1971, with a half enhancement of 6 d. The enhancements were attributed to debris of comets 1961-I and Biela.

Figure 2 shows our results for surface brightness and polarisation during these times. The absolute intensities during the April period are higher than the November period because the ecliptic latitude is lower. The scatter in the data is caused by the difficulties in subtracting out the star background near the galactic plane. It is typical of our relative calibration precision of 10% at low galactic latitudes. From April 15 to 29, 1971, our measurements were taken at $\varepsilon = E90°$, $\beta = 10° \pm 2°$. For comparison, the results of Levasseur and Blamont report an increase of 50% at $\varepsilon = E90°$, $\beta = 0°$, a 50% increase at $\varepsilon = W90°$, $\beta = N90°$, and a 50% increase at $\varepsilon = W90°$, $\beta = N45°$. From November 2 to 16, 1971, our measurements were taken at $\varepsilon = 90°$, $\beta = N30° \pm 2°$. During this period Levasseur and Blamont report an increase of 100% at $\varepsilon = E90°$, $\beta = N0° - 30°$, and no increase in the $\varepsilon = W90°$, directions. In addition, none of the other sizable variations prevalent in the data of Levasseur and Blamont from April 1971 until March 1972 have been found in our data covering this period.

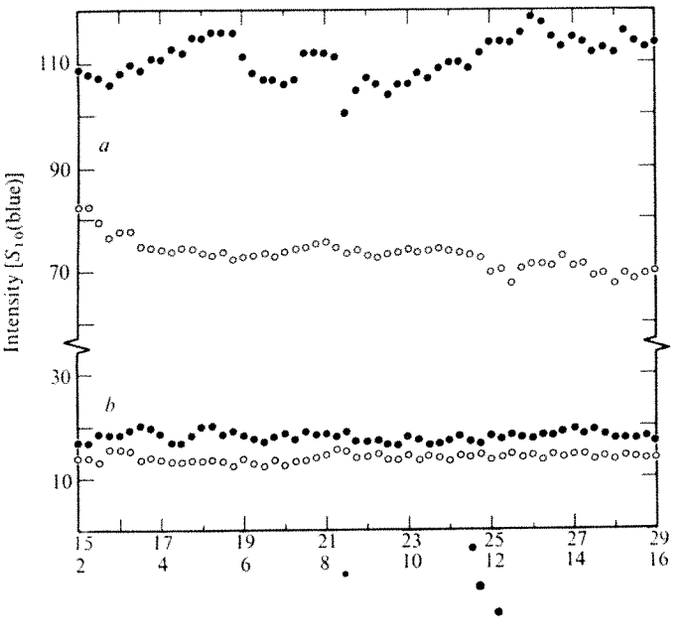


Fig. 2 The brightness (*a*) and polarised intensity (*b*) of the zodiacal light as measured by OSO-V; ●, April 15 to 29, 1971; $\varepsilon = 90°$; $\beta = S10° \pm 2$. ○, November 2 to 16, 1971; $\varepsilon = 90°$; $\beta = N30° \pm 2$. Absolute calibration precision = 30%, relative calibration precision = 10%.

On two occasions, the Lagrangian points L4 and L5 of the Earth–Moon system passed within 2° of the centre of the field of view of our blue zodiacal light telescope. These events took place on January 29, February 7, 1971, and June 25, July 6, 1971. No change in either the intensity or polarisation to the limiting accuracy of the experiment (10%) were observed. This sets a full field upper limit of $10 s_{10}$ (blue) for any change in surface brightness and $2 s_{10}$ (blue) for any change in polarisation due to scattering from dust accrued at these points.

We believe the zodiacal cloud to be a smooth, regular feature of the Solar System as one might expect from a steady influx of particles from the continual breakup taking place in the asteroid belt. There is no evidence for a cloud, with intensity fluctuations of a factor of two, composed of an interwoven maze of comet trails. No variations in intensity or polarisation greater than the limit of accuracy of the experiment—usually 10% arising from comets, solar activity, lunar phase or other events in the Solar System—were observed.

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1 Burnett, G. B., Sparrow, J. G., and Ney, E. P., *Appl. Optics*, **11**, 2075 (1972).
2 Sparrow, J. G., and Ney, E. P., *Astrophys. J.*, **174**, 705 (1972).
3 Sparrow, J. G., and Ney, E. P., *Science*, **181**, 438 (1973).
4 Levasseur, A. C., and Blamont, J. F., *Nature*, **246**, 28 (1973).

f Gravity and gravitational singularities

Trautman has postulated[1] that the usual space–time singularity occurring in classical cosmological models and in the gravitational collapse of massive objects could be averted if intrinsic spin effects are incorporated into general relativity by adding torsion terms to the usual Einstein field equations, that is through the Einstein–Cartan theory. Invoking a primordial magnetic field for aligning all the individual nuclear spins he shows that his universe consisting of 10^{80} aligned neutrons collapses to a minimum radius of the order of 1 cm with a corresponding matter density of 10^{55} g cm^{-3}.

Salam *et al.*[2] have investigated the effect of taking *f* gravity into account in Trautman's model. They consequently obtain profound changes in the numerical values of the parameters, the minimum radius of the Trautman universe now being of the order of 10^{13} cm rather than 1 cm, implying that spin-aligned hadronic matter will not collapse to densities higher than 10^{17} g cm^{-3} — only two or three orders of magnitude higher than that of nuclear matter. But the perfect spin alignment necessary for these results still remains difficult to realise. We now suggest a possible way to avoid this difficulty and still retain all the results.

Two of us have shown[3] that perhaps the easiest and most natural way to include the short range of *f* gravity (mediated by massive spin 2^+ *f* mesons) into Einstein's field equations was to reinterpret the so-called 'cosmological' constant

terms of the inverse Compton length of the f meson

$$\Lambda \simeq \left(\frac{m_f c}{\hbar}\right)^2 \quad (1)$$

m_f being the mass of the f meson.

In the massless (infinitive range) gravity field of conventional general relativity mediated by massless gravitons, $\Lambda = 0$.

Now the usual Friedmann equation with Λ, for a zero pressure Einstein–de Sitter universe is

$$\dot{R}^2 = \left(\frac{8}{3}\right)\pi G\rho R^2 - \left(\frac{2}{3}\right)\Lambda c^2 R^2 \quad (2)$$

with $M = \left(\frac{4}{3}\right)\pi R^3\rho$, being the total mass of the 10^{80} baryons in the Universe. Using $\dot{R} = 0$ at $t = 0$, gives

$$R(0) = \left(\frac{3GM}{\Lambda c^2}\right)^{1/3} \quad (3)$$

Λ is defined as in equation (1), where $m_f \simeq 1{,}500$ MeV. Substitution in equation (3) yields $R(0) \simeq 1$ cm and the corresponding density of matter is $\rho = \left(\frac{\Lambda c^2}{4G}\right) \simeq 10^{55}$ g cm^{-3}. These values are the same as in Trautman's model. It turns out that the Λ term in equation (2) is of the same order of magnitude as the torsion term in Trautman's model. In both cases these additional terms (torsion term in Trautman's, Λ term in ours) in the Friedmann equation are the ones which avert the singularity. For $\Lambda = 0$ (the massless gravity field of infinite range), we recover the old singularity. If we invoke f gravity (and especially as matter in a superdense collapsed state is predominantly composed of hadrons it is reasonable to consider the gravitational interaction between them at short range to be due to exchange of massive f mesons), we must necessarily use the corresponding constant G_f. Therefore, replacing the Newtonian constant G by G_f, the value of which has been estimated[8] to be of the order of $10^{39}G$, we obtain $R(0) \simeq 10^{13}$ cm and the corresponding matter density as $\simeq 10^{17}$ g cm^{-3}. These results are the same as obtained by Salam *et al.* We note in particular that in our formulation the difficult hurdle of finding a suitable mechanism for the alignment of all hadron spins is not encountered. We can understand this result by noting that the Hawking–Penrose[4] singularity theorems for the Einstein field equations $G_{\mu\nu} + \Lambda g_{\mu\nu} = \kappa T_{\mu\nu}$, do not necessarily hold if the cosmological constant Λ is positive, which is implied by our definition in equation (1). Indeed it is remarked in ref. 2 that for all spins aligned in the same direction, the effective spin–spin interaction term in the Lagrangian for the Einstein–Cartan theory which forms the essence of Trautman's torsion term, can be regarded as being of the form $L = -(-g)^{\frac{1}{2}}\,\Lambda_{eff}(x)$, where $\Lambda_{eff}(x)$ is a positive effective cosmological contribution to the Lagrangian. Moreover, as we pointed out earlier, the Λ term we have used in the Friedmann equation is of the same magnitude as Trautman's torsion term.

It is interesting to note that by using G_f instead of G, the absurdly large density $c^5/G^2\hbar \simeq 10^{94}$ g cm^{-3} at which quantum effects of the gravitational field are expected to become important is scaled down to $\simeq 10^{17}$ g cm^{-3}. The corresponding Planck length $(\hbar G/c^3)^{\frac{1}{2}} \simeq 10^{-33}$ cm, at which quantum fluctuations in the space-time curvature are supposed to become significant[5–7], is then scaled up to $\simeq 10^{-14}$ cm. This probably indicates that the quantum effects in f gravity become important during much earlier stages in the collapse, when the density is $\simeq 10^{17}$ g cm^{-3}.

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1 Trautman, A., *Nature phys. Sci.*, **242**, 7 (1973).
2 Isham, C. J., Salam, A., and Strathdee, J., *Nature phys. Sci.*, **244**, 82 (1973).
3 Sivaram, C., and Sinha, K. P., *Lett. Nuovo Cim.*, **8**, 324 (1973).
4 Hawking, S. W., and Penrose, R., *Proc. R. Soc. Lond.*, **A314**, 529 (1970).
5 Wheeler, J. A., in *Relativity Groups and Topology*, (edit. by Dewitt, C. and B.), 328 (Gordon and Breach, New York, 1964).
6 Harrison, E. R., *Phys. Today*, **21**, 31 (1968).
7 Novikov, I. P., and Zeldovich, Ya. B., *A. Rev. Astr. Astrophys.*, **5**, 627 (1967).
8 Sivaram, C., and Sinha, K. P., *Lett. Nuovo Cim.* (in the press).

Trends in the vertical distribution of ozone over Australia

Komhyr *et al.*[1] reported predominantly positive trends in total ozone content over several stations in the 1960s (see also refs 2–4). Here I report the first detailed estimates of trends in the vertical distribution of ozone and discuss some possible causes.

Soundings of the vertical distribution of ozone have been made on a regular weekly basis at Aspendale, Australia (38.0°S, 145.1°E) since June 1965 (ref. 5). Some 432 soundings reached above the 18.1 mbar (27 km) level during the first 8 yr of the programme. Data from these soundings have been corrected to agree with the total amount in a vertical column measured with a Dobson spectrophotometer, by the method[6] in which the ozone concentration above balloon burst is extrapolated along a line of constant mixing ratio for burst heights above the 18.1 mbar level. Extrapolated data are used to calculate the correction factors, but are excluded from the mean data used in trend analysis.

Estimated trends are based on a least squares linear regression against time of 48 successive deviations of 2-month mean ozone partial pressures (from the 8-yr means for the corresponding months) for each of 36 standard levels from 1,000 mbar to 10 mbar. The results are shown in Fig. 1.

Outstanding features are the statistically significant decreases in ozone content—of the free troposphere (chance probability $P < 1\%$ between the 700 and 450 mbar levels), and around the level of the primary ozone maximum ($P << 0.1\%$ at 35 mbar)—and significant increases in ozone content above the 17 mbar level ($P < 0.1\%$ at 12 mbar).

Over the same period of 8 yr the total ozone content as measured by the Dobson spectrophotometer at Aspendale shows an estimated trend of (-3.1 ± 1.1) % per decade.

Table 1 shows these estimated trends in the total ozone content, in the integrated ozone amounts in 10 km thick layers up to 30 km, and in the integrated amount above 30 km (the difference in the trends in the total amount and in the amount below 30 km), all in units of thickness of ozone (reduced to STP) per decade.

Table 1 Ozone trend estimates in various layers of the atmosphere in units of reduced thickness of ozone per decade (data June 1965 to May 1973 inclusive)

Layer (km)	Trend (10^{-3} atm cm per decade)
0 – ∞	−9.9
0 – 10	−2.7
10 – 20	−6.7
20 – 30	−11.2
Above 30	+10.7

The mean total ozone content during the 8-yr period is 0.318 atm cm, and the integrated amount above 30 km is 0.058 atm cm. Thus the mean increase above the 30 km

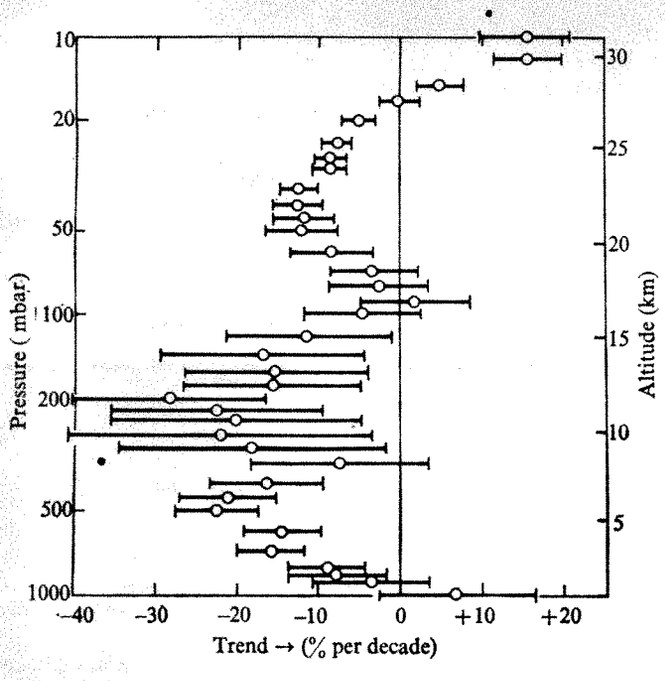


Fig. 1 Estimated trend (% per decade) of ozone partial pressures at 36 standard levels over Aspendale (38°S, 145°E) for 1965–73. Standard errors of the estimates are indicated.

level is 18.5% per decade, in good agreement with the direct trend estimate of $(+15.3 \pm 5.6)\%$ per decade at the 10 mbar (31.1 km) level (Fig. 1).

Trends in the vertical distribution of ozone of such magnitude and statistical significance are of interest in relation to questions of climatic change, global monitoring and pollution, and ozone photochemistry and interacting circulation processes.

Mean annual cycles of ozone partial pressure (nbar) at the 10 mbar and 40 mbar levels are shown in Fig. 2 for Aspendale, with comparable data for Thalwil, Switzerland[6] (47°N) and Boulder/Albuquerque[6,7] (37½°N). As well as the interhemispheric comparison (which will be discussed elsewhere), these curves confirm that the ozone concentration at the 10 mbar level is dominated by photochemistry (maxima in summer) whereas that at 40 mbar is dominated by transport processes (maxima in the late winter and spring).

One might reasonably suggest that the trends at and above the 10 mbar level are due to some process affecting the ozone photochemistry, such as some solar influence or a circulation-induced change in the photochemical process such as a change in the upward flux of NO_x. The trends below the 17 mbar level are most plausibly related to a change in circulation which either reduces the downward flux of ozone from the region of photochemical production or else increases the concentration or flux of some chemical species which destroys ozone in the lower layers. Perhaps the simplest hypothesis is to postulate increased static or dynamic stability at about the 17 mbar level.

Trend analyses of the deviations of 96 consecutive monthly mean temperatures from the 8-yr means for the corresponding months at Laverton, Victoria (the nearest aerological station, some 35 km north-west from Aspendale) show no statistically significant trends at the 10, 15, 50, 100 or 200 mbar levels. Similarly there are no significant trends in the temperature gradients between the 10 and 15, 15 and 50, 50 and 100 or 100 and 200 mbar levels which might have indicated a change in static stability. Estimation of possible trends in the dynamic stability is beyond the scope of the present work, but I note that the available wind data became increasingly inadequate above the 50 mbar level. Any such trend has not resulted in a significant change in the mean temperature or temperature gradient at any of the levels listed above.

Trend analyses of two parameters of the general circulation, the mean latitude of the high pressure belt[8] and the abbreviated southern oscillation index[9], indicate such small trends that they are clearly not related to the observed trends in ozone.

Long term trends in the ozone content above the 30 km level have been tentatively reported[10] and related to the solar cycle. Paetzold *et al.*[11] reported a significant positive correlation between ozone content in the region 20–30 km and sunspot number. They suggest a direct causal link with variations in solar radiation at about 210 μm with no significant phase lag. But the Aspendale data, which span the sunspot maximum of 1968–69, show no evidence of a change in the sign of the trend.

The observed decrease in ozone content below the 17 mbar level could be due to *in situ* destruction by contaminants such as volcanic dust or NO produced by nuclear bomb tests[12]. There is, however, little to support this from the ozone record following the Mt Agung eruption in 1963 (refs 3 and 13) or the resumption of atmospheric bomb testing in 1962–63 (ref. 3), unless the time scale for such effects is so long that no statistically significant departures from normal were to be expected in 1963 or 1964 (ref. 14).

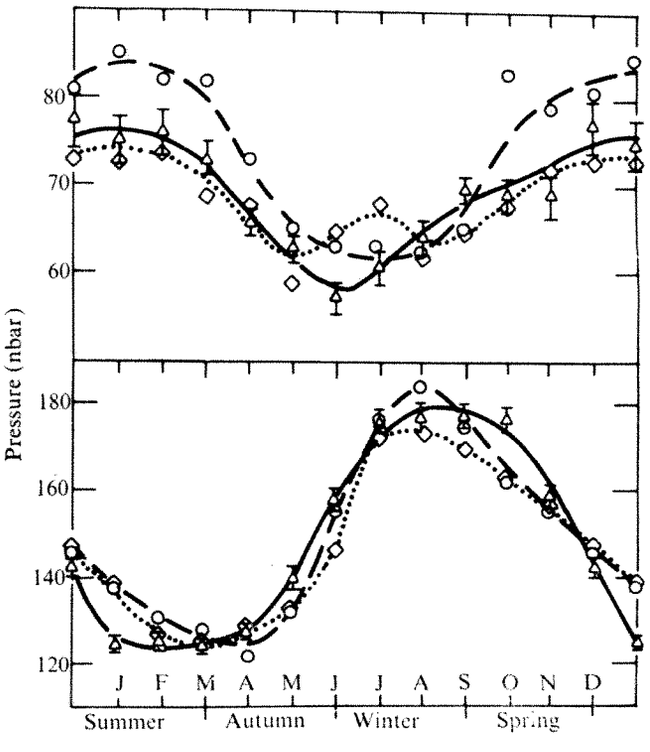


Fig. 2 Mean annual cycle of ozone partial pressures (nbar) at the 10 mbar level (upper diagram) and at the 40 mbar level (lower diagram) for: △, Aspendale (38°S); ○, Boulder/Albuquerque (37½°N); ◇, Thalwil (47°N). Standard errors are shown for the Aspendale results.

I conclude that the cause of the observed ozone trends is at present an open question. These trends are large enough to warrant careful attention in relation to questions of climatic change, global monitoring and ozone photochemistry. More data are needed from more stations and over longer time intervals, and the various possible explanations must be exhaustively tested by all available means.

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[1] Komhyr, W. D., Barrett, E. W., Slocum, G., and Weickmann, H. K., *Nature*, **232**, 390 (1972).
[2] Johnston, H., Whitten, G., and Birks, J., *J. geophys. Res.*, **78**, 6107 (1973).
[3] Goldsmith, P., Tuck, A. F., Foot, J. S., Simmons, E. L., and Newson, R. L., *Nature*, **244**, 545 (1973).
[4] Angell, J. K., and Korshover, J., *Mon. Weather Rev.*, **101**, 426 (1973).
[5] Pittock, A. B., *Q. Jl R. Met. Soc.*, **94**, 563 (1968).
[6] Dutsch, H. U., Zullig, W., and Ling, Ch., *Regular Ozone Observations at Thalwil, Switzerland and at Boulder, Colorado*, LAPETH-I (Lab. f. Atmospharenphysik, ETH, Zurich, 1970).
[7] Hering, W. S., and Borden, T. R., jun., *Ozonesonde Observations over North America, Volume 3*, Environ. Res. Paper No. 133 (Air Force Cambridge Research Laboratory, 1965).
[8] Pittock, A. B., *Q. Jl R. met. Soc.*, **99**, 424 (1973).
[9] Troup, A. J., *Q. Jl R. met. Soc.*, **91**, 490 (1965).
[10] Dutsch, H. U., Boller, P., and Ling, Ch., *Ann. Geophys.*, **25**, 215 (1969).
[11] Paetzold, H. K., Piscalar, F., and Zschörner, H., *Nature phys. Sci.*, **245**, 106 (1972).
[12] Foley, H. M., and Ruderman, M. A., *J. geophys. Res.*, **78**, 4441 (1973).
[13] Pittock, A. B., *J. atmos. Sci.*, **23**, 538 (1966).
[14] Pittock, A. B., *Q. Jl R. met. Soc.*, **100**, 39 (1974).

Radiocarbon and tritium evidence for direct rain recharge to ground waters in the northern Kalahari

THE Kalahari, a flat and sand-covered region of more than 600,000 km² (Fig. 1), has long been classified as a desert because of the virtual absence of surface waters in the form of springs or perennial rivers. The only exception is the inland drainage system in the north, the Okavango delta and Makgadigadi salt pan, which are fed successively by rainwater which drains from the remote highlands of Angola through the Okavango river.

Many investigators[1-6] have subscribed to the view that a sand over-burden exceeding an estimated 6 m would effectively prevent infiltrating rainwater from reaching phreatic water tables. All rainwater retained in the sand would thus be lost by capillary rise and evapotranspiration, chiefly during the dry season. In the area under study, with varying sand cover thickness, the possibility of direct rain recharge was consequently ignored in economic surveys and the ground waters considered to be fossil relics from earlier, much wetter periods.

Table 1 ^{14}C and tritium in phreatic ground water of the northern sand-covered Kalahari

No.	Well	^{14}C (% m.c.)	Tritium (TU)
2	Makgaba 2	120.2±2.6	4.3±0.3
14	Rakops	106.1±4.5	24.4±1.7
54	Khodabis 1231	99.1±2.2	5.1±0.6
60	Toteng 1242	98.7±1.8	16.9±1.7
55	Z 1183	97.7±2.1	15.8±1.3
63	Gweta	97.6±1.5	1.2±0.3
62	Bushman Pits	97.4±2.2	8.0±1.0
1	Makgaba 1	93.7±1.7	5.0±0.5
64	Zoroya 1606	91.9±2.2	10.3±1.1
8	Steinberg's BH	86.2±1.6	2.8±0.4
17	Tshepe	78.4±1.3	1.6±1.6
11	Makoba	78.0±1.4	—
13	Mahata	69.1±1.3	0.7±0.3
58	Tsau	68.3±1.3	0.9±0.2
56	Kuke	66.8±1.7	0.8±0.6
59	Toteng 1320	59.5±1.0	—
57	Sehitwa tribal borehole	54.3±1.2	1.2±0.3

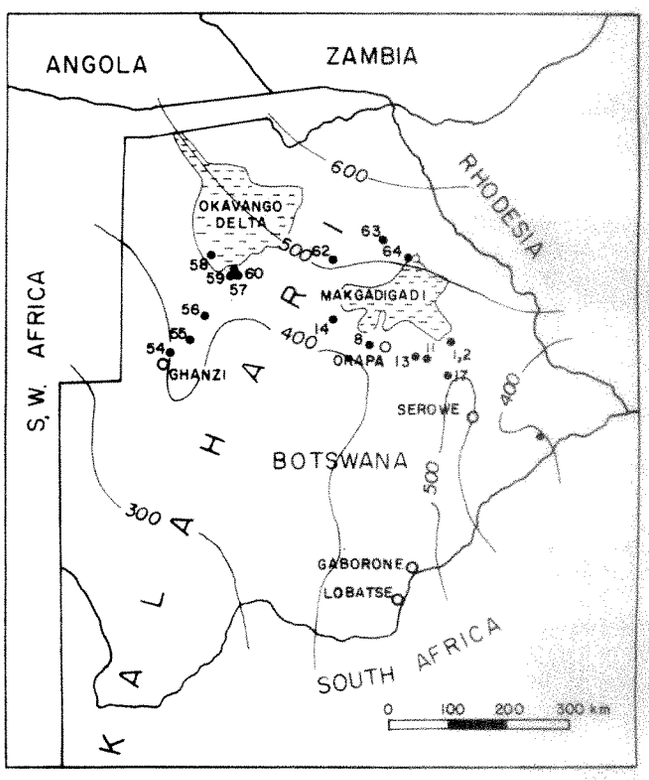


Fig. 1. Sample locations referred to in Table 1.

Recent investigations we have carried out in collaboration with the Geological Survey of Botswana have shown that rain recharge does occur in areas covered with sand. A controlling factor is the annual amount of precipitation, which averages 450 mm in the north, gradually decreasing to 250 mm in the south. In addition, the seasonal variability of annual rainfall with the occasional high rainfall outliers (such as the seasons of 1966–67 and 1973–74) is of extreme importance for recharge.

We sampled several phreatic wells in the northern Kalahari and the environmental ^{14}C ($t_{1/2}$=5,730 yr) and tritium ($t_{1/2}$=

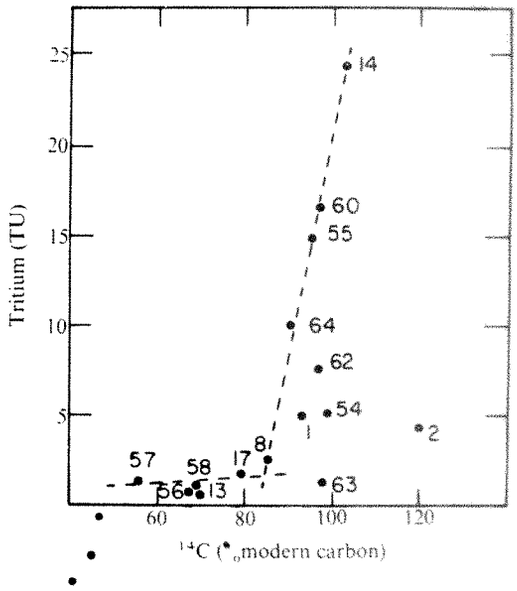


Fig. 2. Radiocarbon-tritium correlation. Samples with values greater than 85% modern carbon radiocarbon and greater than 2 TU of tritium contain a post-nuclear bomb component. Dashed lines, theoretical trends for pre-bomb and post-bomb waters.

12.3 yr) data are reported here (Table 1 and Fig. 1).

Since 1952 nuclear bomb tests have increased the radiocarbon content of atmospheric carbon dioxide above the pre-bomb level of 100% modern carbon (m.c.). Levels in the Southern Hemisphere reached a peak of 165% m.c. in 1963 and decreased to 145% m.c. in 1972 (ref. 7). Biogenic carbon dioxide is dissolved in infiltrating ground water and then reacts with lime free of ^{14}C to produce dissolved bicarbonate. Carbon dioxide at 100% m.c. will produce bicarbonate containing 60 to 90% m.c. through this dilution, with an average of about 85% m.c. (ref. 8). All values above 85% m.c. in Table 1 can therefore be regarded as containing post-bomb recharge, most clearly indicated in the first two cases at 106 and 120% m.c.

The average annual value of tritium in continental rains before the inception of bomb tests in 1952 is estimated at around 5 TU (tritium units). Southern African values reached 60 TU in 1963 and have decreased to about 25 TU at present (ref. 9 and unpublished data). Undiluted recharge at 5 TU in 1952 has decayed to less than 2 TU at present. Therefore, all waters with more than 2 TU must contain a component of recharge from the last 20 yr.

The observations (Table 1 and Fig. 2) corroborate the basic assumptions in the analysis of the tritium and ^{14}C results. The two lines, which tend to define the limits of the results in the pre-bomb and post-bomb eras, intersect at about 2 TU and 85% m.c. The low tritium concentration values to the right of the limit line may indicate different responses to atmospheric bomb tritium and ^{14}C increases. The shallow (1 m) sample no. 2 might present a special case in that roots tapping the water enhance downward transport of ^{14}C.

Ages greater than 20 yr are therefore indicated by values below 85% m.c. The lack of secure theoretical foundations does not permit accurate ^{14}C age calculations. But with an initial value of 85% m.c. waters with 50% m.c. would still be more recent than one ^{14}C half life. Waters in the range of 50 to 85% m.c. may therefore be regarded as having ages from a few hundred to a few thousand years at most, which implies that they are hydrologically active.

Both the radiocarbon and tritium data lead to the unambiguous conclusion that ground waters in the northern Kalahari are directly, and in some cases rapidly, recharged by rain.

Balanced exploitation could make room for additional recharge which is partly lost to evapotranspiration from the often shallow water tables. Even at values of rainwater infiltration as low as 5%, this could still constitute a significant managed ground water supply.

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[1] Boocock, C., and Van Straten, O. J., *Bech. Prot. Rec. geol. Surv.*, 1957/58 (1961).
[2] Boocock, C., and Van Straten, O. J., *Trans. geol. Soc. S. Afr.*, **65**, 125–171 (1962).
[3] Frummurze, H. F., *Rev. Res. Arid Zone Hydrol.*, 58–77 (UNESCO, Paris, 1953).
[4] Martin, H., in *Proc. Inter-Afr. Conf. Hydrol.* (Nairobi, 1961).
[5] Passarge, S., Globus I., *Z. Lander und Volkerkunde*, **190**, 299–302 (1906).
[6] Van Straten, O. J., *Geol. Surv. Bech. Prot. A. Rep.*, 31–35 (1954).
[7] Rafter, T. A. and O'Brien, B. J. in *Proc. eighth int. Conf. Radioactive Dating*. (R. Soc. New Zealand, 1972).
[8] Vogel, J. C. and Ehhalt, D., in *Isotopes in Hydrology*,, (IAEA, Vienna, 1963).
[9] *IAEA Tech. Rept*, Series 96, 117 and 129 (International Atomic Energy Agency, Vienna, 1969, 1970, 1971).

Geomorphology of Siluro-Devonian alluvial plains

The Anglo–Welsh outcrop of the Lower Old Red Sandstone (Siluro–Devonian) reaches approximately 2,000 m in thickness and is largely alluvial[1], consisting of long sequences of fining upwards cyclothems[2]. The fine grade (floodplain) members of the cyclothems commonly include one or more pedogenic carbonate units which are evident in the vertical profile[3–6]. The carbonates seem to represent episodes of soil formation, each of which lasted for about 10^4 yr. Well horizonated palaeosols indicate periods of prolonged weathering, which implies that the alluvial plains on which they were formed remained unflooded for long periods. This could have arisen either because rivers shifted about on the plains, or because of a cyclical river dissection–aggradation coupled with lateral movements. The distribution of Quaternary calcretes[7] suggests that the climate was hot (mean annual temperature 16–20° C) with a low seasonal rainfall (mean annual precipitation 100–500 mm). This is supported by palaeomagnetic results, which indicate a low (southerly) latitude for the Anglo–Welsh area[8].

Four plausible models are considered, which give sequences consistent with known river behaviour and which are applicable to alluvial sequences in the Lower Old Red Sandstone and similar formations.

Model A. Each river, by step-like channel movements combs systematically from side to side of an alluvial cone, in the manner of the Kosi[9] and Indus[10,11]. Soil formation near one side is most intense when the river is active at the opposite side. The resulting palaeosols are discrete, laterally extensive, but non-uniform sheets, each equivalent in time to numerous channel sand-bodies (coarse grade cyclothem members). Provided that the distance moved by the active channel at each step exceeds the width of the channel belt (if braided) or meander belt (if sinuous), vertical profiles show cyclothems with a range of numbers of palaeosols. Fine grade members with no palaeosols result from the erosional emplacement of channel deposits. Control in this model is autocyclic[12], that is, intrinsic to the fluvial regime, and external factors such as climatic change, eustasy and tectonism need not be involved.

Model B. Rivers periodically shift major lengths of their channels by large, almost random distances. This is described as avulsion. It also has an autocyclic control and is well known from modern streams[13,14]. Because the effective zone of the river is laterally restricted, soils can form at sites more distant from the stream. The repeated avulsion gives an alluvial sequence in which palaeosols repeatedly split around bodies of floodplain sediment. The pedogenic carbonates in this model are partly interconnected, either fusing with other palaeosols or having erosional or feather-edge margins. Provided that the lateral distance moved by a river at each avulsion substantially exceeds the width of the channel belt or meander belt, vertical profiles through the alluvium will show cyclothems with contrasted numbers of palaeosols. Each palaeosol is the time equivalent of a number of coarse grade members formed elsewhere. Erosional emplacement of coarse grade members partly explains cyclothems which have no palaeosols. Some floodplain sequences, however, may never have undergone pedogenesis.

Model C. Cyclical river dissection–aggradation occurs because of climatic fluctuations between more and less arid conditions. Following inferred events in the Australian Riverine Plain[15,16], the Lake Torrens Piedmont[17], the Algerian Aurès Mountains[18], and the valley of the Rio Grande[19–22], a shift into relative aridity causes a reduction in the discharge of the river, which leads to channel dissection. The diminished stream lies entrenched in a shallow, possibly terraced, valley, and pedogenesis begins on the terraces and the interfluvial plains, which are no longer flooded by the river. With a climatic amelioration, river discharge again increases and valley filling begins. Soil formation is halted, first on the terraces, and then on the interfluvial plains, as floods become more frequent and widespread.

As soon as the valleys are fully aggraded, the rivers can move laterally by avulsion until the next climatic reversal, by which time they will be in new positions. The alluvial piles thus created have discrete pedogenic carbonates of fairly uniform lateral development, largely embedded in floodplain deposits. Provided that the rivers shift laterally by substantial amounts during each climatic amelioration, the cyclothems show a range of numbers of palaeosols in vertical profile. Fine grade members without palaeosols are largely a result of the erosional emplacement of channel deposits. The main control in this model is allocyclic[12], that is, external to the river regime, though autocyclic avulsion is at times prominent.

Model D. This model is similar to model C but uses a change of base level as the allocyclic control, in combination with avulsion. A fall of the base level causes river entrenchment and promotes soil formation on the exposed interfluvial plains, whereas a rise leads to valley aggradation and river avulsion, and stops pedogenesis in interfluvial areas. For example, rivers crossing the Gulf Coastal Plain to the Gulf of Mexico became entrenched during the Pleistocene glaciations, so that soils formed on the interfluvial plains, but during interglacial periods they aggraded their valleys[23]. The cyclothems and alluvial structure of model D are similar to those of model C. In vertical profile the cyclothems may again contain more than one palaeosol. The erosive emplacement of coarse grade members explains the absence of palaeosols from some cyclothems. model D remains plausible, however, only if the change of base level is less than approximately the channel depth. With bigger ranges, the theoretical coarse grade members are inconsistent with field data, to judge from modern rivers[14]: they are much thicker than the 2–3 m typical of lateral deposits and coarse grade members in the Lower Old Red Sandstone[5], and they are multistorey, with evidence of a change from a braided to a meandering channel pattern during aggradation.

With models A and B, the Siluro–Devonian alluvial plains comprise, at any instant, constructionally active and inactive zones in juxtaposition, with relief arising from alluvial ridges either occupied or recently abandoned by streams. Models C and D provide a more complex picture, though again of juxtaposed active and inactive zones. At one extreme of the controlling cycle, the plains comprise shallow valleys with active rivers and broad interfluvial areas devoid of major drainage. Relief arises from alluvial ridges, active and abandoned, and from valley sides and perhaps river terraces. At the other extreme, the plains are less diverse geomorphologically, with relief provided as in models A and B.

These models provide three alternative possible gross structures for the alluvial sequence. They differ in the three-dimensional fit of the cyclothems, and in the shape, development, and degree of interconnection of the included palaeosols. The structures are all plausible in terms of the essentially one-dimensional vertical profiles observed in the Lower Old Red Sandstone. Finer distinctions demand that an adequate number of cyclothems can be confidently traced laterally over distances of the order of kilometres. This will be possible in the Anglo–Welsh area only through extensive, and expensive, shallow borings. Nonetheless, model D may perhaps be preferred. Recent work shows that the Temeside Shales (?late Silurian), near the base of the Lower Old Red Sandstone, possess palaeosols. As these beds resemble intertidal deposits[24], base level fluctuations could explain the episodes of pedogenesis.

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1 Allen, J. R. L., *in The Upper Palaeozoic and Post-Palaeozoic Rocks of Wales* (University of Wales Press, Cardiff, in the press).
2 Allen, J. R. L., *Sedimentology*, **3**, 163 (1964).
3 Ball, H. W., and Dineley, D. L., *Bull. Br. Mus. nat. Hist.*, **A5**, 177 (1961).
4 Pick, M. C., *Proc. Geol. Ass.*, **75**, 199 (1964).
5 Allen, J. R. L., *Proc. Yorks. geol. Soc.*, **35**, 139 (1965).
6 Allen, J. R. L., *Nature phys. Sci.*, **243**, 84 (1973).
7 Goudie, A., *Duricrusts in Tropical and Subtropical Landscapes* (Clarendon Press, Oxford, 1973).
8 Briden, J. C., Morris, W. A., and Piper, J. D. A., *Geophys. J. R. astr. Soc.*, **34**, 107 (1973).
9 Gole, C. V., and Chitale, S. V., *J. Hydr. Div., Proc. ASCE*, **92** (HY2), 111 (1966).
10 Wilhelmy, H., *Erdkunde*, **20**, 265 (1966).
11 Holmes, D. A., *Geogr. J.*, **134**, 367 (1968).
12 Beerbower, J. R., *Bull. St. geol. Surv. Kansas*, **169**, 31 (1964).
13 Fisk, H. N., *Geological Investigation of the Alluvial Valley of the Lower Mississippi River* (Mississippi River Commission, Vicksburg, 1944).
14 Fisk, H. N., *Fine-grained Alluvial Deposits and their Effects on Mississippi River Activity* (Mississippi River Commission, Vicksburg, 1947).
15 Butler, B. E., *Soil Publ. CSIRO Aust.*, **10**, 1 (1958).
16 Schumm, S. A., *Prof. Pap. US geol. Surv.*, **598**, 1 (1968).
17 Williams, G. E., *Z. Geomorph.*, **17**, 102 (1973).
18 Williams, G. E., *Z. Geomorph.*, **S10**, 40 (1970).
19 Ruhe, R. V., *Ann. Ass. Am. Geogr.*, **54**, 147 (1964).
20 Gile, L. H., *Soil Sci.*, **103**, 265 (1967).
21 Gile, L. H., *Proc. Soil Sci. Soc. Am.*, **34**, 465 (1970).
22 Gile, L. H., and Hawley, J. W., *Proc. Soil Sci. Soc. Am.*, **30**, 261 (1966).
23 Bernard, H. A., and LeBlanc, R. J., *The Quaternary of the United States*, 137, Princeton University Press, Princeton, 1965).
24 Allen, J. R. L., and Tarlo, L. B., *Geol. Mag.*, **100**, 129 (1963).

Orogenic zones in central Australia: intraplate tectonics?

THE plate tectonics model[1,2] has been successful in explaining the orogenic features of the ocean basins and continents associated with the dispersal of Pangaea in post-Triassic time. Dewey and Bird[3] extended these concepts, suggesting that there is a unique relationship between orogenesis and plate-margin tectonism, and various authors have, accordingly, interpreted certain Precambrian and Palaeozoic orogenic zones or 'mobile belts' as plate sutures[4,5]. We argue here that plate-margin tectonism is only one of several possible conditions for the development of an orogenic belt. We suggest that the plate tectonic model of orogenesis needs to be extended and modified, and this suggestion is discussed for Gondwanide Australia. Recent attempts to impose a rift (aulacogene)[6] or subduction[7] model for orogenesis in central Australia are confounded by the observed geological and geophysical facts pertinent to this region.

Three zones of major thrust faulting in crystalline basement rocks have been recognised in central Australia. They bound the Ngalia and Amadeus Basins (Fig. 1) and are characterised by steep gravity gradients[8]. The Amadeus and Ngalia Basins are intracratonic depressions containing latitudinally folded and thrusted Proterozoic and Palaeozoic sedimentary cover rocks[9,10]. The Musgrave Block, south of the Amadeus Basin, was deformed about 600 million years (Myr) ago during the 'Petermann Ranges Orogeny'[11] when the Woodroffe Thrust developed in the basement south of the basin. The Arunta basement complex was deformed during the 'Alice Springs Orogeny'[11] in late Devonian or early Carboniferous time[12]. While the basement rocks of the northern margins of the Amadeus and Ngalia Basins underwent major thrusting and retrograde metamorphism, the overlying Proterozoic cover rocks deformed independently above a decollement horizon[9].

Thick syntectonic molasse-type sediments are associated with the Alice Springs and Petermann Ranges events. Unconformities in the cover sediments, and the thickening and coarsening of facies against the northern margin of the Amadeus Basin[13] indicate that the crystalline basement was

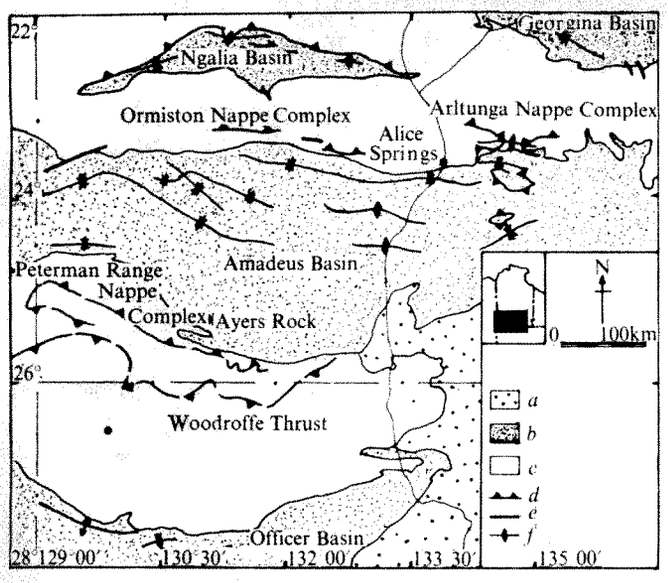


Fig. 1 The location and major structural features of the intracratonic basins and deformed basement zones of central Australia. *a*, Mesozoic strata; *b*, Proterozoic and Palaeozoic cover; *c*, older Precambrian basement rocks; *d*, thrust fault, indicating upper plate; *e*, fault; *f*, major fold.

uplifted at various times between the thrusting episodes.

Features indicating plate-margin tectonism, such as the presence of volcanics, acid intrusives, ophiolites and high pressure, low temperature metamorphics[14], are absent in central Australia; only retrograde metamorphism of the amphibolites and granulites of the basement occurred during the thrusting and folding. The Giles Complex, which contains the only ultramafics in central Australia, is made up of isolated intrusions[15]. The distribution of the various deformed zones and the widespread, relatively undeformed nature of the late Proterozoic and Palaeozoic platform sediments preclude any association of orogenesis with subduction, as does the continuity of contemporaneous structural features across major dislocations. The style of basement thrusting and gravity modelling[16], suggest that deformation was intracratonic and compressive. It was probably confined to the crust.

Palaeozoic deformation of the central Australian shield affected an area that was remote from the nearest continental margin. Lower Palaeozoic palaeomagnetic results from northern[17], central[18], and southern and western Australia[19] suggest that these regions have not experienced large lateral displacements with respect to one another since Cambrian time. Furthermore, the northern and central Australian results indicate that these regions have acted as a single unit at least since the late Proterozoic. The orogenic events should be interpreted in terms of a single central Australian craton that underwent meridional compression while participating in the drift of Gondwanaland[20]. The intermittent, north–south drift of Gondwanaland during the Palaeozoic is now well established[21,20]. We suggest that rapid, intermittent drift of the large Gondwanide plate had intraplate orogenic repercussions, which are manifested in the prominent east-trending basement and cover structures in central Australia. Ancient zones of weakness in the crust imparted a mechanical anisotropy which provided loci for later thrusting; these may have coincided with old east-trending mobile belts now represented by deformed granulite zones. Recurrent thickening and warping of the crust[13] produced the observed unconformities in the Proterozoic and Palaeozoic basins. When Australia drifted from Antarctica, it probably did so along one of the approximately east-trending ancient zones of weakness–perhaps an old mobile belt, as has been suggested for the rifting of South America from Africa[22].

There is clearly a need for a broader plate tectonic theory of orogenesis. Results from the Precambrian cratons of Africa and North America[23,24] are consistent with the concept of intraplate orogeny. It seems that, within the limits of palaeomagnetic resolution, little relative motion has occurred between cratons and the intervening mobile belts have been interpreted as manifestations of ensialic tectonic phenomena. Accordingly, Piper *et al.*[24] suggested that a 'supplement' to the plate theory of Precambrian orogenesis is required. Glikson and Lambert[25] invoked a rift valley (aulacogene) mechanism to explain the development of certain West Australian mobile belts. We have further extended the plate theory of orogenesis to provide a viable basis for understanding the Palaeozoic deformed zones of central Australia.

Finally, it has been shown[26,27] that continental plates, in particular those carrying North America and Australia, are in a state of compression under horizontal maximum principal stresses. Within such continents, earthquake shocks are generally caused by thrust faulting[27,28]. Without invoking a driving mechanism, it is reasonable to expect that large deviatoric stresses existed in Palaeozoic continents and thus accounted for the deformed zones of central Australia.

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1 McKenzie, D. P., and Parker, D. L., *Nature*, **216**, 1276–1280 (1967).
2 Morgan, J., *J. geophys. Res.*, **73**, 1959–1982 (1968).
3 Dewey, J. F., and Bird, J. M., *J. geophys. Res.*, **75**, 2625–2647 (1970).
4 Hamilton, W., *Bull. geol. Soc. Am.*, **81**, 2553–2576 (1970).
5 Gibb, R. A., and Walcott, R. I., *Earth planet. Sci. Lett.*, **10**, 417–422 (1971).
6 Scheibner, E., *Record of the Geological Survey of New South Wales*, **14**, Part 1, 37 (1972).
7 Davidson, D., *Nature*, **245**, 21–23 (1973).
8 Forman, D. J., and Shaw, R. D., *Bull. Bur. Miner. Resour. Geol. Geophys. Aust.*, 144 (1973).
9 Wells, A. T., Forman, D. J., Ranford, L. C., and Cook, P. J., *Bull. Bur. Miner. Resour. Geol. Geophys. Aust.*, 100 (1970).
10 Wells, A. T., Moss, F. J., and Sabitay, A., *Apea*, 144–151 (1972).
11 Forman, D. J., *Rep. Bur. Miner. Resour. Geol. Geophys. Aust.*, 87 (1966).
12 Stewart, A. J., *J. geol. Soc. Aust.*, **17**, 205–211 (1971).
13 Jones, B. G., *J. geol. Soc. Aust.*, **19**, 229–249 (1972).
14 Dickinson, W. R., *Science*, **174**, 107–113 (1971).
15 Nesbitt, R. W., Goode, A. D. T., Moore, A. C., and Hopwood, T. P., *Symposium on the Bushveld Igneous Complex and other Layered Intrusions*, 547 (The Geological Society of South Africa, Special Publication 1, 1970).
16 Anfiloff, W., and Shaw, R. D., *The Earth's Gravitational Field and Secular Variation in Position* (contribution to an international symposium sponsored by the Australian Academy of Science under the auspices of the International Association of Geodesy. V. NSW Sydney).
17 Luck, G. R., *Geophys. J.*, **28**, 475–487 (1972).
18 Embleton, B. J. J., *Earth planet. Sci. Lett.*, **17**, 217–226 (1972).
19 Embleton, B. J. J., and Giddings, J. W., *Earth planet. Sci. Lett.* (in the press).
20 McElhinny, M. W., and Briden, J. C., *Earth planet. Sci. Lett.*, **10**, 407 (1971).

[21] Briden, J. C., *Nature*, **215**, 1334–1339 (1967).
[22] Fyfe, W. S., and Leonardos, O. H., *Nature*, **244**, 501–502 (1973).
[23] Spall, H., *Nature*, **236**, 219–221 (1972).
[24] Piper, J. D. A., Briden, J. C., and Lomax, K., *Nature*, **245**, 244–248 (1973).
[25] Glikson A. Y., and Lambert, I. B., *Earth planet. Sci. Lett.*, **20**, 395–403 (1973).
[26] Fitch, T. J., Worthington, M. H., and Everingham, I. B., *Earth planet. Sci. Lett.*, **18**, 345–356 (1973).
[27] Sykes, L. R., and Sbar, M. L., *Nature*, **245**, 298–302 (1973).
[28] Gordon, F. R., *Royal Society of New Zealand, Bulletin 9*, 85–93 (1971).

Diamond growth by sulphide reduction of CO_2

MARX[1] proposed that natural diamonds were formed by a reduction of CO_2 and other theories have been proposed to explain their formation[2–6]. Carbon dioxide is a common constituent[7,8] and has been found as a gas in natural diamonds[9]. Marx proposed that pyrrhotite is active in the reduction process because it has been found as an inclusion in diamonds[10]. Furthermore, thermodynamic calculations showed such a reduction reaction to be energetically possible:

$$2FeS(solid)+CO_2(gas)=2FeO(solution)+S_2(gas)+C(diamond) \quad (1)$$

Diamond formation by this reaction probably would result in inclusions containing either free sulphur or sulphur compounds, or both. To test this theory experimentally, natural diamond was oxidised by pure oxygen at a high temperature. Any included sulphur would be converted to gaseous SO and SO_2 which could be detected by analysing the products with a research mass spectrometer. The instrument is about 10^5 times more sensitive than any other spectroscopic method[11] and lends itself well to a study of minute quantities of a gaseous substance.

The mass spectrometer used was a 15.25-cm radius 90° sector type magnetic scanning instrument. The instrument, calibration and experimental procedures have been described[12].

Diamond fragments (16 g) were used[9]. The diamond was placed in a quartz tube which was evacuated by an oil diffusion pump to 10^{-7} torr. An oxygen atmosphere of 1 torr was then admitted to the sample tube. The sample was heated to a temperature of 1,050° C and maintained at that temperature for 45 min. The sample container was then placed in a dry ice–acetone bath and noncondensed components were removed by evacuation. Condensable substances were retained in the container. The remaining sample was analysed at room temperature using mass spectromatic techniques. Each experiment was duplicated three times. The weight of diamonds oxidised in each experiment was about 5.3 g.

No evidence for SO and SO_2 in the oxidation products was found in any of the three samples. The 16 g of oxidised diamond fragments comprised several hundred individual diamond crystals, some with visible inclusions, from Africa, Brazil and Arkanas. The detection sensitivity for sulphur was approximately 1 part in 10^9 with respect to other oxidation products. Thus, there was no evidence for the presence of pyrrhotite. This suggests that the reduction of CO_2 by pyrrhotite is not responsible for the formation of all natural diamonds. These limited data do not, however, rule out the possibility of some diamond formation by the reduction of CO_2 by pyrrhotite.

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[1] Marx, P. C., *Mineralog. Mag.*, **38**, 636 (1972).
[2] Meyer, H. O. A., and Boyd, F. R., *Carnegie Inst. Wash. Geophys.* Lab Report to Div. 1967–68 (1969).
[3] Lonsdale, K., and Milledge, H. J., in *Physical Properties of Diamonds* (edit. by Berman, R.), 42 (Clarenden Press, Oxford).
[4] Dawson, J. B., *Phil. Trans. R. Soc.*, **A271**, 297 (1972).
[5] Giardini, A. A., and Tydings, J. E., *Am. Miner.*, **47**, 1393 (1962).
[6] Mitchell, R. H., and Crocket, J. H., *Miner. Deposita*, **6**, 392 (1971).
[7] Hearn, B. C., jun., *Science*, **159**, 622 (1968).
[8] Kennedy, G. C., and Nordlie, B. E., *Econ. Geol.*, **63**, 495 (1968).
[9] Melton, C. E., Solotti, C. A., and Giardini, A. A., *Am. Miner.*, **57**, 1518 (1972).
[10] Sharp, W. E., *Nature*, **211**, 402 (1966).
[11] Melton, C. E., *Principles of Mass Spectrometry and Negative Ions* (Dekker, New York, 1970).
[12] Melton, *J. phys. Chem.*, **74**, 582 (1970).

BIOLOGICAL SCIENCES

E. coli lactose operon ribosome binding site

IN *Escherichia coli*, protein synthesis is initiated with formylmethionine, coded by the triplet AUG. As the first step in translation, ribosomes bind to the AUG initiator codon in the presence of initiation factors, charged fMet-tRNA and GTP. The sequence or structure of a messenger RNA molecule must signal that a particular AUG triplet is an initiation codon, and the cell's ribosomes must recognise this region as containing a signal for initiation of translation. In a reaction suitable for *in vitro* protein synthesis, except that it contains only one species of charged tRNA, fMet-tRNA, ribosomes bind to and protect initiation regions from nuclease digestion. The ribosomes bind at the AUG initiator codon and cannot proceed any further due to the lack of charged tRNA species other than fMet-tRNA. In the hope of determining the characteristics peculiar to an initiation region in a mRNA molecule, a number of ribosome binding sites have been sequenced[1–8].

I have previously reported the sequence of the first 63 bases of the lactose operon mRNA transcribed from the (CAP-independent) UV5 promoter mutant[9]. Figure 1 shows this sequence. The first AUG triplet occurs at positions 39–41, and the succeeding bases, read in triplets, code for the first seven amino acids of β-galactosidase[10]. This was highly suggestive that translation might initiate at position 39 of the UV5 *lac* message, and that ribosomes should bind to and protect the surrounding region of RNA from nuclease digestion.

To verify that the region around the AUG codon at position 39 is a ribosome binding site, I have isolated the portion of the UV5 *lac* mRNA which ribosomes protect from digestion by RNase A (which cuts after C and U) and RNase T_1 (which cuts after G). Table 1 lists the

Table 1 RNase T_1 oligonucleotides recovered after protection of a protein synthesis initiation complex from RNase A or T_1 digestion

Oligonucleotide	Molar yield after digestion with RNase A	RNase T_1
G(A)	2-3	2-3
AUAACAAUUUCACACAG(G)	1/4–1/2	1
AAACAG(C)	1	1
CUAUG(A)	1	1
ACCAUG(A)	1	1
AUUACG(G)	1	1
AUUCACUG(G)	1/4	1/3–1/2

RNA was synthesised *in vitro* from UV5 *lac* DNA fragment template as previously described[9]. Synthesis was for 13 min at 25° C. After phenol extraction and two ethanol precipitations to remove unincorporated triphosphates, the ^{32}P-labelled RNA was taken up in 20 μl of 0.5 mM EDTA and incubated at 37° C for 15 min. Initiation complexes were formed in a 40 μl reaction mix similar to that described by Steitz[1] containing: 0.1 M Tris, *p*H 7.4, 0.05 M NH_4Cl, 5 mM $MgOAc_2$, 0.2 mM GTP, 2.5 mM β-mercaptoethanol, 6.6 A_{260} units ribosomes, about 100 μg crude initiation factors, and 1.0 A_{260} unit mixed tRNA charged with ^{35}S-formylmethionine. Ribosomes and initiation factors were prepared from *E. coli* B/r as described by Anderson *et al.*[15], except that: the preparative buffer contained 1 mM dithiothreitol instead of 6 mM β-mercaptoethanol; ribosomes were made up to 0.05 M Tris, *p*H 7.4 before the 2 M NH_4Cl wash; and initiation factors were dialysed into preparative buffer containing 5% glycerol, and made up to approximately 10% glycerol before storage at −70° C in plastic tubes. *E. coli* K12 mixed stripped tRNA (General Biochemicals) was charged with ^{35}S-methionine and formylated. Charging was assayed by precipitation in cold and hot TCA. After 12 min incubation at 37° C the reaction was cooled to 25° C, 75 μg ml^{-1} RNase A or T_1 was added and incubation was continued at 25° C for 15 min. The reactions were layered on to linear 3.8 ml 5-20% sucrose gradients in the buffer described by Kondo *et al.*[16], except that the $MgOAc_2$ concentration was raised from 5 to 10 mM. After centrifugation for 1 h at 55 K in the IEC SB405 rotor, about 20 fractions were collected directly into 3.5 ml scintillation vial inserts and the peaks of radioactivity determined by Cherenkoff counts. Typically about 1–3% of the counts sedimented with the ribosomes after RNase digestion. These fractions were pooled, precipitated with 2.5 volumes ethanol and 1/10 volumes 20% NaAc at −20° C overnight, extracted with phenol, and precipitated with ethanol twice. The protected fragment was digested with RNase T_1 and fingerprinted by standard procedures described by Barrell *et al.*[17]. The data above are compiled from three separate experiments with A, C and U-labelled RNA.

oligonucleotides found in RNase T_1 fingerprints of this protected region. These oligonucleotides fit into the portion of the UV5 *lac* message surrounding the presumed initiator codon.

Table 2 compares the sequence of protected regions isolated after digestion with RNase A or RNase T_1. Ribosome protection does not preserve a unique sequence from nuclease attack: the size of the protected fragment depends on which nuclease accomplished the digestion, and on its concentration and activity. At the 3′ end of the *lac* ribosome binding site, the T_1 fragment AUUCACUG is sometimes protected and sometimes clipped during digestion with either RNase A or RNase T_1. At the 5′ end the long T_1 fragment, AUAACAAUUUCACACAG, is always protected from RNase T_1 digestion, so its 3′-terminal G must be well shielded in the protected region. RNase A usually cleaves within this fragment, so some C and U residues at its 5′ end must be exposed. It seems to have no preferred cleavage site in this region.

Sequences of nine other ribosome binding sites have been published[1–7], as well as that of an anomalous site on Qβ RNA which *B. stearothermophilus* ribosomes bind and protect[8]. A long stretch at the 5′ end of MS2 RNA has also been sequenced, and this includes a region which probably contains the MS2 A protein initiation site; its sequence is very similar to that of the initiation region for R17 A protein, although the initiation codon is GUG[11,12].

Common features of initiation or ribosome binding regions are an AUG initiator codon, the sequence AGGA, the sequence PuPuUUUPuPu (Pu stands for purine), and one or more nonsense codons near the initiation site. Table 3 lists the sequences of the 11 known initiation regions, drawn so that similar sequences can be easily compared. All the initiation regions (or presumed initiation regions) which have been sequenced, except that for the MS2 A protein, include an AUG initiator codon. The *lac* ribosome binding site shares the sequence AGGA (positions 28 to 31 of the mRNA) with seven other ribosome binding sites[1–4,8,11] and the *lac* site has the sequence AAUUUCA (positions 18 to 24) which, with C substituted for a purine, resembles the sequence PuPuUUUPuPu found in six other initiation regions[1,4–6,11]. All but the *lac* site have a nonsense codon (UAA, UGA or UAG) within 15 bases of the initiator AUG; in *lac* the nearest nonsense codon (UAA) is 22 bases away. In some regions a stable conformation can be drawn with the AUG protruding at the 'ear' of a loop of RNA, but no such stable secondary structure is apparent in that of the *lac* operon. Table 3 also shows the site which *B. stearothermophilus* ribosomes protect on Qβ RNA[8]. This site probably contains the sequence AGGA, but it shares no other features with known initiation sites.

Though all these regions have a certain resemblance to one another, none of these common features alone, not even the AUG initiator codon, is an absolute requirement for ribosome binding. There is a GUG codon in the initiator region of the MS2 A protein[12]. Reinitiations occur *in vivo* in mutants of the *lac i* gene (which codes for *lac* repressor) which carry early amber mutations. Three different mutant

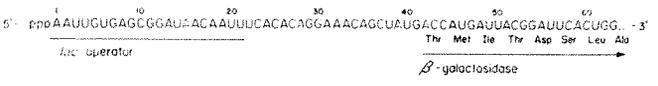


Fig. 1 The first 63 bases of the m RNA transcribed from the UV5 *lac* promoter[9]. The *lac* operator sequence is copied into bases 1 to 21. β-galactosidase initiates at the AUG codon at position 39, and the amino-terminal sequence of β-galactosidase[10] is matched to the triplets on the message which code for those amino acids.

Table 2 Ribosome binding region of the *lac* message

Maximum RNase T_1-protected fragment:
5′-AUAACAAUUUCACACAGGAAACAGCUAUGACCAUGAUUACGGAUUCACUG
Minimum RNase T_1-protected fragment:
5′-AUAACAAUUUCACACAGGAAACAGCUAUGACCAUGAUUACG
Minimum RNase A-protected fragment:
5′-AGGAAACAGCUAUGACCAUGAUUACGGAU

Oligonucleotides recovered from ribosome-protected fragments of the UV5 *lac* message were fitted into the sequence of the first 63 bases of this messenger (shown in Fig. 1). This table compares the minimum site protected by RNases A and T_1 with the maximum protected region. Gel electrophoresis of protected fragments cleaved by RNase T_1 reveals two main species. Sequence analysis of these species show that they differ by a single oligonucleotide, AUUCACUG, which is reflected in the low recovery of this oligonucleotide after protection and digestion with RNase T_1 (Table 1). But gel electrophoresis of fragments protected from RNase A digestion reveals a range of species between 30 and 40 bases long, and no preferred cutting sites are evident in fingerprints of protected RNA.

proteins all have fMet as their amino-terminal amino acid, but these restarts occur at amino acid residues which are normally translated as formylmethionine (AUG), valine (GUX) or leucine (CUX, UUA or UUG), so ribosomes seem to bind to codons which resemble the normal AUG initiation site, and then mistranslate[13,14]. Steitz has reported that *B. stearothermophilus* ribosomes bind *in vitro* to the RNA genome of the coliphage Qβ and protect a region

Table 3 Sequences of ribosome binding sites

AAACA*UG* AGGA UUACCCAUGUCGAAGACAACAAAG	R17 replicase[1]
(AAC,U)A AGGA *UGA*AAUGCAUGUCUAAGACAGC	Qβ replicase[3]
AGAGCC(C)UCAACCGGGGUU*UGA*AGCAUGGCUUCUAACUUUACUCAG	R17 coat protein[1]
...AGAGCCUCAACCG(G,A)GUU*UGA*AGCAUGGCUUCCAACUUUACUCAG	f2 coat protein[5]
AAUU*UGA*UCAUGGCAAAAUUAGAGAC	Qβ coat protein[2]
CCU AGGA GGUU*UGA*CCUAUGCGAGCUUUUAGUG	R17 A protein[1]
...CCU AGGA GGUU*UGA*CCUGUG	MS2 5′ end[11,12]
GAGUA*UAA*G AGGA CAUAUGCCUAAAUUACCGCGUG	Qβ A protein[4]
AGGTTTTCTG,CTT AGGA TTT*AA*TCATGTTTCAGACTTTTATTTCTCGCCAC	ΦX174 spike protein[6]
AACA*UGA*GG*UAA*CACCAAAUGAUUUUCACUAAAGAGCCUGCGAACG	a T7 *in vitro* RNA[7]
A*UAA*CAAUUUCACAC AGGA AACAGCUAUGACCAUGAUUACGGAUUCACUG	*lac* operon
AAACUG(AAAG,AG,G,G,G)AUUACACUCGAACGCCGUCAUCG	Qβ – *B. stearothermophilus* ribosomes' site[8]

Sequences of ribosome binding sites, from indicated sources, aligned with respect to their AUG or GUG initiator codons. Nonsense codons *UAA*, *UGA* and *UAG* are in italics. The popular sequence PuPuUUUPuPu is underlined, and AGGA is boxed. Parentheses and commas mark uncertainties in fragment order.

which has no initiator codon, although it probably does include the sequence AGGA[8].

The *lac* operon ribosome binding site is the first to be sequenced from an *E. coli* operon. It is pleasing to find a close resemblance between phage and host protein synthesis initiation regions. But while there are similarities of sequence between many of the known ribosome-binding sites, the resemblance among all these sites is too casual to allow any conclusions about how ribosomes recognise initiation regions.

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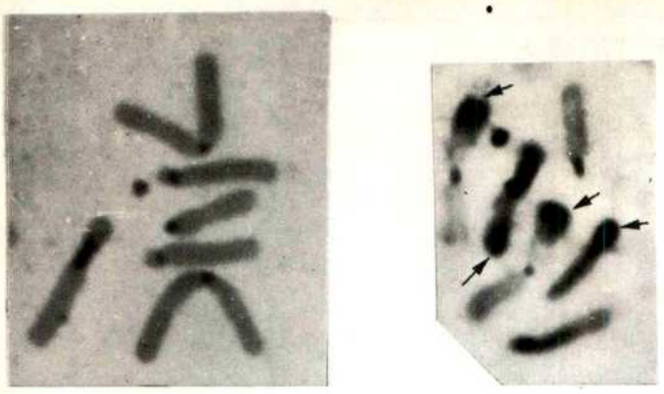
1 Steitz, J. A., *Nature*, **224**, 957–964 (1969).
2 Staples, D. H., and Hindley, J., *Nature new Biol.*, **234**, 211–212 (1971).
3 Staples, D. H., Hindley, J., Billeter, M. A., and Weissmann, C., *Nature new Biol.*, **234**, 202–204 (1971).
4 Robertson, H. D., Barrell, B. G., Weith, H. L., and Donelson, J. E., *Nature new Biol.*, **241**, 38–40 (1973).
5 Hindley, J., and Staples, D. H., *Nature*, **224**, 964–967 (1969).
6 Gupta, S. L., Chen, J., Schaefer, L., Lengyel, P., and Weissman, S. M., *Biochem. biophys. Res. Commun.*, **39**, 883–888 (1970).
7 Arrand, J. R., and Hindley, J., *Nature new Biol.*, **244**, 10–13 (1973).
8 Steitz, J. A., *J. molec. Biol.*, **73**, 1–16 (1973).
9 Maizels, N. M., *Proc natn. Acad. Sci. U.S.A.*, **70**, 3585–3589 (1973).
10 Zabin, I., and Fowler, A. V., *J. biol. Chem.*, **247**, 5432–5435 (1972).
11 DeWachter, R., Merregaert, J., Vandenberghe, A., Contreras, R., and Fiers, W., *Eur. J. Biochem.*, **22**, 400–414 (1971).
12 Volckaert, G., and Fiers, W., *FEBS Lett.*, **35**, 91–96 (1973).
13 Ganem, D., Miller, J. H., Files, J. G., Platt, T., and Weber, K., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3165–3169 (1973).
14 Files, J. G., Weber, K. K., and Miller, J. H., *Proc. natn. Acad. Sci. U.S.A.* (in the press).
15 Anderson, J. S., Bretscher, M., Clark, B. F. C., and Marcker, K. A., *Nature*, **215**, 490–492 (1967).
16 Kondo, M., Eggerston, G., Eisenstadt, J., and Lengyel, P., *Nature*, **220**, 368–370 (1968).
17 Barrell, B. G., in *Procedures in Nucleic Acid Research* (edit. by Cantoni, G. L., and Davies, D. R.), **2**, 751–779 (Harper and Row, New York, 1971).

Incorporation of exogenous DNA into mammalian chromosomes

MANY reports[1–4] have shown that exogenous DNA can be taken up and incorporated into the nuclei of cells in culture as well as *in vivo;* but no morphological evidence has been presented to suggest that such foreign DNA actually becomes associated with the chromosomes of inoculated cells. The Indian barking deer, *Muntiacus muntjak*, has the lowest number of chromosomes yet reported for mammals, ♀ = 6; ♂ = 7 (ref. 5). The combination of such a low chromosome number and the unique morphology of each pair of chromosomes in the diploid set make this animal particularly attractive for comparative cytogenetic studies. The C- and G-banding patterns[6–8] have also been established for this animal which allow even more detailed comparisons to be made than those based solely on gross chromosome morphology.

The purpose of this study was to determine if sequences of highly repetitive exogenous DNA could be incorporated into the muntjak chromosomes and demonstrated cytologically. Cells were inoculated with the synthetic polynucleotide poly (dA)·poly(dT) and then chromosome preparations were carried through the C-banding procedure which demonstrates repetitive DNA sequences[9]. The results suggest that, on the basis of new C bands, poly (dA) · poly (dT) can be incorporated into the muntjak genome (Table 1). The normal C-banding pattern is contrasted in Fig. 1 with a representative example of a cell showing extra C bands which presumably are due to new areas of highly repetitious DNA in the chromosome. Control cells (inoculation conditions 1 and 2) consistently showed a pattern of staining confined to the centromere regions of the autosomes, the centromere and neck of the X chromosome, and the entire Y chromosome. The new C bands were usually observed at the terminal portion of chromosome pair 1.

Seventy-two per cent of metaphases from cells inoculated with only Earle's balanced salt solution (EBSS) had the diploid set of chromosomes. The deviation from diploidy was due primarily to the loss of the small Y chromosome in many metaphases. This was also true for cells inoculated with DEAE · dextran poly(dA) · poly(dT), or the combination of the two. Extra C bands were observed only in the cells treated with poly (dA) · poly (dT) alone or in combination with DEAE · dextran and they occurred with approximately equal frequency in the two conditions.



a *b*

Fig. 1 C-banding patterns of *Muntiacus muntjak*. Cells were treated as described in Table 1. Chromosome spread (*a*) represents control cells inoculated with EBSS only (condition 1), and (*b*) was taken from cells inoculated with poly (dA) · poly (dT) (condition 3). Arrows point to extra C-bands as compared with controls.

Various studies[1,3,4] have shown that exogenous DNA is taken up by mammalian cells and becomes intranuclear to the extent of from 0.5 to 5% of the host cell DNA, but breakdown of the exogenous DNA followed by resynthesis in the nucleus would lead to an apparent nuclear incorporation of the foreign DNA. It has been estimated that the amount of donor DNA that actually becomes incorporated into the chromosomal material of the inoculated cells is less than 0.3% of the host cell DNA[3].

Our study avoids many of the inherent technical problems associated with previous biochemical and biophysical approaches to studying the incorporation of exogenous DNA into mammalian cells. Breakdown and reutilisation of the added poly (dA) · poly (dT) would not be expected to alter the C-banding pattern of the chromosomes, and only the portion of poly (dA) · poly (dT) that becomes associated with the chromosome would be detected by the C-banding technique. Since synthetic homoribopolynucleotides can be taken up by intact mammalian cells[11] without the addition of DEAE·dextran, it is not surprising that there was no difference in the frequency of extra C bands between cells treated with poly (dA) · poly (dT) alone or in combination with DEAE · dextran. The appearance of the extra C bands only at the terminal portion of the chromatids of specific chromosomes is of particular interest in that it suggests new DNA is added at specific sites and that certain chromosomes are more likely to be involved than others.

Table 1 Effect of synthetic DNA on chromosomes of *Muntiacus muntjak*

Inoculation	No. of cells examined	No. diploid (%)	No. with extra C bands (%)
1. EBSS	132	95(72)	0
2. DEAE·dextran	104	60(58)	0
3. poly(dA)·poly(dT)	263	162(62)	30(11·4)
4. DEAE·dextran+ +poly(dA)·poly(dT)	190	150(79)	19(10·0)

Fibroblast cells of the male muntjak were grown in medium MAB 87/3 (ref. 10) with 5% foetal bovine serum screened for coliphages. Slide culture chambers (Bio-Tek) were inoculated with cells and allowed to incubate at 37° C for 2 d to reach log phase of growth and about 1/2 confluency. Inoculation conditions included: (1) EBSS only; (2) DEAE-dextran (DEAE·D) (Pharmacia) (100 μg ml^{-1}) in EBSS; (3) poly (dA) · poly (dT) (PL Biochemicals) (2 μg ml^{-1}) in EBSS; and (4) DEAE·D (100 μg ml^{-1}) and poly (dA) · poly (dT) (2 μg ml^{-1}) in EBSS. Each culture was incubated with one of the above solutions for 15 min at room temperature, then rinsed once with Tris-buffered saline, and fed with 2.5 ml of complete growth medium. After about 36 h at 37° C, colcemide was added to a final concentration of 0.025 μg ml^{-1} and cultures incubated an additional 16 h. Medium was then replaced by a hypotonic solution consisting of medium without serum:H_2O (1:2) for 20 min. Subsequent fixation was in acetic:methanol (1:3), and C-band staining was according to the procedure of Arrighi and Hsu[9]. C-band differentiation was enhanced by a rapid rinse in 50% methanol or a 5 min rinse in 10% methanol followed by a distilled H_2O rinse and air-drying. Metaphase spreads from each culture were then examined at 1,000× for exact count and C-banding patterns. The poly (dA) · poly (dT) used in these studies had a buoyant density of 1.644 in neutral CsCl, $S_{20,w} = 7.9$, and molecular weight = 5×10^5.

The observations reported here are consistent with the hypothesis that under certain conditions exogenous DNA can be taken up by cells and incorporated into the cell genome. An alternative explanation for the extra C bands is that poly (dA) · poly(dT) has indirectly induced the changes in banding patterns. Direct evidence for the incorporation of poly (dA) · poly (dT) into the chromosomes will require *in situ* hybridisation studies. If the new C bands prove to be of poly (dA) · poly (dT) origin and if they are heritable, it should be possible to use synthetic nucleic acids in gene therapy rather than viruses which carry unnecessary and often unwanted genetic information in addition to that which may be therapeutically useful.

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1 Kay, E. R. M., *Nature*, **191**, 387 (1961).
2 Ledoux, L., and Charles, P., *Expl Cell Res.* **45**, 498 (1967).
3 Robins, A. B., and Taylor, D. M., *Nature*, **217**, 1228 (1968).
4 Watters, C., and Gullino, P. M., *Cancer Res.*, **31**, 1231 (1971).
5 Wurster, D. H., and Benirschke, K., *Science*, **168**, 1364 (1970).
6 Hsu, T. C., and Arrighi, F. E., *Chromosoma*, **34**, 243 (1971).
7 Patterson, R. M., and Petricciani, J. C., *Heredity*, **64**, 80 (1973).
8 Brown, J. A., and Cohen, M. M., *Can. J. genet. Cytol.*, **15**, 145 (1973).
9 Arrighi, F. E., and Hsu, T. C., *Cytogenetics*, **10**, 81 (1971).
10 Gorham, L. W., and Waymouth, C., *Proc. Soc. exp. Biol. Med.*, **119**, 287 (1965).
11 Schell, P. L., *Biochim. biophys. Acta*, **240**, 472 (1971).

Site of attachment of J chain to human immunoglobulin M

In spite of a remarkable similarity of the general polypeptide chain structure of immunoglobulins, their molecular weights vary from 150,000 to 950,000 because some occur in monomeric (IgG, IgD and IgE) and others in monomeric or polymeric forms (IgA and IgM) (ref. 1). The largest immunoglobulin, IgM, appears in a stellate configuration of five disulphide-linked subunits (IgM_5), each consisting of two pairs of covalently linked heavy (μ) and light (κ or λ) chains[2]. Recent evidence shows that polymeric immunoglobulins, whether composed of dimers, tetramers or pentamers, contain one molecule of an additional polypeptide, termed J chain[3-5]. Produced within the same cells as immunoglobulins, J chain becomes attached to polymeric IgM and IgA during the final stages of intracellular assembly[6-8]. Available results indicate that this polypeptide is essential for polymerisation of monomeric immunoglobulins[9,10], has a molecular weight of about 15,000 (refs 11–13), and is covalently attached by disulphide bonds to the Fc region of IgM (refs 14–16). Although the covalent structure of μ chain has been determined[17], the exact location of the disulphide bond(s) connecting J chain to μ chain has not been known. To investigate this question, we used available information concerning fragmentation of IgM and of μ and J chains by cleavage with cyanogen bromide (CNBr) (refs 17–20). Because

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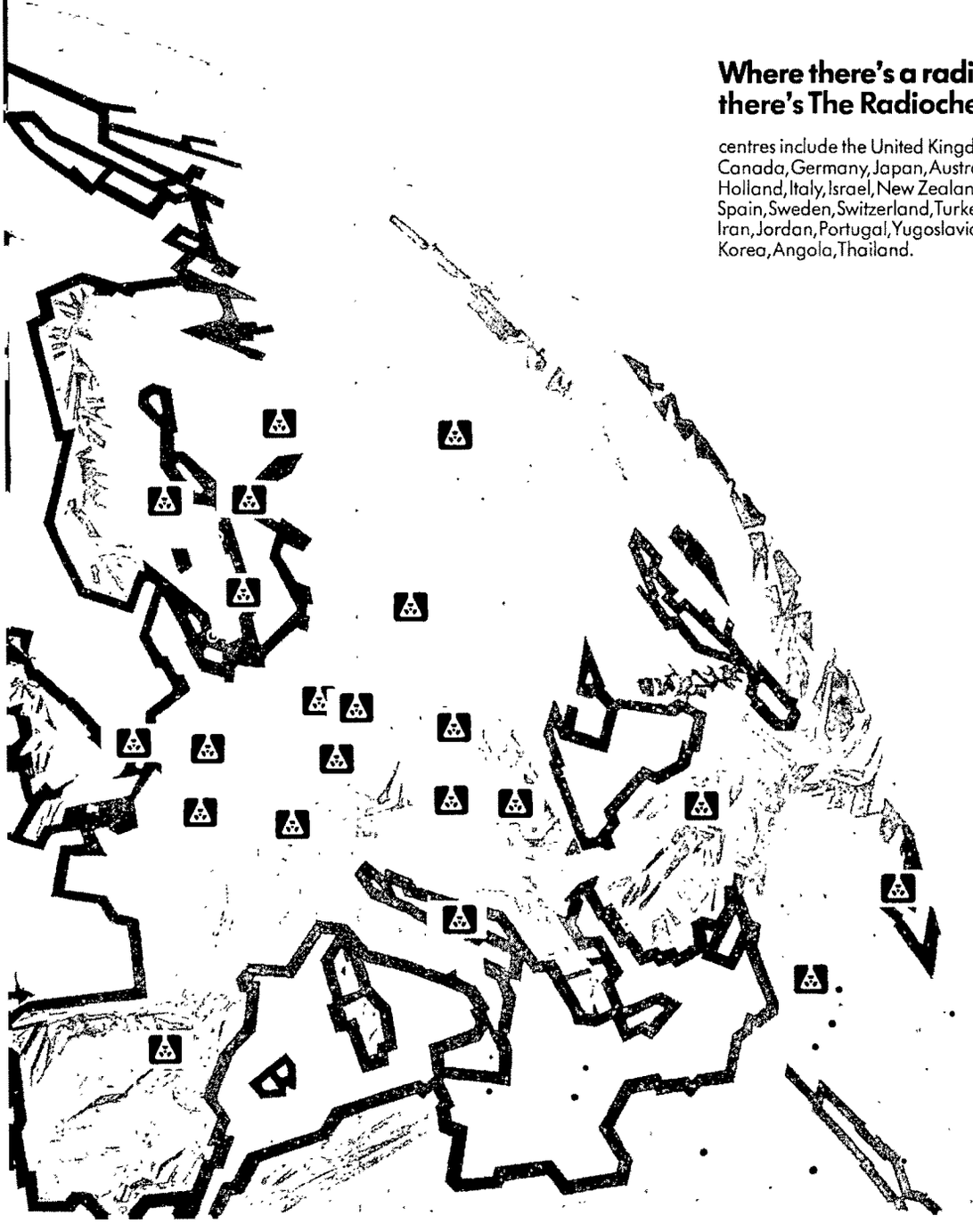
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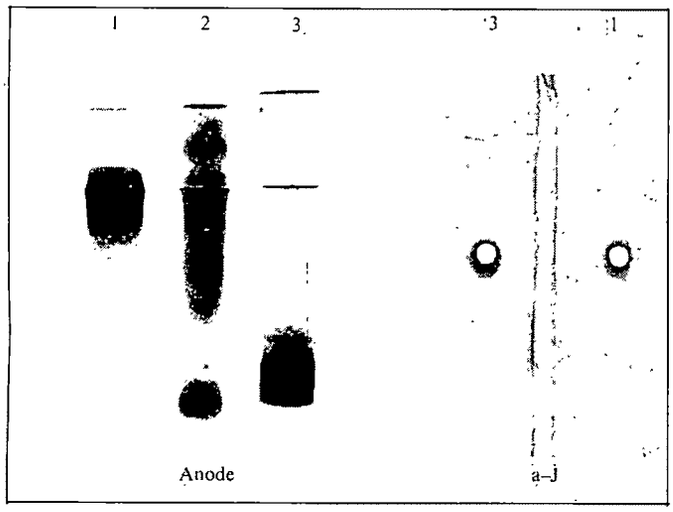


Fig. 1 Disc electrophoresis[22] and immunoelectrophoresis with an anti-J chain serum (a-J) (ref. 23) of CNBr cleaved IgM (1), fraction A Fig. 2 (2), and fraction A purified by DEAE-ion exchange chromatography[23] (3). Lyophilised IgM was dissolved in 70% formic acid at 2% protein concentration and cleaved with CNBr for 4 h at room temperature. The amount of CNBr was double that of the protein. After dilution with ten volumes of distilled water, the material was lyophilised.

this procedure leaves disulphide bridges intact we reasoned that the portion of μ chain(s) linked by these bridges to J chain would be obtainable as a CNBr fragment.

IgM isolated from serum of a patient with Waldenström's macroglobulinaemia[21] was cleaved with CNBr (ref. 20), and the products were examined by disc electrophoresis at *p*H 9.4 (ref. 22) and by immunoelectrophoresis with an antiserum to S-sulphonated J chain[23]. CNBr treatment released a fragment with the electrophoretic mobility and antigenic determinants of J chain (Fig. 1). Purification of this fragment was achieved by extracting CNBr-cleaved IgM with a 1% ammonium bicarbonate buffer brought to neutrality by bubbling with CO_2. Whereas most large fragments are insoluble in this buffer and are removable by centrifugation, fragments containing J chain are soluble and can be further purified by gel filtration through Sephadex G-200 (Fig. 2) and subsequent DEAE-ion exchange chromatography[23]. Disc electrophoresis at *p*H 9.4 and polyacrylamide electrophoresis in the presence of sodium dodecylsulphate[24] showed the purity of this fragment, which reacted with an anti-J chain serum but not with antisera to IgM, or κ and λ chains (Meloy Laboratories).

The molecular weight of a purified J chain-containing fragment was established by sedimentation equilibrium ultracentrifugation in 5 M guanidine-HCl[12]. The average molecular weight of three samples (A_{280nm} = 0.4–0.7) was 15,700 (range 15,400–15,900). Peptide maps and amino acid analyses[20] strongly resembled those of isolated J chain. This fragment, extensively reduced and alkylated, was applied on a column of Sephadex G-25, to separate individual components that were connected by disulphide bonds (Fig. 3). Resulting fractions were desalted, lyophilised, and their amino acids were analysed (Table 1). The first and largest fraction (B1) contained amino acids in proportions resembling those of J chain and its large N-terminal fragment[20]. The second fraction (B2) contained amino acids found in a fragment obtained by CNBr cleavage of isolated J chain[19,20]. The presence of a homoserine residue in B1 and its absence from B2 indicated that the former corresponded to the N-terminal and the latter to the C-terminal parts of J chain. The third fraction (B3) lacked homoserine, was eluted in the same position on Sephadex G-25 as the C-terminal octapeptide of α chain[20], and had an electrophoretic mobility and amino acid composition like that of the C-terminal octapeptide of μ chain[18,25]. These results indicate that the peptide in fraction B3 originated from the C-terminal end of the μ chain and that J and μ chains are connected by a disulphide bridge between the penultimate cysteine residue of μ chain and a cysteine residue in the J chain. The molecular weight of the J chain-containing fragment implies the presence of one, perhaps two, octapeptides.

Cysteine residues involved in inter-IgM_s disulphide bonds are located at position 414 from the N-terminal end of the μ chain[17,26,27]. Attachment of J chain to the penultimate cysteine residue (position 575) of only one or two C-terminal octapeptides indicates that J chain does not join individual IgM_s to form a pentameric molecule of IgM. If J chain, as well as previously described inter-IgM_s disulphide bridges, were to join five IgM_s to form a pentameric molecule, at least five of the ten C-terminal octapeptides would appear in association with J chain. In such a case a considerably increased molecular weight would be evident. Moreover, since at least two cysteine residues of J chain are involved in an intra-chain disulphide bridge[20], only four cysteine residues would be available to interact with IgM_s.

Of five known classes of human immunoglobulins, partial or complete amino acid sequences of the C-terminal parts of heavy chains have been determined for two monomeric immunoglobulins, IgG (ref. 28) and IgE (ref. 29), and for the two polymeric

Table 1 Amino acid compositions of J chain, purified fragment A and its components

Amino acids	J Chain IgM*	Fragment A	B2	JCNBr†	B3
	in residues per 100		residues per chain		
Asp	16.5	16.1	2.0	2.0	1.0
Thr‡	9.0	9.1	2.1	1.9	2.0
Ser‡	5.8	6.4			0.9
Hse		0.7			
Glu	11.6	12.6	1.1	1.0	
Pro	6.6	5.8	2.3	1.9	
Gly	2.2	3.4			1.2
Ala	4.7	4.6	2.2	2.0	1.0
Val	7.1	6.5	0.8	0.8	
Met	0.7				
Ile	5.9	5.1			
Leu	6.5	6.6	1.2	1.0	
Tyr	4.2	3.1	0.7	0.8	0.6
Phe	1.2	1.3			
Lys	4.5	5.3			
His	0.9	1.1			
Arg	7.5	7.2			
Cys§	5.0	5.1	0.6	0.7	0.6

Amino acid analyses were performed on a Beckman 120 C amino acid analyser modified for single column, high speed analyses. Samples were hydrolysed in constant boiling HCl at 108° C for 24 h. All values represent the average of two or more analyses.

* Compiled from previously reported values[4,13].

† Obtained by CNBr cleavage of isolated J chain; small fragment separated on Sephadex G-25 (ref. 20).

‡ Corrected for losses due to acid hydrolysis.

§ Determined as cysteic acid[35].

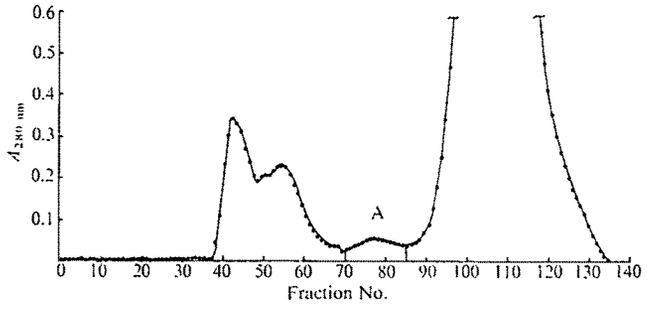


Fig. 2 Gel filtration of material obtained by extraction of CNBr-cleaved IgM with 1% ammonium bicarbonate solution saturated with CO_2 to neutrality. Extracted material was lyophilised, dissolved in 5 M guanidine-HCl, and applied on a column (2.6 × 100 cm) of Sephadex G-200 in 5 M guanidine-HCl. Material with electrophoretic mobility and antigenic determinants of J chain was present in fraction A (Fig. 1). Further purification was performed on DEAE Sephadex column[23].

immunoglobulins, IgM (ref. 17) and IgA (refs 30, 31). A comparison of the C-terminal portions of the heavy chains of these four classes revealed that α and μ chains extend beyond the C-terminal ends of the γ and ε chains by an additional 19 amino acids. The C-terminal octapeptides released by CNBr cleavage of μ and α chains are at the end of this extension[17,30,31]. In polymeric IgA, J chain is also disulphide-linked to the penultimate cysteine residue of the α chain[20,32]. The correspondence in sites of J chain attachment to μ and α chains and the pronounced homology of the two latter chains indicate that this region of both heavy chains is associated with the tendency of IgA and IgM molecules to polymerise.

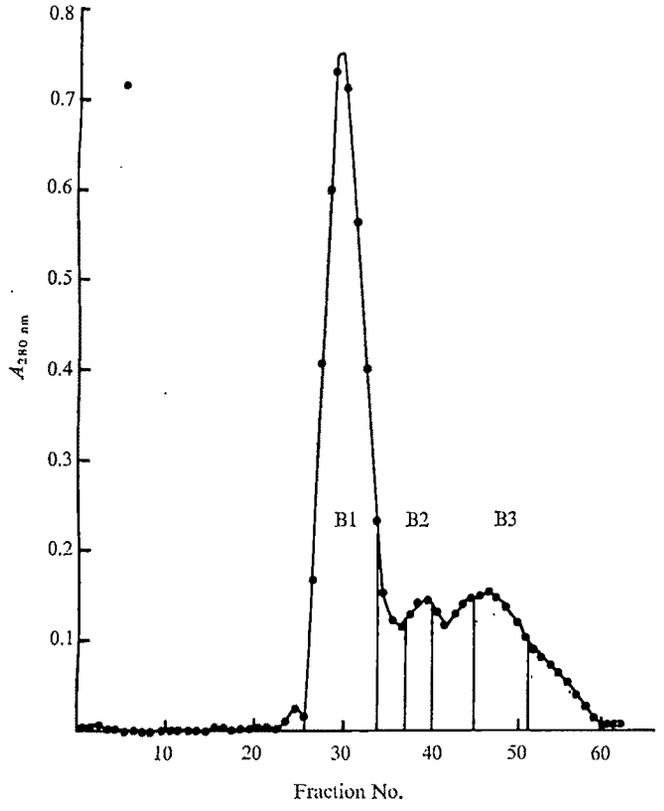


Fig. 3 Gel filtration of purified fraction A, reduced with 0.2 M 2-mercaptoethanol and alkylated with iodoacetamide[20]. A column (1.5 × 35 cm) of Sephadex G-25 fine was equilibrated in 5 M guanidine-HCl. Fraction B2 was further purified by rechromatography on the same column. Desalting was performed on a Sephadex G-10 column in 1% ammonium bicarbonate buffer.

Nevertheless, the mechanism of polymerisation remains speculative. A successive disulphide bond interchange reaction initiated by J chain has been suggested[5,13,33,34]. The incongruity between the necessity of J chain for polymerisation[9,10] and the variant positions of the cysteine residues involved in the inter-IgM$_s$ disulphide bonds (residue 414) and that of J chain attachment (residue 575) may be resolved by postulating a labile disulphide bond between these residues in a monomeric molecule as suggested for IgA (ref. 30). CNBr cleavage failed to release two disulphide-linked C-terminal octapeptides that were released only after subsequent disulphide bond cleavage. This finding led to the conclusion that, rather than being mutually connected by a disulphide bond, the penultimate cysteine residues of the two α chains of monomeric IgA apparently form an intra-α or inter-α chain disulphide bond with the cysteine residue located elsewhere on the α chain. Possibly the penultimate cysteine residues of intracellular monomeric IgM are a part of an analogous labile disulphide bond that, if cleaved in the presence of J chain, might initiate the release of -SH groups that particpate in inter-IgM$_s$ disulphide bonding. With the polymerisation completed, J chain remains attached to the penultimate cysteine.

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1 Edelman, G. M., *Science*, **180**, 830–840 (1973).
2 Metzger, H., *Adv. Immun.*, **12**, 57–116 (1970).
3 Halpern, M. S. and Koshland, M. E., *Nature*, **228**, 1276–1278 (1970).
4 Mestecky, J., Zikan, J., Butler, W. T., *Science*, **171**, 1163–1165 (1971).
5 Koshland, M. E., and Wilde, III, C. E., in *The Immunoglobulin A System* (edit. by Mestesky, J., and Lawton, III, A. R.,) 129–138 (Plenum, New York, 1974).
6 Parkhouse, R. M. E., *Nature new Biol.*, **236**, 9–11 (1972).
7 Halpern, M. S., and Coffman, R. L., *J. Immun.*, **109**, 674–680 (1972).
8 Raam, S. V., and Inman, F. P., *J. Immun.*, **110**, 1044–1050 (1973).
9 Parkhouse, R. M. E., and Della Corte, E., *Biochem. J.*, **136**, 607–609 (1973).
10 Della Corte, E., and Parkhouse, R. M. E., *Biochem. J.*, **136**, 597–606 (1973).
11 O'Daly, J. A., and Cebra, J. J., *Biochemistry*, **10**, 3843–3850 (1971).
12 Schrohenloher, R. E., Mestecky, J., Stanton, T. H., *Biochim. biophys. Acta*, **295**, 576–581 (1973).
13 Wilde, III, C. E., and Koshland, M. E., *Biochemistry*, **12**, 3218–3224 (1973).
14 Mestecky, J., Kulhavy, R., Kraus, F. W., *Fedn Proc.* **30**, Abstr. 1527 (1971).
15 Meinke, G. C., and Spiegelberg, H. L., *Fedn Proc.* **30**, Abstr. 1526 (1971).
16 Inman, F. P., and Ricardo, M. J., *J. Immun.*, **112**, 229–233 (1974).
17 Putnam, F. W., Florent, G., Paul, C., Shinoda, T., and Shimizu, A., *Science*, **182**, 287–291 (1973).
18 Zikan, J., and Bennett, J. C., *Biochim. biophys. Acta*, **317**, 447–461 (1973).
19 Mestecky, J., Kulhavy, R., Stanton, T. H., Wright, G. P., and Bennett, J. C., *Fedn Proc.*, **32**, Abstr. 4203 (1973).
20 Mestecky, J., Schrohenloher, R. E., Kulhavy, R., Wright, G. P., and Tomana, M., *Proc. natn. Acad. Sci., U.S.A.*, **71**, 544–548 (1974).
21 Schrohenloher, R. E. and Bennett, J. C., *J. Immun.*, **107**, 870–880 (1971).
22 Reisfeld, R. A., and Small, Jun., P. A., *Science*, **152**, 1253–1254 (1966).
23 Mestecky, J., Zikan, J., Butler, W. T., and Kulhavy, R., *Immunochemistry*, **9**, 883–890 (1972).
24 Weber, K., and Osborn, M., *J. biol. Chem.*, **244**, 4406–4412 (1969).
25 Wikler, M., Köhler, H., Shinoda, T., and Putnam, F. W., *Science*, **163**, 75–78 (1969).
26 Mihaesco, C., Frangione, B. and Franklin, E. C., *Fedn Proc.*, **29**, Abstr. 2222 (1970).
27 Frangione, B., Prelli, F., Mihaesco, C., and Franklin, E. C., *Proc. natn. Acad. Sci., U.S.A.*, **68**, 1547–1551 (1971).
28 Edelman, G. M., Cunningham, B. A., Gall., W. E., Gottlieb, P. D., Rutishauser, V., and Waxdal, M. J., *Proc. natn. Acad. Sci., U.S.A.*, **63**, 78–85 (1969).
29 Bennich, H., Milstein, C., and Secher, D. S., *FEBS Lett.*, **33**, 49–53 (1973).
30 Prahl, J. W., Abel, C. A., and Grey, H. M., *Biochemistry*, **10**, 1808–1812 (1971).
31 Chuang, C-Y., Capra, D. J. and Kehoe, J. M., *Nature*, **244**, 158–160 (1973).
32 Mendez, E., Prelli, F., Frangione, B., and Franklin, E. C., *Biochem. biophys. Res. Comm.*, **55**, 1291–1297 (1973).
33 Mestecky, J., Schrohenloher, R. E., Kulhavy, R., Wright, G. P., and Tomana, M., in *The Immunoglobulin A System* (edit. by Mestecky, J., and Lawton, III, A. R.), 99–109 (Plenum, New York, 1974).
34 Tomasi, jun., T. B., and Hauptman, S., in *The Immunoglobulin A System* (edit. by Mestecky, J., and Lawton, III, A. R.), 111–120 (Plenum, New York, 1974).
35 Hirs, C. H. W., *Methods Enzymol.*, **11**, 59–62 (1967).

Proportional increase of bursa-derived cells in chickens of the Obese strain

CHICKENS of the Obese strain (OS) spontaneously develop severe autoimmune thyroiditis during the first weeks of life, with clinical signs of hypothyroidism[1,2]. Serological tests reveal both circulating and bound thyroglobulin autoantibodies[3]. The lymphoid infiltration of the thyroid gland is characterised by a predominance of plasma cells and the formation of a large number of germinal centres[4]. In view of the histological and serological features, the spontaneous autoimmune thyroiditis of OS chickens can be considered as an appropriate animal model for human Hashimoto thyroiditis[2].

The major issue in research on autoimmune diseases is the question whether a primary autoantigenic stimulation (for example, by antigenically altered autologous material) of an essentially normal immune system leads to the development of disease, or if an abnormal immune system is incapable of maintaining the state of self tolerance. A combination of both mechanisms may also be involved in pathogenesis.

Investigations in the OS have provided data which support both a primary alteration of the thyroid glands before any signs of lymphoid infiltration do occur[5], and a disturbed function of the immune system as well[6,7]. With regard to the latter, it is noteworthy that the development of spontaneous autoimmune thyroiditis can be suppressed by neonatal or, even better, *in ovo* bursectomy[8], whereas neonatal thymectomy leads to an increase of frequency and severity[9] and also entails a significant augmentation of non-thyroid autoantibodies[6,7,9]. In contrast to experimentally induced autoimmune diseases, the spontaneous autoimmune thyroiditis is therefore considered to be 'B dependent'. From these and other studies[10], a new functional concept for the classification of autoimmune disease into B and T dependent extremes with intermediate forms has been proposed[11], and the diagnostic and therapeutic implications of this concept were discussed.

Table 1 Immunofluorescence tests on 10 NWL and 12 OS chickens at 19 d of embryonic life

Organ	Strain	% of lymphocytes reacting with: ABS	ATS	aIg
Bursa	OS	70	0	70
	NWL	71	0	57
Thymus	OS	2	92	0
	NWL	6	91	0
Spleen	OS	58	43	4
	NWL	29	64	1
Blood	OS	3	12	2
	NWL	ND	7	3

aIg, Anti-whole-chicken immunoglobulin antiserum raised in rabbits. Figures indicate mean % of cells reactive with respective antisera. The lymphoid cells of each organ were pooled from all OS or NWL chickens. ND, not done

Here we describe studies on the distribution of B and T cells in various lymphoid organs of OS chickens by means of immunofluorescence tests with specific turkey anti-chicken bursa cell (ABS) and anti-thymus cell (ATS) sera[12]. Antisera to chicken immunoglobulin have also been employed for the demonstration of immunoglobulin surface determinants as a further B-cell marker[13]. We thought that a postulated hyper-reactivity of the B-dependent portion of the immune system in the OS may be reflected in an altered distribution of B and T cells in the chickens' lymphoid tissues.

Table 2 Immunofluorescence tests on 5 NWL and 4 OS chickens 1.5 weeks after hatching

Organ	Strain	% of lymphocytes reacting with: ABS	ATS	aIgM	aIgG
Bursa	OS	85± 3	8± 2	67± 8	22± 9
	NWL	74± 7	10± 6	62±11	17±14
Thymus	OS	28± 5	63±11	ND	ND
	NWL	11± 6	87± 8	ND	ND
Spleen	OS	58± 6	41± 5	50±14	5± 1
	NWL	19± 4	56± 9	12± 4	6± 6
Blood	OS	27± 2	63± 5	19± 6	4± 3
	NWL	21± 5	68± 3	18± 5	1± 2

aIgM, Anti-chicken-IgM-serum raised in rabbits; aIgG, anti-chicken-IgG-serum raised in rabbits. Cell suspensions were not pooled. Figures indicate mean and s.d. ND, not done.

Lymphoid cells from the bursa of Fabricius, thymus and spleen were prepared by consecutively passing tissue fragments through 80 and 200 mesh screens and washing the cell suspensions in phosphate buffered saline (*p*H 7.2). Blood lymphocytes were obtained by sedimentation in polyvinylpyrrolidone (PVP) (refs 12, 13). The viability of the cells was always >90%. The reaction of cells with ABS or ATS was visualised in an immunofluorescent staining procedure on living cells[14]. The FITC-labelled antiserum to turkey immunoglobulin used in the indirect immunofluorescence tests was produced in our laboratories and characterised as described earlier[12]. Appropriate control experiments to exclude nonspecific staining were also carried out[12,13]. The conjugate was absorbed with chicken immunoglobulins. All readings were done on coded slides and with epi-illumination on a Reichert Zetopan microscope equipped with a HBO-200 mercury vapour lamp and suitable exciter and barrier filters[12]. After the immunofluorescent staining procedure the cells were counterstained with Evans blue to facilitate counting. IgM and IgG determinants on the surface of lymphocytes were detected in an indirect immunofluorescent test using specific rabbit antisera prepared as described elsewhere (B.A. and G.W., unpublished).

Table 1 gives the results obtained with cell suspensions of 19-d-old embryos. At this age there is a greater percentage of B cells in the spleen of OS chickens than in the spleen of NWL controls. No such feature was found in the other lymphoid populations tested. Using an anti-whole-immunoglobulin serum, there seems to be an increase of cells with immunoglobulin surface determinants (ISD) in the OS chicken bursa as compared with NWL birds.

Table 2 summarises the data from chickens 1.5 weeks after hatching. At this age an increase of B cells is not only found in the spleen but also in the thymus of OS as compared to NWL birds. Analysis of the cell populations

Table 3 Immunofluorescence tests on three NWL and three OS chickens 4 weeks after hatching

Organ	Strain	% of lymphocytes reacting with: ABS	ATS	aIg	aIgM	aIgG
Bursa	OS	91± 6	1± 3	76± 2	65±15	29± 3
	NWL	86± 7	4± 2	91± 9	44± 4	42±11
Thymus	OS	25± 6	68± 5	20± 3	15± 3	7± 1
	NWL	3± 3	93± 4	4± 2	1± 1	1± 2
Spleen	OS	57± 4	33± 6	53± 7	33± 8	15± 2
	NWL	28± 7	52± 9	35± 5	15± 2	18± 8
Blood	OS	21± 3	68± 7	16± 7	11± 5	9± 2
	NWL	23± 5	66± 6	19± 3	14± 9	10± 6

See legend for Tables 1 and 2

with chain specific anti-immunoglobulin sera revealed a preponderance of IgM over IgG-carrying B cells in the spleen of OS chickens. The additive values obtained with anti-IgM and anti-IgG were in good agreement with the figures obtained by incubating the cells with ABS. Four weeks after hatching a similar pattern was obtained (Table 3), but now the ratio of IgM to IgG carrying cells was also higher in the bursa of the OS as compared to NWL controls.

Table 4 shows that in 9-week-old OS chickens the proportion of B cells is increased in spleen, thymus and blood, and that here again most of these cells bear IgM on their surface. From the data obtained with chain-specific anti-immunoglobulin sera it seems reasonable to assume that the increment in B cells in lymphoid organs of the OS chickens is made up of IgM–ISD carrying cells. As in the 4-week-old animals, this IgM shift is also reflected in the bursal cell population.

A crucial observation in these studies was the altered splenic proportion of B and T cells already in OS embryos, that is, well before any signs of thyroiditis can be found. Thus, this increase of B cells in OS birds as compared with NWL chickens seems to be independent of the thyroiditis-induced metabolic or endocrine status. The occurrence of nearly 30% of B cells in the thymus of 1.5-week-old OS birds again emphasises this point. In NWL chickens such high numbers of B-cells in the thymus are only rarely attained, and if they do occur at all in healthy, unimmunised birds, they are found much later in life[15].

It may be hypothesised that the abnormal invasion of the OS thymus by B cells may impair normal maturation and regulatory processes in it, or that some primary derangement of the thymus function is a prerequisite for such an abnormal immigration of B cells. The late appearance of an increase in B cells in OS chicken blood may be explained either by the fast migration of 'pathological' B cells from blood to the tissues and thus the maintenance of normal levels of B cells in the blood for a longer period until the capacity of the peripheral lymphoid tissues for B cells is saturated, or by a selective microenvironment specifically attracting B cells into the prospective lymphoid organs early in the animals development.

Table 4 Immunofluorescence tests of four NWL and four OS chickens at 9 weeks of age

Organ	Strain	% of lymphocytes reacting with: ABS	ATS	aIgM	aIgG
Bursa	OS	92± 6	7± 3	71±12	35± 6
	NWL	91± 8	6± 5	48± 9	55± 6
Thymus	OS	32± 6	67±13	22± 5	9± 4
	NWL	12± 8	86± 5	4± 2	7± 3
Spleen	OS	60± 9	40± 6	39± 3	26± 8
	NWL	38± 5	58± 8	15± 6	22± 4
Blood	OS	56± 7	26± 8	34± 7	14± 5
	NWL	23± 6	70±11	12± 4	10± 3

See legend for Tables 1 and 2

Attempts to delineate B and T cells in NZB mice, the other well established model of spontaneous autoimmune disease, gave ambiguous results[16,17]. An alteration of the proportion of T cells to B cells has only been observed very late in the animals life, after the onset of clinical symptoms of the disease.

Our results are the first step towards a diagnostic application of the concept of B and T dependent autoimmune diseases[10,11]. As stated above, this concept is only concerned with the effector cells, and, in the case of OS chickens, the postulated hyper-reactivity of the B cell system does not exclude the posssibility that the initial aetiological factor in this strain may be a deficient T-cell depressor compartment[2]. In addition, the present approach may not only provide information about diagnostic aspects of autoimmune disease but also entail the use of appropriate T or B-cell specific immunosuppressive procedures. In the case of the OS, this point has already been substantiated by suppressing the spontaneous autoimmune thyroiditis with anti-bursa cell[18] or anti-immunoglobulin sera (G.W., B.A. and W. Johnson, unpublished). That this concept may also be true for human autoimmune diseases was confirmed in recent studies by Chused *et al.*[19] who demonstrated a significant increase of B cells in Sjögrens syndrome.

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1 Cole, R. K., Kite, J. H., jun., and Witebsky, E., *Science*, **160**, 1357 (1968).
2 Wick, G., Sundick, R. S., and Albini, B., *Clin. Immun. Immunopath.*, (in the press).
3 Wick, G., Witebsky, E., Kite, J. H., jun., and Beutner, E. H., *Clin. exp. Immun.*, **7**, 173 (1970).
4 Witebsky, E., Kite, J. H., jun., Wick, G., and Cole, R. K., *J. Immun.*, **193**, 708 (1969).
5 Sundick, R. S., and Wick, G., *Gen. comp. Endocr.* (in the press).
6 Wick, G., *Clin. exp. Immun.*, **7**, 187 (1970).
7 Albini, B., and Wick, G., *Immunology*, **21**, 957 (1971).
8 Wick, G., Kite, J. H. jun., Cole, R. K., and Witebsky, E., *J. Immun.*, **104**, 45 (1970).
9 Albini, B., and Wick, G., *Immunology*, **24**, 545 (1973).
10 Wick, G., and Steiner, R., *J. Immun.*, **109**, 471 (1972).
11 Wick, G., and Albini, B., in *Immunopathology, 7th Int. Symp.*, (edit. by Miescher, P.A.) (in the press).
12 Wick, G., Albini, B., and Milgrom, F., *Clin. exp. Immun.*, **15**, 237 (1973).
13 Albini, B., and Wick, G., *Int. Arch. Allergy*, **44**, 804 (1973).
14 Möller, G., *J. exp. Med.*, **114**, 415 (1961).
15 Albini, B., and Wick, G., *J. Immun.*, **112**, 444 (1974).
16 Stobo, J. D., Talal, N., and Paul, W. E., *J. Immun.*, **109**, 701 (1972).
17 Waksman, B. H., Raff, M. C., and East, J., *Clin. exp. Immun.*, **11**, 1 (1972).
18 Wick, G., and Witebsky, E., *Int. Arch. Allergy*, **40**, 603 (1971).
19 Chused, T. M., Hardin, J. A., Frank, M. M., and Green, I., *J. Immun.*, **112**, 641 (1974).

Reversal of immunological tolerance by cyclophosphamide through inhibition of suppressor cell activity

IT has been shown in mice that specific immunological unresponsiveness to contact sensitisation with picryl chloride induced by pretreatment with picryl sulphonic acid is a positive phenomenon rather than an absence of specific immunocompetent cells[1]. In this system it was shown that transfer of lymph node cells from unresponsive to normal mice would limit the recipients' contact sensitivity response to picryl chloride and that these cells had characteristics suggesting that they were T as opposed to B cells[2]. We should like to report that a similar state of unresponsiveness to the dinitrophenyl group in guinea pigs can be broken by treatment with cyclophosphamide (CY) just before sensitisation and this is associated with the return of the ability of T cells to proliferate in the draining lymph node as part of the immune response.

The guinea pigs used were of the outbred white spotted Himalayan strain (400–450 g). Tolerance was induced by two intravenous injections of 500 mg kg^{-1} dinitrobenzene-

Table 1 Reversal of tolerance to dinitrophenyl hapten by cyclophosphamide

Experimental protocol		Mean skin reactivity				PCA titre*
	Time after sensitisation (d)	14	28	56	70	
1 SO_3–SO_3	DNFB (CFA)	0 (6)	0 (6)		0 (6)	0 (6)
2 SO_3–SO_3 CY (3 d)	DNFB (CFA)	1.25 (6)	1.0 (5)		1.0 (5)	1:22 (6)
3 SO_3–SO_3 CY (14 d)	DNFB (CFA)	0 (6)		0 (6)		1:7 (6)
4	DNFB (CFA)	1.9 (6)				1:30 (7)
5 SO_3–SO_3	DNCB (contact)	0 (6)	0 (6)		0 (6)	
6 SO_3–SO_3 CY (3 d)	DNCB (contact)	1.1 (5)	0.5 (5)		0.75 (4)	
7	DNCB (contact)	1.3 (8)				

* 21 d after sensitisation.

SO_3–SO_3 indicates $DNBSO_3$ given at intervals of 14 d. In experiments 1, 2, 5 and 6 sensitisation was with DNFB in CFA or contact with DNCB 14 d after the last dose of $DNBSO_3$. In experiments 2 and 6 sensitisation was preceded by CY, 3 d previously. In experiment 3 sensitisation was with DNFB in CFA 28 d after the last dose of $DNBSO_3$ and was preceded by CY, 14 d previously. The figures in parentheses refer to the number of animals in each experiment.

sulphonic acid sodium salt ($DNBSO_3$) with an interval of 14 d, the last dose being 14 or 28 d before an attempt at sensitisation with 500 μg of 2, 4-dinitrofluorobenzene (DNFB) in complete Freund's adjuvant (Difco) (CFA) or contact with 50% 2, 4-dinitrochlorobenzene (DNCB) in acetone on the ear. Sensitivity was tested by contact with 0.09, 0.05 and 0.03% DNCB in acetone on the flank. The intensity of skin reactions was assessed 24 h later as described previously[3]. Animals were injected intraperitoneally with 250 mg kg^{-1} CY either 3 or 14 d before sensitisation. Antidinitrophenyl antibodies were detected three weeks after sensitisation by passive cutaneous anaphylaxis[4]. Auricular lymph nodes draining the site of application of a sensitising dose of DNCB 4 d previously were examined for immunoblast (large pyroninophilic cell) proliferation in paracortical areas by counting the number of these cells in a microscopic field of diameter 450 μm. At least 10 lymph nodes were examined in each group.

Table 1 shows that CY given 3 d before sensitisation, 14 d after the last dose of $DNBSO_3$ effectively reverses the unresponsiveness of guinea pigs to DNFB or DNCB. Reversal of unresponsiveness was found both with contact sensitivity (a cell-mediated immune phenomenon) and in the production of humoral antibodies detectable by PCA. Reversal of unresponsiveness in contact sensitivity persisted beyond 70 d after immunisation and therefore was not a temporary phenomenon. But if sensitisation was not attempted until 14 d after CY, no reversal of tolerance could be detected in contact sensitisation although low titres of PCA antibody were produced. This would indicate that sensitisation had to be within a limited period after CY to be fully effective.

A comparison of the effect of these various measures on T-cell proliferation in the lymph node draining the site of sensitisation is shown in Table 2. It can be seen that the development of unresponsiveness by treatment with $DNBSO_3$ is associated with an eight-fold reduction in the number of immunoblasts seen 4 d after sensitisation with DNCB. Treatment of these DNCB unresponsive animals with CY 3 d before sensitisation was associated with a four-fold rise in the number of immunoblasts to the level found in control animals given CY 3 d before sensitisation. But if sensitisation was delayed to 14 d after CY, the level of immunoblasts in the draining node did not return to even half the level found in control animals sensitised 14 d after CY, which had not been previously made unresponsive with $DNBSO_3$. These findings seem to parallel the peripheral manifestations of the immune response. The return of ability of unresponsive animals to react is associated with a reversal of the depression of the ability of T cells to proliferate in the draining lymph nodes following sensitisation. The failure to show reversal of unresponsiveness if sensitisation is delayed 14 d after CY corresponds with an inability to obtain a level of immunoblast proliferation nearer to that found in control sensitised animals.

The increased proliferation of T cells in tolerant animals treated with CY just before sensitisation cannot be simply a rebound phenomenon, since in normally sensitised animals pretreatment with CY results in a significant decrease in immunoblasts in the draining node.

The preferential inhibition of suppressor cell activity by CY has been demonstrated previously in normal immune responses. These include contact sensitivity and the Jones–Mote type of delayed hypersensitivity in the guinea pig[5–7]. Cyclophosphamide seems to act on immunological mechanisms by depressing B lymphocytes in preference to T lymphocytes and is selectively cytotoxic to those cells which are short lived and have a more rapid rate of turnover in peripheral lymphoid tissues[8–10]. These changes have been demonstrated both histologically and by the use of techniques to detect the θ antigen on lymphocytes on the mouse as well as by labelling studies using ^{125}IUDR and ^{51}Cr. As it can be reversed by treatment with CY, specific immunological tolerance might be considered to be the result of active suppression of the ability of T cells to proliferate in lymphoid tissue. These observations also indicate that, in this model, active suppression of T-cell function is on the afferent rather than the efferent side of the immune arc. Moreover we have observed that tolerance to DNCB can be reversed in syngeneic animals by transfusion of cells from actively sensitised donors. This is a further indication that suppression does not occur on the efferent side of the arc[11]. The finding that CY reverses tolerance if animals are sensitised 3 d later effectively excludes the role of enhancing antibody or circulating immune complexes in this system, as antibody levels would not be significantly reduced such a short time after treatment.

It seems therefore that CY has a preferential action on clones of suppressor cells, releasing effector cells for proliferation in cell-mediated immunity and in antibody production. Our results also show that suppressor cells can regenerate within 2 weeks of CY treatment, so that sensitisation by DNFB at this time fails. In spite of previous observations that CY acts preferentially on B rather than

Table 2 Cytology of lymph nodes draining site of application of 50% DNCB 4 d previously

Experimental protocol		Immunoblasts in paracortical area field* 450 μm diameter
1 SO_3–SO_3	DNCB	24.5± 9.9
2 SO_3–SO_3 CY (3 d)	DNCB	108 ±48
3 SO_3–SO_3 CY (14 d)	DNCB	64.5±29
4 CY (3 d)	DNCB	134 ±46
5 CY (14 d)	DNCB	169 ±59.4
6	DNCB	194 ±57

* Arithmetical mean ± s.d.

Significance of differences 1+2, $P<0.001$; 2+4, P: no significant difference; 3+5 $P<0.001$; 4+6, P: $0.01>P>0.002$.

• Notation of experimental protocol is as in Table 1.

T cells, our present observations do not exclude a role for short lived rapidly proliferating suppressor T cells that might be affected by CY in the same way as B cells and differently from effector T cells. At present one cannot say whether in this system the suppressor cells belong to a T or a B cell population.

A similar reversal of homograft tolerance has been described following irradiation in mice[12]. But in this and other reports the effect can only be obtained in weak or already terminating states of tolerance[13].

In conclusion we suggest that CY can break immunological tolerance by inhibition of a population of suppressor cells that effectively prevent T-cell proliferation in the draining lymphoid tissue and thus prevent the release of specifically sensitised cells into the periphery. Whether these suppressor cells act by preventing antigenic recognition or by direct inhibition of the proliferative phase of the immune response has not as yet been determined.

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1 Asherson, G. L., Zembala, M., and Barnes, R. M. R., *Clin. exp. Immun.*, **9**, 111 (1971).
2 Zembala, M., and Asherson, G. L., *Nature*, **244**, 227 (1973).
3 Frey, J. R., de Weck, A. L., and Geleick, H., *J. invest. Derm.*, **42**, 41 (1964).
4 Polak, L., Frey, J. R., and Turk, J. L., *Immunology*, **25**, 451 (1973).
5 Turk, J. L., Parker, D., and Poulter, L. W., *Immunology*, **23**, 493 (1972).
6 Turk, J. L., and Parker, D., *Immunology*, **24**, 751 (1973).
7 Katz, S. I., Parker, D., Sommer, G., and Turk, J. L., *Nature*, **248**, 612 (1974).
8 Turk, J. L., and Poulter, L. W., *Clin. exp. Immun.*, **10**, 285 (1972).
9 Poulter, L. W., and Turk, J. L., *Nature new Biol.*, **238**, 17 (1972).
10 Turk, J. L., and Poulter, L. W., *Int. Arch. Allergy*, **43**, 620 (1972).
11 Polak, L., Geleick, H. and Frey, J. R., *Monogr. Allergy*, **8**, 168 (1974).
12 Mäkelä, O., and Nossal, G. Z. V., *J. Immun.*, **88**, 613 (1962).
13 Hraba, T., *Monogr. Allergy*, **3**, 66 (1968).

Augmentation of lymphocyte reactivity in guinea pigs, mice, monkeys and humans sensitised to BCG, dinitrochlorobenzene or nitrogen mustard

NONSPECIFIC augmentation of tumour immunity has been achieved through the use of Bacillus Calmette-Guérin (BCG), *Corynebacterium parvum*, *Toxoplasma gondii*, *Besnoitia jellisoni* and other intracellular parasites[1-4]. Nonliving preparations, such as cell walls from BCG, also cause nonspecific augmentation of tumour immunity[5]. The mechanisms underlying these effects are not clear. It has been postulated that amplification of macrophage or reticuloendothelial function is responsible for the enhancement of tumour immunity by these agents. In the mouse, BCG causes a change in the rate of activation of macrophages, as well as a change in their lysosomal content and bacteriolytic power[6,7]. In the guinea pig, there is an initial granulomatous reaction at the BCG inoculation site, followed by a peripheral histocyte-mediated destruction of tumour cells[8]. We have now examined the effect of intradermal BCG on the 'specific' and 'nonspecific' responsiveness of circulating lymphocytes. We also investigated the ability of two chemical agents to produce similar or additive effects.

Strain 2 guinea pigs were inoculated intradermally with either BCG or dinitrochlorobenzene (DNCB), or nitrogen mustard, or all three of these agents. Each agent produced a local delayed hypersensitivity reaction. BCG was given as an 0.1 ml inoculation of *Mycobacterium bovis* vaccine (Research Foundation, Chicago, Illinois). DNCB was given as an 0.1 ml intradermal inoculation containing 2,000 μg dissolved in acetone. Nitrogen mustard was administered as an 0.1 ml intradermal inoculation of 100 μg of Mustargen (Merck, Sharpe and Dohme) in physiological saline. Animals that received all three agents received three separate intradermal inoculations.

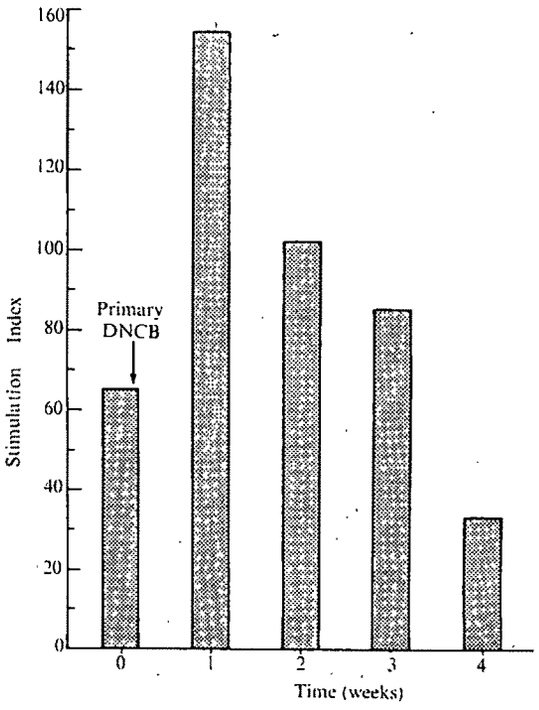


Fig. 1 Effect of primary DNCB exposure on lymphocyte responsiveness to PHA in 13 patients

Six weeks later, blood was drawn by cardiac puncture and lymphocytes were separated on Ficoll-Hypaque gradients and tested for reactivity against phytohaemagglutinin (PHA) and tuberculin purified protein derivative (PPD) using a microtechnique[9]. Two hundred thousand lymphocytes per well were incubated in a Falcon Microtest plate for 48 h with 10 μg of PHA or PPD, followed by a 24 h incubation with 2 μCi of ^{3}H-thymidine, and then liquid scintillation counting of the d.p.m. of the ^{3}H-thymidine taken up by cellular DNA. The same assay was used to test guinea pig, monkey and human circulating lymphocytes. Slightly different conditions were used to test mice, since lymphocytes were of splenic origin, and were incubated with PHA for shorter periods[10].

Table 1 shows a typical experiment in which guinea pigs treated with BCG had a higher responsiveness to PHA than did guinea pigs not treated with BCG. The same is true for reactivity against PPD. Pigs treated with a combination of BCG, DNCB and nitrogen mustard had the highest reactivities to PHA and PPD. Other studies showed that intradermal DNCB or nitrogen mustard alone also increased PHA responsiveness. Similarly, in mice, increased PHA reactivity of lymphocytes was observed in animals inoculated subcutaneously 10 d previously with 1,000–2,000 μg of DNCB.

Table 1 Effect of intradermal BCG, DNCB and nitrogen mustard on guinea pig lymphocyte stimulation by PHA and PPD

Sensitisation	^{3}H-Thymidine (d.p.m.) Media	PHA	PPD	Stimulation index of circulating lymphocytes PHA	PPD
Nothing	3,478 ± 428	4,869 ± 308	2,670 ± 74	1.4	0.8
BCG	3,769 ± 726	11,852 ± 1,845	6,834 ± 1,300	3.1	1.8
BCG, DNCB and nitrogen mustard	4,774 ± 1,282	17,023 ± 832	11,385 ± 3,797	3.6	2.4

The possibility of enhancement by DNCB and nitrogen mustard of BCG induced-lymphocyte responsiveness was then examined in seven rhesus monkeys. Two monkeys had received no previous BCG and were PPD negative. Five monkeys had been inoculated intradermally with 10^7 viable BCG organisms every 2 to 3 weeks for 4 months. On repeated skin testing with second strength PPD, two of the latter five had developed a positive PPD reaction, and two were negative to intradermal PPD in spite of repeated BCG administration. Blood lymphocytes from all these animals were tested for PHA and PPD reactivity (Table 2).

Table 2 Effect of BCG treatment on monkey lymphocyte stimulation by PHA and PPD

Monkey no.	BCG treatment every 2-3 weeks × 4 months	Skin reactivity to PPD	Stimulation index of circulating lymphocytes PHA	PPD
50	No	Neg.	6.0	0.86
605	No	Neg.	8.2	1.6
248	Yes	Neg.	21.3	6.0
922	Yes	Neg.	33.0	4.0
676	Yes	±	18.0	7.3
730	Yes	Pos.	8.5	4.3
708	Yes	Pos.	14.1	19.0

As expected, the PPD stimulation index was lowest in monkeys receiving no BCG (0.86–1.6), and considerably higher in treated monkeys (4.0–19.0). As was true in other species, the PHA reactivity was also enhanced by BCG, varying from 8.5 to 33.0, compared with 6.0 and 8.2 in the two control monkeys.

Four of the monkeys were then randomly chosen for intradermal inoculation with 0.2 ml of DNCB and 0.2 ml of nitrogen mustard. The preparations were the same as described above for the guinea pigs. When blood lymphocytes were tested 1 week later, there was an average increase of 106% in the PHA stimulation index and 221% in the PPD stimulation index. In two animals not treated with DNCB and nitrogen mustard, the average PHA and PPD stimulation indices fell 11% and 24% respectively. These data are summarised in Table 3.

Table 3 Effect of DNCB and nitrogen mustard on BCG-induced enhancement of lymphocyte responsiveness

Monkey no.	DNCB and nitrogen mustard	Stimulation index PHA	PPD	% Change in stimulation index PHA	PPD
730	No	3.4	2.5	− 60%	− 42%
605	No	11.4	1.5	+ 39%	− 6%
248	Yes	84.0	41.3	+ 300%	+ 583%
708	Yes	27.3	10.0	+ 93%	− 47%
922	Yes	26.7	9.8	− 19%	+ 145%
676	Yes	27.1	22.1	+ 51%	+ 203%

In the final monkey experiment, three monkeys (Nos 708, 922 and 676) which had previously received BCG, DNCB and nitrogen mustard were given all three agents again. Their average PHA stimulation index 1 week later was 63, representing average increases of 133% over the immediately previous values and 190% over the initial values. The largest increase of the three was in monkey 708, which was the only PPD positive animal in that subgroup.

In patients with or without previous BCG treatment, we have observed that topical application of 2,000 μg of DNCB increases PHA reactivity of circulating lymphocytes. The data from 13 patients with melanoma, sarcoma and adenocarcinoma are presented in Fig. 1. Although no patient was tested at every point, the composite of all the values shows a doubling of PHA responsiveness after the first exposure to DNCB. The average stimulation index 4 weeks after primary sensitisation was less than the average value before sensitisation. This may be due to an undershoot following the peak stimulation, or may reflect a lack of stability in this population of cancer patients, several of whom underwent surgery, radioisotopic scans or other diagnostic procedures during the interval of study. These factors are being evaluated. Patients exposed a second time to DNCB also showed enhancement of PHA responsiveness.

Our studies support the conclusion that agents that produce a delayed hypersensitivity reaction increase PHA responsiveness of the host's circulating lymphocytes. This enhancement of peripheral lymphocyte reactivity may be a primary pathway by which BCG is effective in tumour immunotherapy. As in some forms of chemotherapy, the combination of agents seems more effective than either agent alone. The application of this observation to immunotherapy and tumour-specific immunity is being examined.

During immunological monitoring the effect of one test on the results of another should be considered, as well as the effect of the test on the patient's immune status. Reactivity to DNCB has been found to be a favourable prognostic sign in patients undergoing surgery for malignancy[11]. It is also conceivable that the nonspecific stimulation of circulating lymphocytes produced by DNCB sensitisation has some adjunctive therapeutic value

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[1] Mathé, G., *et al.*, *Lancet*, i, 697 (1969).
[2] Mathé, G., Pouillart, P., and Lapeyraque, F., *Br. J. Cancer*, **23**, 814 (1969).
[3] Currie, G. A., and Bagshawe, K. D., *Br. med. J.*, **1**, 541 (1970).
[4] Nauts, H. C., Fowler, G. A., and Bagatko, F. H., *Acta med. scand.*, Suppl. **276**, 1 (1953).
[5] Bartlett, G. L., and Zbar, B., *J. natn. Cancer Inst.*, **50**, 1385 (1973).
[6] Collins, F. M., and Mackaness, G. B., *Cell. Immun.*, **1**, 253 (1970).
[7] Collins, F. M., and Mackaness, G. B., *Cell. Immun.*, **1**, 266 (1970).
[8] Hanna, M. G., Zbar, B., and Rapp, H. J., *J. natn. Cancer Inst.* **48**, 1441 (1972).
[9] Parker, J. W., and Lukes, R. J., *Am. J. clin. Path.*, **56**, 174 (1971).
[10] Strong, D. M., Ahmed, A. A., Thurman, G. B., and Sell, K. W., *J. Immun. Methods*, **2**, 279 (1973).
[11] Eilber, F. R., and Morton, D. L., *Cancer*, **25**, 362 (1970).

Transplantation of allogeneic lymphoid cells specifically depleted of graft *versus* host reactive cells

GRAFT *versus* host (GvH) disease has been a major problem in the transplantation of allogeneic haematopoietic or immunocompetent lymphoid cells. In man, attempts to reduce the GvH effect of transplanted allogeneic cells, including HL-A matching[1,2], cell separation techniques[3], anti-lymphocyte globulin[4], and injection of small doses of bone marrow cells[5], have aided successful bone marrow transplantation. Various techniques have been used in animal systems[6-14] to reduce the GvH reaction by removing reactive cells from populations of lymphoid cells. So far, however, there has been no attempt to assess the potential value of such specifically depleted cells in transplantation.

Recently we have shown that one of these techniques, adsorption of immune cytotoxic thymus-derived (T) cells onto monolayers of target cells bearing the sensitising antigens, is greatly improved by centrifugation[15], thus giving 70–90% depletion of cytotoxic cells. These results encouraged us to apply this fractionation technique to deplete nonimmune lymphoid precursor cells carrying surface receptors for mouse alloantigens. We have now found that normal BALB/c ($H\text{-}2^d$) spleen cells can be depleted specifically of GvH reactive cells (against histoincompatible C57BL/6 ($H\text{-}2^b$) alloantigens), and that lethally irradiated C57BL/6 mice transplanted with the fractionated BALB/c allogeneic cells survive with no apparent signs of GvH disease.

Fractionation of normal BALB/c spleen cells on C57BL/6 spleen cell monolayers abolished their capacity to become sensitised *in vivo* against C57BL/6 alloantigens as measured *in vitro* by the ^{51}Cr release assay using EL-4 ($H\text{-}2^b$) labelled target cells (Table 1). The specificity of the adsorption was investigated using monolayers prepared with antigenically unrelated cells. BALB/c spleen cells which had been adsorbed on either BALB/c or SJL/J ($H\text{-}2^s$) monolayers or on empty dishes (not shown) retain the ability to become sensitised against C57BL/6 and elicit cell-mediated cytotoxicity (CMC) *in vitro* (Table 1, experiment 2). These results are consistent with the concept that precursor cells for CMC, known to be T cells[15,17,18], possess receptors on their surface which can recognise and bind specifically to the corresponding cell surface antigens.

We next investigated whether the fractionated spleen cells can reconstitute lethally irradiated allogeneic recipients in the absence of developing fatal GvH disease. C57BL/6 mice injected with: (1) unfractionated BALB/c spleen cells; (2) BALB/c spleen cells adsorbed either on BALB/c or on SJL/J spleen cell BALB/c monolayers, and (3) spleen cells adsorbed on empty plastic dishes, all developed GvH disease characterised by runting, and died within 2–4 weeks of inoculation. On the other hand, about 70% of the C57BL/6 mice that received 4–6 $\times$ 10^7 BALB/c spleen cells adsorbed on C57BL/6 monolayers survived more than 6 months with no signs of disease (Table 2). Control lethally irradiated SJL/J mice injected with 4 $\times$ 10^7 BALB/c splenocytes either unfractionated or depleted on C57BL/6 monolayer all died within 3 weeks (not shown).

Table 1 Failure of *in vivo* sensitisation of BALB/c spleen cells adsorbed onto C57BL/6 spleen cell monolayer

Experiment	No. of BALB/c donor cells	Spleen cell monolayer used for adsorption	Cell-mediated cytotoxicity at effector target ratio of 100:1	25:1
1	6×10^7	None	45.8 ± 0.9	39.5 ± 1.6
		C57BL/6	−0.2 ± 0.4	−0.06 ± 0.01
2	5×10^7	None	30.0 ± 0.04	23.2 ± 1.8
		C57BL/6 ($H\text{-}2^b$)	1.4 ± 0.04	0.02 ± 0.6
		BALB/c ($H\text{-}2^d$)	29.2 ± 0.8	15.2 ± 1.4
		SJL/J ($H\text{-}2^s$)	25.2 ± 0.4	21.9 ± 2.4
	None	——	−0.8 ± 0.27	−1.63 ± 0.4

Donor cells from 10–12-week-old male BALB/c mice were teased and a cell suspension was prepared in Eagle's minimal essential medium (MEM). Various numbers of fractionated or unfractionated lymphoid cells were injected intravenously into lethally irradiated (750–800 r., using a ^{60}Co source, 35 r. min^{-1}) male C57BL/6 mice of the same age. The mice were irradiated 2 h before inoculation and injected intraperitoneally with heparin (50 U) 15 min before cell administration. Antibiotics were included in the drinking water for the first 3 weeks after inoculation. Six (experiment 1) or 7 d (experiment 2) after inoculation mice were killed and their spleens were tested *in vitro* for cell-mediated cytotoxicity against ^{51}Cr-labelled EL-4 ($H\text{-}2^b$) target cells as described before[18]. Spleen cell monolayers used for adsorption were prepared by the technique described by Stulting and Berke[16], using poly-L-lysine (Sigma)-coated polystyrene Petri dishes (60 $\times$ 15 mm, Falcon No. 3002). Briefly, 8 $\times$ 10^7 to 10 $\times$ 10^7 (2 ml) spleen cell leukocytes suspended either in Dulbecco's phosphate-buffered saline (PBS) or in RPMI 1640 medium were added to each plate. The plates were incubated at room temperature for 45 min and then washed thoroughly with PBS. Adsorption was performed as described before[17]. 2 $\times$ 10^7 (2 ml) BALB/c spleen cells were added to each monolayer and incubated at 37° C for 45 min in a humidified atmosphere of 5% CO_2 and 95% air. The plates were then centrifuged at 80*g* for 5 min at 24° C in a PR-J (IEC) centrifuge using rotor No. 259 (IEC) and cups No. 384 (IEC). The nonadherent cells were collected after a brief agitation, washed once in MEM, counted, and used for inoculation. Cell-mediated cytotoxicity was measured essentially as described before[18]: 0.1 ml containing 2.5 $\times$ 10^4 ^{51}Cr-EL-4 cells in MEM + 10% foetal calf serum was mixed with 0.2 ml containing the appropriate number of spleen lymphocytes to yield the desired effector to target cell ratio. The cell mixtures were incubated at 37° C in a humidified atmosphere of 5% CO_2 and 95% air for 3 h on a rocking platform. After incubation, 1 ml of medium was added, the cells were centrifuged at 400*g* for 10 min, and the supernatant was counted for radioactivity. The results are expressed in specific percentage lysis ± s.e. and represent the mean of tests run in triplicate. The percentage specific lysis was calculated as follows:

$$\frac{\text{Test counts—background counts}}{\text{Total releasable counts—background counts}} \times 100$$

where background counts were released in the presence of normal spleen and total releasable counts represent approximately 80% of total counts.

At first, we thought that the fractionated BALB/c spleen cells used to inoculate the irradiated C57BL/6 mice could have been contaminated with C57BL/6 cells eluted off the monolayers during the adsorption procedure, so that the syngeneic C57BL/6 cells were actually responsible for protection and survival of the irradiated mice. This was ruled out as follows. Elution of C57BL/6 cells from the monolayers was estimated not to exceed 1.5 $\times$ 10^6 cells, or roughly 10% of the BALB/c spleen cells recovered from each monolayer. Mice inoculated with a mixture of 5 $\times$ 10^7 unadsorbed BALB/c cells and 5 $\times$ 10^6 C57BL/6 spleen cells (Table 2, experiment 2) all died, as did control mice inoculated with 5 $\times$ 10^7 unadsorbed BALB/c spleen cells. Moreover, BALB/c spleen cells fractionated on mitomycin C-treated (40 μg ml^{-1} containing 5 $\times$ 10^7 lymphocytes for 30 min at 37°C) C57BL/6 spleen cell monolayers could also reconstitute irradiated C57BL/6 mice.

The mice that survived the allogeneic transplant of fractionated cells were examined 30 d later for lymphoid cell chimaerism by complement-dependent cytotoxic assays using specific allogeneic antisera. The spleens were composed predomi-

Table 2 Survival of lethally irradiated C57BL/6 mice inoculated with fractionated allogeneic BALB/c spleen cells

Experiment	No. of BALB/c donor cells	Spleen cell monolayer used for adsorption	No. of mice alive* / No. of mice used
1	None	None	0/10
	2×10^7	None	0/10
	4×10^7	None	0/10
	2×10^7	C57BL/6	5/9
	4×10^7	C56BL/6	7/10
	6×10^7	C57BL/6	3/4
2	5×10^7	None	0/5
	5×10^7	BALB/c	0/5
	5×10^7	SJL/J	1/6
	5×10^7 BALB/c + 5×10^6 C57BL/6	None	0/5
	5×10^7	C57BL/6	4/6

* These results were derived 120 d after inoculation. A few animals died 1–5 d after inoculation and were omitted from the group.

nantly of host lymphoid cells (Table 3). These results, however, were not unexpected, for spleen cells have been considered to be a poor source of stem cells. The exact mechanism by which the animals remain alive is not known. Donor allogeneic spleen cells, incapable of triggering a GvH reaction, may serve as transient cells and help the host to recover from the immediate effects of irradiation.

These experiments show that: first, fractionated allogeneic spleen cells inoculated into lethally irradiated recipients permit the host to recover from the combined fatal effects of irradiation and GvH disease; and second, a specific compartment of immunocompetent GvH reactive cells can be depleted from a normal spleen lymphoid cell preparation by adsorption on allogeneic cell monolayers. Previous failures to demonstrate

Table 3 Origin of spleen lymphoid cells obtained from irradiated C57BL/6 mice that survived allogeneic spleen cell transplants*

Treatment	% Dead cells† Normal C57BL/6 spleen	Normal BALB/c spleen	Reconstituted spleen
Complement alone	15	15	15
Anti-BALB/c serum (1:10)	15	15	15
Anti-BALB/c serum (1:10) + complement (1:5)	15	85	20
Anti-C57BL/6 serum (1:10)	15	15	15
Anti-C57BL/6 serum (1:10) + complement (1:5)	70	15	65

* This group of mice was killed 30 d after inoculation of BALB/c spleen cells fractionated on C57BL/6 spleen cell monolayers.

† The values represent the percentage of lymphoid cells killed in a complement-mediated cytotoxic assay as determined by the trypan blue (0.2%) dye exclusion technique. Fresh rabbit serum was the source of complement after being absorbed with normal BALB/c and C57BL/6 spleen cells (2 volumes of serum + 1 volume of cells at 4° C for 1 h). Anti-BALB/c and anti-C57BL/6 sera were prepared in C57BL/6 and BALB/c mice, respectively, by three biweekly injections of 2×10^7 spleen cells. The spleen cells used in the cytotoxic assays were treated first with ammonium chloride (0.14 M) in Tris buffer to remove red blood cells. Spleen cells (3×10^5, 0.1 ml) were incubated with 0.1 ml of antiserum for 30 min at 24° C, then 0.1 ml complement was added and the mixture was incubated for a further 30 min at 37° C.

depletion of T helper cell[19] or of GvH reactive cells from sensitised[20] or nonsensitised[21] cells may have been due to technical problems and not to the absence of antigen-binding receptors on the precursor cells. It seems likely that the centrifugation step used by us in the adsorption process allows firm binding of the receptor-bearing cells to the membrane antigens. Our results indicate that unsensitised precursor cells carry antigen-binding receptors on their surface, and agree with the findings of Lonai *et al.*[13] and Altman *et al.*[14]. We believe that the techniques used in our study could be applied in bone marrow transplantation, immunodeficiency diseases, and the immunotherapy of cancer.

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[1] Santos, G. W., Sensenbrenner, L. L., Burke, J., Colvin, O. M., Owens, A. H., Bios, W., and Slavkin, R., *Exp. Hematol.*, **20**, 78 (1970).
[2] Graw, R. G., Brown, J. A., Yankee, R. A., Leventhal, B., Wang-Peng, J., Rogentine, G. N., and Henderson, E. S., *Blood*, **36**, 736 (1970).
[3] Guyer, R. J., Kay, M. E. M., Knapton, P. J., and Dicke, K. A., *Exp. Hematol.*, **20**, 123 (1970).
[4] Mathe, G., Amiel, J. L., Schwarzenberg, L., Schneider, M., Choay, J., Trolaid, P., Hayat, M., Schlemberger, J. R., and Jasmin, C., *Brit. med. J.*, **2**, 131 (1970).
[5] Dicke, K. A., *Science*, **180**, 168 (1973).
[6] Elves, M. W., *Transplantation*, **16**, 403 (1973).
[7] Bruz, H., Lindenmann, J., and Wigzell, H., *Nature*, **246**, 146 (1973).
[8] Kiger, W., Florentine, I., and Mathe, G., *Transplantation*, **16**, 393 (1973).
[9] Bach, M. L., Bach, F. H., and Zoschke, D., *Israel J. med. Sciences*, **9**, 344 (1973).
[10] Ford, W. L., and Atkins, R. C., *Nature new Biol.*, **234**, 178 (1971).
[11] Lonai, P., Wekerle, H., and Feldman, M., *Nature*, **235**, 235 (1972).
[12] Wekerle, H., Lonai, P., and Feldman, M., *Proc. natn. Acad. Sci., U.S.A.*, **69**, 1620 (1972).
[13] Lonai, P., Eliraz, A., Wekerle, H., and Feldman, M., *Transplantation*, **15**, 368 (1973).
[14] Altman, A., Cohen, I. R., and Feldman, M., *Cell. Immun.*, **7**, 134 (1973).
[15] Kedar, E., Ortiz de Landazuri, M., Bonavida, B., and Fahey, J. L., *J. immun. Methods* (in the press).
[16] Stulting, R. D., and Berke, G., *J. exp. Med.*, **137**, 932 (1973).
[17] Kedar, E., Ortiz de Landazuri, M., and Bonavida, B., *J. Immun.*, **112**, 1231 (1974).
[18] Bonavida, B., *J. Immun.*, **112**, 926 (1974).
[19] Wigzell, H., *Transplantn Rev.*, **5**, 76 (1970).
[20] Mage, M. G., and McHugh, L., *J. Immun.*, **111**, 652 (1973).
[21] Clark, W. R., and Kimura, A. K., *Transplantation*, **16**, 110 (1973).

Subunit complex III-IV as the nucleotide binding site of Q_β replicase

OF the four subunits of Q_β replicase, I, III and IV are bacterial proteins and II is coded for by the phage genome[1,2]. Among host-derived subunits, I is identified as factor i, a protein which controls cistron-specific translation in *Escherichia coli*[3,4], and III and IV are identified as protein synthesis elongation factors, EFTu and EFTs (ref. 5), respectively.

Several lines of evidence indicate that EFTu and EFTu–EFTs interact with GDP or GTP (refs 5–8) and that EFTs stimulates the exchange of GDP bound to EFTu with unbound GTP (refs 9–12), but does not interact with these nucleotides. It has not been known whether EFTu and EFTs in the Q_β replicase perform functions analogous to those in protein synthesis.

Recent work in our laboratories (ref. 13 and unpublished results) has shown that GTP binding to EFTu–EFTs from *E. coli* HAK 88, a mutant with altered EFTs (ref. 14), is thermolabile and that this is also the case for Q_β replicase formed in infected cells. These results support the suggestion that subunit complex III–IV is concerned with nucleotide binding, or at least GTP binding.

I report here results concerning the nucleotide-binding site of Q_β replicase, which indicate that subunit complex III–IV can account for the ability of the enzyme to bind all four nucleotides, whereas I–II cannot, and that there are two distinct nucleotide-binding sites on the enzyme and its subunit

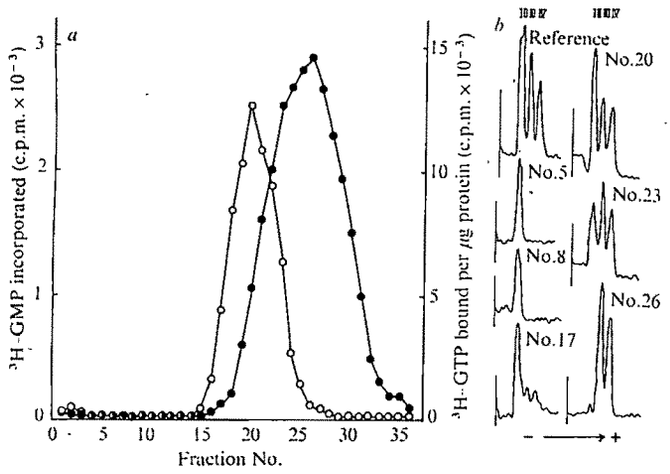


Fig. 1 Sedimentation pattern of Q_β replicase subunits carrying enzyme activity and GTP binding ability. Q_β replicase was purified as described previously[16]. The glycerol density gradient fraction with a specific activity of 5,500 U mg^{-1} protein was used and revealed only four bands corresponding to the enzyme subunits I, II, III and IV[1,2] using sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis[17]. Q_β replicase (200 U) was dissociated into its subunit complexes I-II and III-IV by dialysis for 4 h at 0° C against a buffer containing 0.01M Tris HCl (*p*H 7.6), 1 mM Mg acetate, 2×10^{-4} M EDTA, 1×10^{-4}M dithiothreitol and 5% glycerol. The dialysate was centrifuged on 5 ml of 10–30% glycerol density gradient made up in the same buffer for 17 h at 37,000 r.p.m. at 6° C in the RPS-40 rotor of Hitachi ultracentrifuge 55P-2. A fraction (0.15 ml) was collected from the bottom of the tube and aliquots were used for analysis of enzyme components by SDS-polyacrylamide gel electrophoresis[17], determination of protein concentration[18], Q_β replicase assay (○) with poly(C) as template[16] and ^{3}H–GTP-binding (●). The reaction mixtures for the GTP-binding assay contained in 0.25 ml: 25 μmol Tris HCl (*p*H 7.6), 2.5 μmol $MgCl_2$, 1 μmol 2-mercaptoethanol, 0.22 μCi ^{3}H–GTP (12 Ci mmol^{-1}) and enzyme components from the gradient. After incubation for 20 min at 30° C, the reaction mixture was passed with gentle suction through a nitrocellulose membrane filter (Sartorius MF 50, 0.45 μm, 12 mm) presoaked in a buffer containing 0.1M Tris HCl (*p*H 7.6), 0.01M $MgCl_2$, 0.025M NaCl and 4×10^{-4} M 2-mercaptoethanol and washed with 0.25 ml of the same buffer. The SDS-polyacrylamide gels (acrylamide 7.5% *bis*-acrylamide 0.2%, SDS 0.1%, sodium phosphate 0.1M, *p*H 7.5) were run for 3 h at 7.5 mA per gel, stained with Coomassie brilliant blue and destained[17]. The gels were scanned at 600 nm in a Toyo DMU-2 scanner. *a*, Q_β replicase and GTP-binding activities of fractions from the gradient; *b*, SDS-polyacrylamide gels of fractions from the gradient and the purified Q_β replicase as reference.

complex III–IV, one of which is GTP-specific and the other is probably common to nucleotides other than GTP or to all four.

To examine the binding of nucleotides to Q_β replicase and its subunit complexes I–II and III–IV, ^{3}H-nucleotide was incubated with protein, filtered through a nitrocellulose membrane filter and washed with a buffer as described in the legend to Fig. 1. ^{3}H-radioactivity bound to the membrane filter was then measured as described previously[15]. Q_β replicase was dissociated into subunit complexes I–II and III–IV as described by Kamen[2]. The two complexes were separated by centrifugation on a glycerol density gradient (Fig. 1). Subunit complex I–II sedimented in fractions 4 to 14 and subunit complex III–IV in fractions 25 and 26 of the gradient; replicase activity, assayed with poly(C) as template, appeared in fractions 17 to 24 with a main peak in fraction 20.

^{3}H-GTP binding was also determined in an aliquot of each fraction of the gradient. Essentially no ^{3}H binding was observed with fractions 2 to 14. Binding, which was first seen in fraction 16, reached a maximum in fraction 26 and declined in subsequent fractions. Fraction 26 contained the largest quantity of the III–IV complex. The amount of ^{3}H-GTP bound per μg of protein in fraction 20 was about 40% of that in fraction 26. The molecular weight of Q_β replicase and its subunit complex III–IV has been assumed to be 210,000 and 80,000 (refs 1, 2), respectively, and I found that the amount of ^{3}H-GTP bound per molecule of protein was about the same for these two components.

Binding of ^{3}H-GTP to Q_β replicase and its subunit complexes I–II and III–IV was also compared using various concentrations of protein and constant GTP (Fig. 2). The amount of ^{3}H-GTP bound increased linearly with increasing quantities of protein when the replicase or its subunit complex III–IV was used. The amount of ^{3}H-GTP bound per mole of subunit compex III–IV was about 85% of the amount bound per mole of enzyme. On the contrary, there was no detectable binding of ^{3}H-GTP when up to 5 pmol of subunit complex I–II was added.

Since no detectable protein, including replicase and its subunit complexes I–II and III–IV, pass through a membrane filter even in the presence of nucleoside triphosphates, it seems unlikely that they form a nucleotide-protein complex which could pass through the membrane. Further, since Q_β replicase activity was reconstituted from subunit complexes I–II and III–IV and from I–II and EFTu–EFTs of *E. coli* when incubated as described by Kamen[2], subunit complex I–II does not seem to be inactivated during preparation and storage. Thus it is concluded that, unlike Q_β replicase and its subunit complex III–IV, subunit complex I–II cannot bind ^{3}H-GTP under the conditions used.

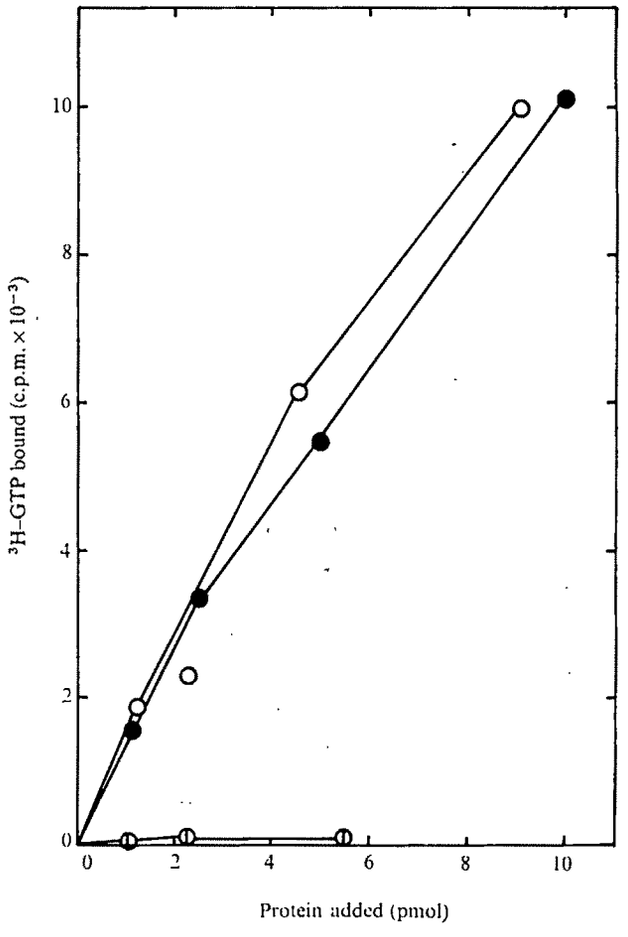


Fig. 2 Comparison of ^{3}H–GTP-binding ability of Q_β replicase and its subunit complexes I-II and III-IV. The complexes were prepared as described in Fig. 1. ^{3}H–GTP-binding was carried out as described in Fig. 1 with various quantities of the replicase (○), subunit complex I-II (Φ) or subunit complex III-IV (●) and 0.22 μCi of ^{3}H–GTP (12 Ci mmol^{-1}). The amount of protein added was calculated by assuming that the molecular weight of enzyme subunits I, II, III and IV are 70,000, 65,000, 45,000 and 35,000 (ref. 2), respectively, and that one molecule of enzyme, subunit complexes I-II and III-IV consist of one molecule each of their respective components.

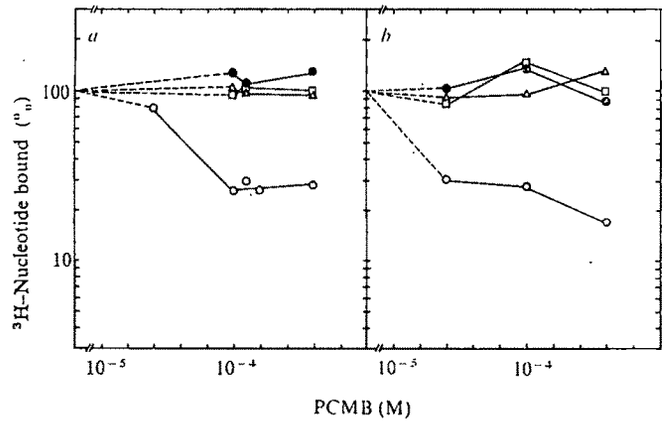


Fig. 3 The effect of PCMB on the binding of ^{3}H-nucleotides to Q_β replicase and its subunit complex III-IV. Q_β replicase and subunit complex III-IV, previously dialysed against 0.05M Tris HCl (*p*H 7.6) containing 0.01M $MgCl_2$ and 20% glycerol were used. The nucleotide-binding assay was carried out for 20 min at 25° C after preincubation of 4.5 pmol of the enzyme (*a*) or 2.5 pmol of subunit complex III-IV (*b*) with the indicated concentrations of PCMB for 10 min at 30° C. In these assays, 0.46 μCi of ^{3}H-ATP (33 Ci mmol^{-1}), 0.35 μCi of ^{3}H-CTP (30 Ci mmol^{-1}), 0.15 μCi of ^{3}H-GTP (12 Ci mmol^{-1}) or 0.49 μCi of ^{3}H-UTP (27 Ci mmol^{-1}) was added to each tube. The amount of ^{3}H-nucleotide bound was measured as described in Fig. 1 and is given as the percentage of the nucleotide bound to enzyme or subunit complex in the absence of PCMB. The amounts of ^{3}H-labelled ATP, CTP, GTP and UTP bound to the control enzyme were 2,558, 2,449, 5,404 and 2,388 c.p.m., respectively, and to the control subunit complex III-IV, 2,612, 1,643, 2,821 and 2,825 c.p.m., respectively. ●, ^{3}H-ATP; △, ^{3}H-CTP; ○, ^{3}H-GTP; □, ^{3}H-UTP.

To localise sites for other nucleotides, I have examined the binding of ^{3}H-ATP, CTP and UTP to these protein components. Each nucleotide reacted with subunit complex III–IV as well as with Q_β replicase (Table 1), although, in either case, bindings of ATP, CTP and UTP were about one order of magnitude weaker than that of GTP. By contrast, significant binding with subunit complex I–II was not observed by either nucleotide-binding assay. These results support the idea that all four nucleotide-binding sites of Q_β replicase are located in subunit complex III–IV rather than I–II. The nucleotide-binding of complex III–IV seems to be somewhat low compared with those of Q_β replicase (Table 1), but can account for about 70% of those of the enzyme. Thus, it seems that subunit complex III–IV needs to interact with other subunit(s) in order to function normally.

Table 1 Binding of ^{3}H-nucleotides to Q_β replicase and its subunit complexes I-II and III-IV

^{3}H-nucleotide added	pmol	^{3}H-nucleotides bound, pmol per pmol protein I-II-III-IV	I-II	III-IV
	2.6	0.05	<0.001	0.03
^{3}H-GTP	5.2	0.09	<0.001	0.06
	13.2	0.18	0.001	0.16
	26.4	0.33	0.002	0.21
	13.2	0.04	<0.001	0.03
^{3}H-ATP	26.4	0.06	<0.001	0.05
	69.0	0.14	0.001	0.12
	139.0	0.29	0.001	0.21
	57.9	0.10	<0.001	0.06
^{3}H-CTP	116.0	0.17	<0.001	0.11
	174.0	0.27	0.001	0.17
	17.9	0.04	<0.001	0.02
^{3}H-UTP	89.0	0.16	<0.001	0.12
	179.0	0.36	0.002	0.21

Q_β replicase and its subunit complexes I-II and III-IV were prepared as described in Fig. 1. The binding assay was carried out for 20 min at 30° C as described in Fig. 1 with ^{3}H-ATP (33 Ci mmol^{-1}), ^{3}H-CTP (30 Ci mmol^{-1}), ^{3}H-GTP (12 Ci mmol^{-1}) or ^{3}H-UTP (27 Ci mmol^{-1}) in the presence of 4.5 pmol of Q_β replicase, 3.84 pmol of subunit complex I-II or 5.0 pmol of subunit complex III-IV. Protein concentration was calculated as described in Fig. 2. The purity of ^{3}H-nucleotides used was estimated to be 99% by paper chromatography.

In an attempt to obtain more information about the nucleotide binding site, the effect of the sulphydryl blocking reagent, *p*-chloromercuribenzoate (PCMB), on nucleotide-binding to the enzyme was examined. PCMB inhibits the enzyme completely at a concentration of 1×10^{-5} M (ref. 19). About 70% of ^{3}H-GTP binding of the enzyme was blocked by 1×10^{-4} M PCMB, whereas the binding of ^{3}H-ATP,CTP and UTP to the enzyme was insensitive to the reagent even at a concentration of 6×10^{-4} M (Fig. 3*a*). A similar correlation was observed with the effect of PCMB on nucleotide-binding to subunit complex III–IV (Fig. 3*b*). These results strongly suggest that there are two different types of nucleotide-binding sites on the enzyme and its subunit complex III–IV; one of which is a PCMB-sensitive site which is of the GTP specific and the other, a PCMB-insensitive site. This possibility is supported in part by evidence that neither ATP, CTP nor UTP interfere with the binding of ^{3}H-GTP to subunit complex III–IV[5]. At present it is still obscure whether the second type of binding site, or PCMB-insensitive site, is common to nucleotides other than GTP and whether PCMB-sensitive GTP binding site is involved, throughout all the processes, in Q_β RNA replication.

Using Q_β replicase with a temperature-sensitive subunit IV, subunit IV has been found to be involved in an early stage of the RNA replication but not in RNA chain elongation when Q_β RNA is the template (my unpublished results). Furthermore, this protein was shown to stimulate specifically the binding of GTP to EFTu–GDP. It is more likely therefore that subunit III *per se* carries binding sites for all four ribunucleoside triphosphates, while subunit IV acts as an effector protein controlling the affinity of nucleotide-binding to the enzyme, particularly the binding of GTP, which is required for initiation of RNA synthesis[20]. As Blumenthal *et al.*[5] pointed out, it is possible that these components in Q_β replicase also take part in a specific interaction with RNA in order to initiate replication since subunit complex III–IV can form complex with Q_β RNA retainable on a membrane filter (unpublished observation).

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1 Kondo, M., Gallerani, R., and Weissmann, C., *Nature*, **228**, 525–527 (1970).
2 Kamen, R., *Nature*, **228**, 527–533 (1970).
3 Groner, Y., Pollack, Y., Berissi, H., and Revel, H., *Nature new Biol.*, **239**, 16–19 (1972).
4 Groner, Y., Sheps, R., Kamen, R., Kolakofsky, D., and Revel, M., *Nature new Biol.*, **239**, 19–20 (1972).
5 Blumenthal, T., Landers, T. A., and Weber, K., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1313–1317 (1972).
6 Ertel, R., Redfield, B., Brot, N., and Weissbach, H., *Archs Biochem. Biophys.*, **128**, 331–338 (1968).
7 Miller, D., and Weissbach, H., *Archs Biochem. Biophys.*, **141**, 26–37 (1970).
8 Kawakita, M., Arai, K., and Kaziro, Y., *Biochem. biophys. Res. Commun.*, **42**, 475–481 (1971).
9 Miller, D., and Weissbach, H., *Biochem. biophys. Res. Commun.*, **38**, 1016–1022 (1970).
10 Weissbach, H., Miller, D., and Hachmann, J., *Archs Biochem. Biophys.*, **137**, 262–269 (1970).
11 Hachmann, J., Miller, D., and Weissbach, H., *Archs Biochem. Biophys.*, **147**, 457–466 (1971).
12 Lucas-Lenard, J., and Lipmann, F., *Ann. Rev. Biochem.*, **40**, 409–448 (1971).
13 Kuwano, M., Kamiya, T., Endo, H. and Hori, K., *J. molec. Biol.* (in the press).

[14] Kuwano, M., Ono, M., Yamamoto, M., Endo, H., Kamiya, T., and Hori, K., *Nature new Biol.*, **244**, 107–109 (1973).
[15] Hori, K., *Biochim. biophys. Acta*, **217**, 394–407 (1970).
[16] Mitsunari, Y., and Hori, K., *J. Biochem.*, 263–271 (1973).
[17] Weber, K., and Osborn, M., *J. biol. Chem.*, **224**, 4406–4412 (1969).
[18] Bücher, T., *Biochim. biophys. Acta*, **1**, 292–314 (1947).
[19] Ohki, K., and Hori, K., *Biochim. biophys. Acta*, **281**, 233–243 (1972).
[20] August, J. T., Banerjee, A. K., Eoyang, L., Franze de Fernandez M. T., Hori, K., Kuo, C. H., Rensing, U., and Shiparo, L., *Cold Spring Harbor Symp. quant. Biol.*, **33**, 73–81 (1968).

Effect of ATP on release of intracellular enzymes from damaged cells

THE use of serum enzyme measurements as aids to the diagnosis of disorders of the heart, liver and other organs is established practice in clinical laboratories everywhere, but little is known of the mechanisms whereby intracellular enzymes are discharged from damaged cells. In necrotic states, complete disruption of the cell will lead to release of its contents, but in reversible inflammatory states the cause of the increased membrane permeability remains obscure.

In an attempt to devise a model for the study of the factors affecting the liberation of intracellular enzymes, we recently described the effects of phospholipases A and C, on enzyme release in rat lymphocytes and human leukocytes[1]. In these experiments the porosity of the cell membrane was increased leading to the efflux of cytosolic, and to a lesser extent mitochondrial, enzymes into the medium. We have now investigated the actions of a number of substances which might protect the cell against the effects of phospholipases as judged by a reduction in the leakage of intracellular lactate dehydrogenase. One of these was adenosine triphosphate, ATP, and its effects are described here.

Rat lymphocyte suspensions were prepared in Krebs–Ringer buffer solution (*p*H 7.4) containing glucose (8.3 mmol l^{-1}) and were subjected to phospholipase A as previously described[1]. The experiments were carried out in flasks which were gently shaken in the water-bath (37° C) of a Warburg apparatus. In parallel experiments, ATP was incorporated into the reaction medium 10 min before the addition of the phospholipase. Incubation at 37° C was continued for a further 30 min, after which samples were removed and centrifuged. Lactate dehydrogenase activities were determined in the supernatant and also in sonicated homogenates of the cell suspension to provide an estimate of the total activity. The spectrophotometric method of Wróblewski and LaDue[2] with pyruvate as substrate was used in 25° C.

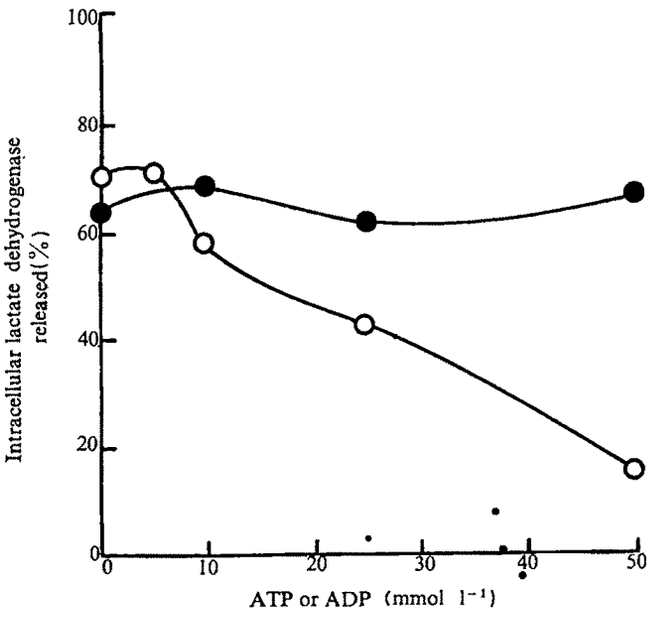


Fig. 1 Effect of ATP (O) and ADP (●) on the leakage of lactate dehydrogenase from rat lymphocytes evoked by phospholipase A (50 mU ml^{-1}).

The results shown in Fig. 1, indicate that at concentrations greater than 5 mmol l^{-1}, ATP reduces the amount of lactate dehydrogenase released from suspensions of rat lymphocytes as the result of the action of phospholipase A. No such effect was observed with ADP in similar concentrations. The protective effect was proportional to the concentration of ATP. In control preparations from which the phospholipase was omitted only small amounts of lactate dehydrogenase were released, and it was uncertain whether ATP exerted a protective effect in these also. We excluded the possibility that ATP had an inhibitory effect on lactate dehydrogenase by demonstrating comparable activities in homogenates containing and omitting ATP, and also by adding ATP to some of the samples during enzyme determination.

Table 1 Effect of ATP on the release of lactate dehydrogenase from rat lymphocytes

ATP concentration (mmol l^{-1})	No. of experiments	Enzyme activity lost from cells (% of control)	
		Mean ± s.d.	Significance
5	7	83 ± 24.9	$P < 0.05$
10	7	74 ± 21.6	$P < 0.005$
50	7	32 ± 19.1	$P < 0.001$

Cells from different rats in each experiment were incubated for 3 h at 37° C in Krebs-Ringer solution (*p*H 7.4).

Similar effects were observed when the output of aspartate transaminase, malate dehydrogenase and glucose 6-phosphate dehydrogenase activities was measured.

A second series of experiments was carried out in which rat lymphocytes were incubated for 3 h at 37° C in the absence of phospholipase A. During this period the intracellular ATP concentration of a control preparation, determined by the method of Adam[3], declined from 1.75 mmol l^{-1} at 30 min to <0.01 mmol l^{-1} after 3 h, and the release of lactate dehydrogenase increased from 15% to 67±s.d. 16%. Incorporation of ATP into the medium led to significant reductions in the enzyme activity lost from the cells (Table 1).

The protective effect ATP in physiologically relevant concentrations observed here is consistent with the view that maintenance of cell membrane integrity is associated in some way with the energy content of the cell. This indicates that any condition which leads to a diminution of intracellular ATP is likely to result in leakage of enzymes and other proteins. Such a relationship was suggested by Sweetin and Thomson[4] to explain their observation that human erythrocytes did not release significant amounts of enzymes until the cells had metabolised all the glucose in the medium. Our work provides experimental evidence in support of this suggestion.

As well as acting as a reserve of energy, ATP may have a direct effect in maintaining the integrity of the cell membrane, as it is known to be concerned in the biosynthesis of phosphatidic acids[5], intermediates in the synthesis of phospholipids, the main components of the membrane. The belief that ATP is almost exclusively an intracellular material and that it cannot enter the cell from extracellular fluids is difficult to reconcile with our findings. The protective action may be associated with a direct effect on the cell membrane or with the uptake of ATP from the medium. We point out that such a process has been observed in rat muscle by Chaudry and Gould[6].

The concept that intracellular enzyme release is secondary to reduction in the ATP content of the cell provides an explanation for the efflux of aldolase from intact rat muscles in response to anoxia, deprivation of glucose and exposure to high potassium ion concentrations reported by Zierler[7]. Glucose is the primary source of ATP synthesis, anoxia leads to accumulation of NADH and failure of the tricarboxylic acid cycle and thus to the inhibition of ATP synthesis, while the high potassium concentration interferes with the action of the Na^+/K^+ pump, the energy for which is provided by the hydrolysis of ATP by ATPase.

Discharge of intracellular enzymes into the circulation occurs in tissue damage due to a variety of causes, such as virus infection, trauma, chemical toxicity, hypoxia, invasion by a malignant process and so on. The suggestion that failure to synthesise ATP in adequate amounts may be a common factor seems to merit further investigation.

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1 Robinson, J. M., and Wilkinson, J. H., *Clin. chim. Acta*, **47**, 347–356 (1973).
2 Wróblewski, F., and LaDue, J. S., *Proc. Soc. exp. Biol., N.Y.*, **90**, 210–213 (1955).
3 Adam, H., in *Methods of Enzymatic Analysis*, first ed. (edit. by Bergmeyer, H. U.), 539 (Verlag Chemie, Weilheim, 1962).
4 Sweetin, J. C., and Thomson, W. H. S., *Clin. chim. Acta*, **48**, 403–411 (1973).
5 Kornberg, A., and Pricer, W. E., jun., *J. biol. Chem.*, **204**, 345–357 (1953).
6 Chaudry, I. H., and Gould, M. K., *Biochim. biophys. Acta*, **196**, 320–326 (1970).
7 Zierler, K. L., *Am. J. Physiol.*, **190**, 201–205 (1958).

Uptake of the neurotransmitter candidate glutamate by glia

GLUTAMIC acid is the primary candidate for a role as an excitatory transmitter at synapses mediating the primary afferent pathway and cortical pathways in mammals[1]. Seeking to gain evidence for this, various laboratories have looked for a mechanism for the efficient removal of glutamic acid from the synaptic cleft. Following the paradigm of monoamine synapses, in which high affinity uptake systems apparently localised in the presynaptic membrane remove transmitter after its action at the postsynaptic membrane[2], high affinity uptake systems for glutamate removal have been sought. Logan and Snyder[3] described a high affinity transport system for glutamate in a synaptosomal preparation, and postulated that it might serve to localise glutamate-mediated synapses. Recent evidence has suggested that the transport systems involved in the removal of putative amino acid transmitters from the synaptic cleft are not localised exclusively in the synapse.

Bulk isolated glial cells have a high affinity uptake system for γ-aminobutyrate (GABA)[4] that seems suitable for participation in the removal of GABA from the synaptic cleft. Autoradiography of a lobster nerve-muscle[5] preparation and rat retina[6] also suggests that GABA is accumulated by glial cells. Similar studies[7] implicated glia in the removal of glycine in spinal cord preparations, glutamate in cortical areas and GABA in the cerebellum.

We have investigated further the ability of glial cells, either obtained as bulk cell fractions or from the C–6 line of cultured rat astrocytoma (given by Dr G. Sato), to accumulate glutamate. We consider that the use of both these preparations can most reliably suggest *in vivo* properties of glial cells: with bulk isolated glia alone there are problems due to cell damage and contamination, and with cultured cells dedifferentiation and the expression of anomalous metabolic properties can be troublesome.



Fig. 1 Positive phase contrast photomicrograph of living rat C-6 glioma cells in normal growth medium 48 h after a 40 h pulse in 10^{-7} M amethopterin. Astroblastic cells soon begin to resemble normal astrocytes. The multipolar processes become very long and develop broad end feet. Bar represents 50 μm.

Bulk isolated glial cell fractions were prepared as described before[4] and were examined by electron microscopy and by the addition of labelled synaptosomes to the initial tissue suspension; both methods indicated less than 10% synaptosomal contamination in the glial fractions. The C–6 clonal line grows in our hands as morphologically differentiated cells, with multiple processes. A 24 h exposure to amethopterin (10^{-7} M) during the third day of logarithmic growth, however, produced cells which appeared to be even further morphologically differentiated (Fig. 1). In addition, the glia-specific protein S–100 increased after amethopterin treatment[9] further indicating that these cells can be induced to express the properties of differentiated glial cells.

Uptake experiments were carried out as described before[3]. Under the conditions used less than 10% of the amino acid is metabolised when examined by paper chromatography. Samples were oxidised in a Packard oxidiser and counted in a Packard liquid scintillation counter. The results are expressed as nmol of substrate accumulated per mg dry weight in 4 min.

The uptake of glutamate by cultured glial cells is illustrated in Fig. 2. Table 1 shows that these results, including the demonstration of high and low affinity uptake systems, correspond remarkably to values reported for the uptake of glutamate by synaptosomal preparations. As a further check on the correspondence between the ability of the synaptosomal fraction to accumulate glutamate and that of the cultured glial cell, the Na^+ dependence of glutamate accumulation was investigated. Solutions containing sucrose or choline chloride were substituted for sodium chloride solutions. The results indicate that the high affinity uptake

Table 1 K_m values for glutamate uptake

Bulk isolated glial cells	$1.2 \pm 0.4 \times 10^{-5}$ M	high affinity
	$1.3 \pm 0.7 \times 10^{-4}$ M	low affinity
Cultured glial cells	$6.6 \pm 1.2 \times 10^{-5}$ M	high affinity
	$1.3 \pm 0.8 \times 10^{-3}$ M	low affinity
Synaptosomal fraction[3]	$3.6 \pm 1.3 \times 10^{-5}$ M	high affinity
	$1.5 \pm 0.9 \times 10^{-3}$ M	low affinity

system was inhibited 81±4% in the presence of choline chloride, while the low affinity system was inhibited 22±3% under identical conditions. This compares with the recently published Na^+ requirement for glutamate uptake by a synaptosomal fraction[10] in which 95.8% inhibition was obtained for the high affinity system.

To evaluate the specificity of the high affinity uptake system two further experiments were performed. The uptake of alanine, a neutral amino acid, was studied in cultured glial cells. A single uptake system was found over the concentration range of 10^{-3} to 10^{-7} M. This system had a K_m of $1.0\pm0.5\times10^{-3}$ M. A line of cultured HeLa cells exhibited only a single uptake system for glutamate, with a K_m of $3.8\pm1.3\times10^{-3}$ M. This suggests that high affinity uptake systems are not involved in the accumulation of all amino acids by glia, and that glutamate uptake mediated by high affinity transport systems is not a general property of other cell types.

To investigate the possibility that glial elements contribute significantly to glutamate uptake by the synaptosomal fraction, it is necessary to show that glia can form small vesicles which can accumulate glutamate. Following a report by Cotman *et al.*[11], we attempted to isolate a gliasome fraction from a sample of cultured cells using the standard Whittaker[12] procedure for isolation of synaptosomes. These

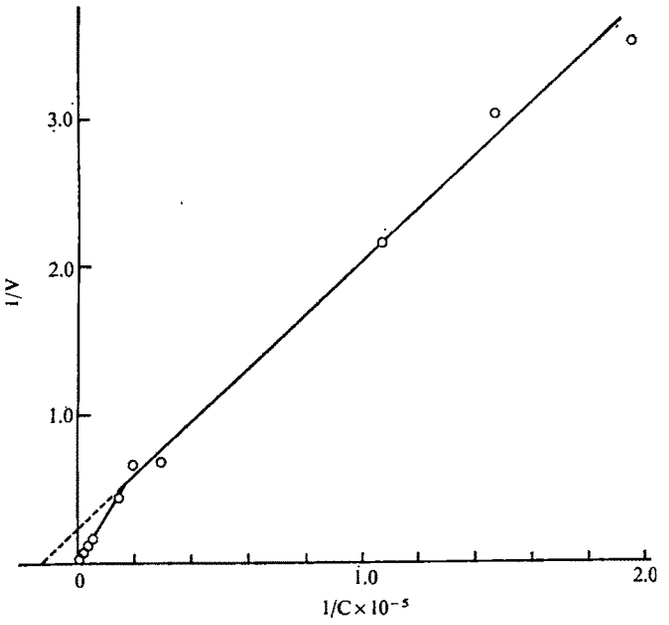


Fig. 2 A double reciprocal plot of the velocity of L-glumatic acid accumulation into glia from the C-6 line of rat glioma at 37°C and varying glumatic acid concentrations. The uptake media contained 140 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 3 mM $CaCl_2$, 10 mM dextrose and 10 mM Tris HCl at *p*H 7.4. High affinity $K_m=6.7\times10^{-5}\pm1.3$; low affinity $K_m=1.3\times10^{-3}\pm0.8$.

gliasomes were incubated with 10^{-8} M glutamate and achieved tissue to medium ratios consistently in excess of 24 in 4 min. This uptake was temperature-sensitive and seemed to correspond to the high affinity uptake system characterised in intact cultured cells. This suggests that material isolated by the Whittaker procedure may contain appreciable non-neuronal elements.

Our results indicate that mammalian glial cells, prepared either as a bulk isolated fraction or from the C-6 rat glioma cell culture, have a high affinity transport system for the removal of extracellular glutamate. Glia have also been found to be involved in the uptake of glutamate by the neuromuscular junction of arthropods. This synapse is almost certainly mediated by glutamate[13] and Feeder and Salpeter[14] showed that most labelled cells in an arthropod neuromuscular junction of arthropods. This synapse is cells, a glial element surrounding the synapse. Our demonstration of a high affinity uptake system for glutamate localised in glial cells and the findings of a similar system for GABA in glial cells together with autoradiographic evidence for glial accumulation of these compounds[7] strongly suggests that if these amino acids are neurotransmitters, perisynaptic glial elements are involved in their inactivation.

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1 Hammerschlag, R., and Weinreich, D., in *Adv. Biochem. Psychopharmac.* (edit. by Costa, E., and Greengard, P.), **6**, 165–180 (Raven Press, New York, 1972).
2 Iversen, L. L., *The uptake and storage of noradrenaline in sympathetic nerves* (Cambridge University Press, London, 1967).
3 Logan, W. J., and Snyder, S. H., *Nature*, **234**, 297–299 (1971); *Brain Res.*, **42**, 413–21 (1972).
4 Henn, F. A., and Hamberger, A., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2686–2690 (1971).
5 Orkand, P. M., and Kravitz, E. A., *J. Cell Biol.*, **49**, 75–89 (1971).
6 Neal, M. J., and Iversen, L. L., *Nature new Biol.*, **235**, 217–218 (1972).
7 Hökfelt, T., and Ljungdahl, A., in *Adv. Biochem. Psychopharmac.* (edit. by Costa, E., and Greengard, P.), **6**, 1–36 (Raven Press, New York 1972).
8 Bloomstrand, C., and Hamberger, A., *J. Neurochem.*, **16**, 1401–1407 (1969).
9 Silbert, S. W., and Goldstein, M. N., *Cancer Res.*, **32**, 1422–1427 (1972).
10 Bennett, J. P., Logan, W. J., and Snyder, S. H., *Science*, **178**, 997–999 (1972).
11 Cotman, C. W., Herschman, H., and Taylor, D., *J. Neurobiol.*, **2**, 169–180 (1971).
12 Grey, E. G., and Whittaker, V. P., *J. Anat.*, **96**, 79–88 (1962).
13 Usherwood, P. N. R., *Neurosci. Res. Prog. Bull.*, **10**, 136–143 (1972).
14 Feeder, I. R., and Salpeter, M. M., *J. Cell Biol.*, **46**, 300–307 (1970).

Involvement of brain cholinergic mechanisms in the action of chlorpromazine

THE function of cholinergic neurones in ganglia[1], striatum and other brain areas is normally regulated by dopamine (DA)[2] which, applied microiontophoretically, reduces the increased activity of caudate neurones elicited by acetylcholine[3]. Moreover, DA iontophoresis on caudate neurones mimics the responses elicited by electrical stimulation of the substantia nigra[4,5].

Chlorpromazine and haloperidol, neuroleptics which increase the turnover rate of DA in striatum[6,7], presumably block DA receptor function[8]. Such an action has been credited as an explanation for the antagonism by chlorpromazine of certain behavioural effects induced by amphetamine[9]. It is pertinent to remember that these amphetamine effects are thought to be mediated through the release of transmitter from dopaminergic neurones[10].

Table 1 Turnover rate of ACL in rat striatum after haloperidol and chlorpromazine

Treatment (μmol kg^{-1} i.p.)	ACh (nmol g^{-1})	Choline (nmol g^{-1})	ACh (d.p.m. nmol^{-1})	Choline (d.p.m. nmol^{-1})	ACh turnover rate (μmol g^{-1} h^{-1})
Saline	48 ± 3.2	39 ± 2.8	228 ± 20	286 ± 33	0.98 ± 0.091
Haloperidol (5.2)	45 ± 4.1	42 ± 3.1	296 ± 32	299 ± 25	1.2 ± 0.10
Haloperidol (10.4)	47 ± 3.7	35 ± 1.1	330 ± 19*	227 ± 17	1.8 ± 0.15*
Chlorpromazine (9.3)	41 ± 6.0	37 ± 2.7	260 ± 23	312 ± 34	1.1 ± 0.06
Chlorpromazine (18.6)	50 ± 4.1	41 ± 4.9	370 ± 17*	285 ± 30	1.4 ± 0.10*

Phosphorylcholine-methyl-^{14}C was infused intravenously at a constant rate for 6 min (30 μCi kg^{-1} min^{-1}). Drugs were injected 1 h before beginning the infusion. The figures represent the mean values of four experiments ± s.e.m.
* $P < 0.05$ when compared with saline-treated animals.

We have investigated whether the action of chlorpromazine or haloperidol is associated with a change in the activity of brain cholinergic neurones. Since the amount of transmitter synthesised per unit of time is believed to be a good index of its *in vivo* utilisation[11] and since transmitter utilisation may change with neuronal activity[12], we have decided to monitor the effect of chlorpromazine and haloperidol on the turnover rate of acetylcholine (ACh) in occipital cortex and striatum of rats to assess whether when DA receptors are blocked, the synthesis of ACh in various areas of rat brain is increased. We have measured ACh turnover rate in these two brain structures because cholinergic neurones are present in various brain areas and dopaminergic endings are present in neocortex, limbic system, hypothalamic nuclei and striatum[13,14]. However, in striatum a link between the two neuronal systems is suggested by the present understanding of the role of DA in Parkinson's disease.

Tables 1 and 2 show the specific activities of ACh and choline in the striatum and occipital cortex of rates receiving saline, chlorpromazine or haloperidol 1 h before the constant rate infusion of radioactive phosphorylcholine for 6 min. Steady state concentrations of ACh and choline were not changed by the infusion of phosphorylcholine. Moreover, the choline concentrations in the two brain areas studied were comparable whereas the ACh concentrations in

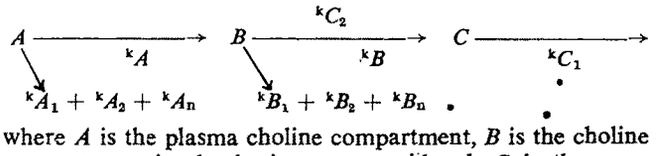
Table 2 Turnover rate of ACL in rat occipital cortex after haloperidol and chlorpromazine

Treatment (μmol kg^{-1} i.p.)	ACh (nmol g^{-1})	Choline (nmol g^{-1})	ACh (d.p.m. nmol^{-1})	Choline (d.p.m. nmol^{-1})	ACh turnover rate (μmol g^{-1} h^{-1})
Saline	15 ± 1.1	30 ± 2.1	522 ± 53	1186 ± 120	0.14 ± 0.014
Haloperidol (5.2)	14 ± 2.1	29 ± 3.2	570 ± 69	1140 ± 104	0.15 ± 0.012
Haloperidol (10.4)	14 ± 1.8	33 ± 1.4	500 ± 73	1094 ± 81	0.14 ± 0.011
Chlorpromazine (9.3)	13 ± 2.6	37 ± 3.9	504 ± 80	1154 ± 137	0.15 ± 0.014
Chlorpromazine (18.6)	16 ± 1.2	35 ± 4.5	474 ± 78	1206 ± 97	0.14 ± 0.012

Phosphorylcholine-methyl-^{14}C was infused intravenously at a constant rate for 6 min (30 μCi kg^{-1} min^{-1}). Drugs were injected 1 h before beginning the infusion.
The figures represent the mean values of four experiments ± s.e.m.

Sprague-Dawley male rats (125–150 g) were infused at a constant rate with 0.65 μmol kg^{-1} min^{-1} of phosphoryl choline-methyl-^{14}C (44.0 mCi mmol^{-1}; New England Nuclear Co.) and killed at various times afterwards by microwave radiation focused on the skull (75 mW cm^2). The total power delivered down the wave guide was 1.5 kW at a frequency of 2.45 GHz. We found that when the heads of the rats were exposed to this radiation for 2–3 s, the brain choline and ACh concentrations were stabilised. The brain was dissected and processed for the assay of choline and ACh specific radioactivity as described by Hanin *et al.*[15]. As Fig. 1 shows, the specific activity of striatal ACh and choline increased with infusion time, and the rate of increase proceeded parallel with infusion time in both compounds. The percentage of the ACh store that was renewed per unit of time (fractional rate constant) was estimated at various times of infusion by the finite difference method (ref. 16 and Fig. 1). The fractional rate constant (k) multiplied by the steady state concentration of brain ACh yields an estimation of the amount of ACh made per unit of time.

Brain ACh turnover rate was calculated according to a method described in detail elsewhere[15]. In brief, this method assumes the following kinetic model



where A is the plasma choline compartment, B is the choline compartment in the brain area considered, C is the compartment of ACh in the same area and k_A, k_B and k_C denote the fractional rate constants of A, B and C. respectively. To measure k_C, which is the percentage of ACh pool metabolised per unit of time, and at steady state is equal to the rate of synthesis, the ratio of the specific activity of ACh and choline was calculated at 6 min of infusion and this value was multiplied by the concentration of ACh (in μmol g^{-1}). This value expressed by a given time unit gives an estimate of the turnover rate of Ch.

striatum were three times larger than in occipital cortex. The specific activity of choline in cortex was three times that in striatum, suggesting that each brain area includes several choline compartments. The greater specific activities of choline in cortex than in striatum suggests that the choline compartment that is in rapid equilibrium with blood-borne choline in cortex is smaller than that of striatum. The data presented in the tables also suggest that the brain choline pool that is in rapid equilibrium with the plasma pool is that involved in the synthesis of ACh, because the specific activities of ACh and choline were greater in cortex than in striatum. The amount of ACh synthesised per unit of time was greater in striatum than in cortex: this difference exceeded the three-fold difference in pool size present in these tissues. The amount of ACh synthesised in striatum of rats receiving chlorpromazine (18.6 μmol kg^{-1} intraperitoneally) or haloperidol (10.4 μmol kg^{-1} intraperitoneally) 1 h before the infusion of phosphorylcholine was increased; there was no similar increase in the occipital cortex. These findings minimise the possibility that nonspecific effects of the two drugs are operative in explaining the increase of ACh turnover rate; actually, they suggest that these two drugs affect some neuronal mechanism that is important in the control of striatal ACh synthesis and less important in the control of the synthesis of ACh in occipital cortex. Since these two neuroleptics block striatal dopaminergic receptors[8] and since DA concentrations in striatum are very high[13] the action of haloperidol and chlorpromazine on dopaminergic and cholinergic mechanisms of striatum might be interrelated. A blockade of striatal DA receptors in striatum may account

for the extrapyramidal side effects evoked by the two drugs in several animals species, including man. This possibility is supported by reports that haloperidol is more potent than chlorpromazine in eliciting extrapyramidal side effects[17] and in increasing striatal DA turnover rate (A. Revuelta, personal communication). The finding that chlorpromazine increases the rate of synthesis of brain ACh is in keeping with a report by Stadler *et al.*[18] showing that chlorpromazine increases the release of ACh from the perfused striatum of rats. Chlorpromazine is credited for exciting an inhibitory action on cholinergic receptors when given in large doses.

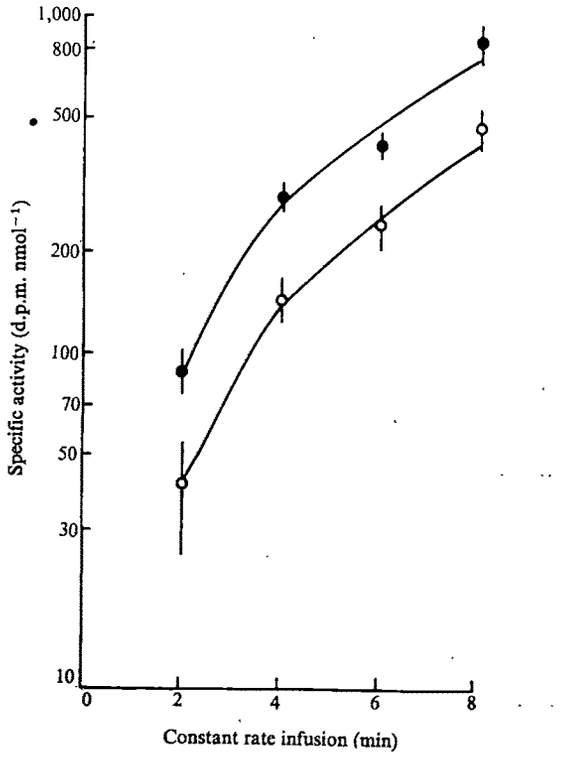


Fig. 1 ACh (○) and choline (●) specific radioactivities in rat striatum after phosphorylcholine(methyl-^{14}C) (30 μCi kg^{-1} min^{-1}) was infused for various minutes and the rats were killed immediately after microwave irradiation.

$$* k_{ACh} = 2\,(S_{Ach\ t2} - S_{Ach\ t1})/\Delta_t\,(S_{ch\,t1} - S_{Ach\,t1} + (S_{ch\,t2} - S_{Ach\,t2})$$

Δmin	$*k_{ACh}$
3-2	0.44
4-3	0.42
5-4	0.34
6-5	0.32
7-6	0.31
8-7	0.32

Such an action cannot explain the increased synthesis of striatal ACh elicited by chlorpromazine because haloperidol, which is more active than chlorpromazine in stimulating the synthesis of ACh, is less active in blocking cholinergic receptors[17]. Similar considerations can be made to exclude the fact that the anticholinesterase action of chlorpromazine is involved in the stimulation of striatal ACh synthesis[17]. In interpreting the physiological implications of the increase of ACh synthesis rate reported in the Tables, it would be important to know whether an increase of striatal ACh synthesis corresponds with an increase in the activity of cholinergic neurones. A direct answer to this question cannot be given at this time because the correlations between ACh turnover rate and the firing rate of cholinergic neurones has not been studied directly. Presumptive indirect evidence, however, supports the belief that the rate of ACh synthesis reflects the activity of cholinergic neurones (D. Cheney and M. Trabucchi, unpublished work).

Finally, it would be interesting to establish whether this increase of ACh synthesis in striatal cholinergic neurones is required to elicit an antipsychotic action. We are currently working to explore several factors involved. We found that clozapine, a neuroleptic with antipsychotic activity comparable with chlorpromazine but lacking the extrapyramidal side effects[19], fail to increase the rate of ACh synthesis in striatum. Although, the present level of understanding would indicate that the increased biosynthesis of striatal ACh relates to the extrapyramidal side effects of neuroleptics rather than to their antipsychotic properties, more work is needed to reach a definite conclusion on this point.

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1 Libet, B., and Tosaka, T., *Proc. natn. Acad. Sci. U.S.A.*, **67**, 667–673 (1970).
2 Portig, P. J., and Vogt., M., *J. Physiol., Lond.*, **204**, 687–715 (1969).
3 Salmoiraghi, G. C., Costa, E., and Bloom, F. E., *A. Rev. Pharmac.*, **5**, 213–234 (1965).
4 Connor, J. D., *Science*, **160**, 899–900 (1968).
5 Connor, J. D., *J. Physiol. Lond.*, **208**, 691–703 (1970).
6 Anden, N. E., Roos, B. E., and Werdinius, B., *Life Sci.*, **3**, 149–158 (1964).
7 Neff, N. H., and Costa, E., in *Antidepressant drugs* (edit. by Garattini, S., and Dukes, M. N. G.) 28–34 (Excerpta Medica Foundation, Amsterdam, 1967).
8 Kebabian, J. W., Petzold, G. L., and Greengard, P., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2145–2149 (1972).
9 Van Rossum, J. M., in *Neuropsychopharmacology* (edit. by Brill, H., Cole, J. O., Deniker, P., Hippius, H., and Bradley, P. B.) 321–329 (Excerpta Medica Foundation, Amsterdam, 1967).
10 Costa, E., and Groppetti, A., in *Proceedings of an international symposium on amphetamines and related compounds; proceedings of the Mario Negri Institute for Pharmacological Research* (edit. by Costa, E., and Garattini, S.) 231–255 (Raven Press, New York, 1970).
11 Costa, E., and Neff, N. H., *Handbook of neurochemistry* (edit. by Lajtha, A.), **4**, 45–90 (Plenum, New York, 1970).
12 Sedvall, G., and Kopin, I. J., *Life Sci.*, **6**, 45–51 (1967).
13 Fuxe, K., Hokfelt, T., and Ungerstedt, U., *Int. Rev. Neurobiol.*, **13**, 93–126 (1970).
14 Ungerstedt, U., *Acta. physiol. scand.*, **367**, 1–122, Suppl. (1971).
15 Hanin, I., Cheney, D. L., Trabucchi, M., Massarelli, R., Wang, C. T., and Costa, E., *J. Pharmac. exp. Ther.*, **187**, 68–77 (1973).
16 Neff, N. H., Spano, P. F., Groppetti, A., Wang, C. T., and Costa, E., *J. Pharmac. exp. Ther.*, **176**, 701–709 (1971).
17 Snyder, S. H., Banerjee, S. P., Yamamura, H. I., Greenbay, D., *Science* (in the press).
18 Stadler, H., Lloyd, K. G., Gadea-Ciria, M., and Bartholini, G., *Brain Res.*, **55**, 476–480 (1973).
19 DeMaio, D., *Arzneimittel-Forsch.*, **22**, 919–923 (1972).

Effect of histamine H-receptor blockade on fasting serum gastrin and fasting gastric acid secretion in the dog

Histamine receptors which are blocked by conventional antihistamines, such as mepyramine maleate, have been termed H_1-receptors. Receptors which are not blocked by these drugs have been found in the cardiac atrium, the uterus and the gastric parietal cell[1] and have been termed H_2-receptors. An antagonist of these receptors (burimamide) has recently been discovered[2]. Histamine H_2-receptor antagonists inhibit gastric acid secretion stimulated by

histamine, pentagastrin, hypoglycaemia and food in the dog (M. I. Grossman and S. J. Konturek, unpublished) and by histamine and pentagastrin in cat (S. J. Konturek, unpublished), pig (G.O.B., M. W. Waterworth, M. D., and S. B., unpublished), and man[3]. Studies have shown that some hormonal inhibitors of gastric acid secretion, namely secretin[4] and glucagon[5,6], depress fasting serum gastrin levels in man and a common mechanism of action through suppression of gastrin-mediated acid release has been postulated. This study attempts to determine whether burimamide has any effect on gastric acid secretion and gastrin release in basal conditions in the dog.

Two mongrel dogs weighing 17 and 25 kg were prepared with gastric fistulae, and subsequent studies were performed at least 3 months after surgery. Not more than one experiment was carried out every 48 h, and dogs were fasted for 16 h before each study. Gastric juice was collected every 15 min for 1.25 h after gastric collections had reached basal levels. After the first 15 min injections lasting 60 s of either burimamide (8 mg per kg body weight in a solution containing 15 mg ml^{-1}) or 0.15 M NaCl ml^{-1} were given into a leg vein. In both dogs burimamide injections

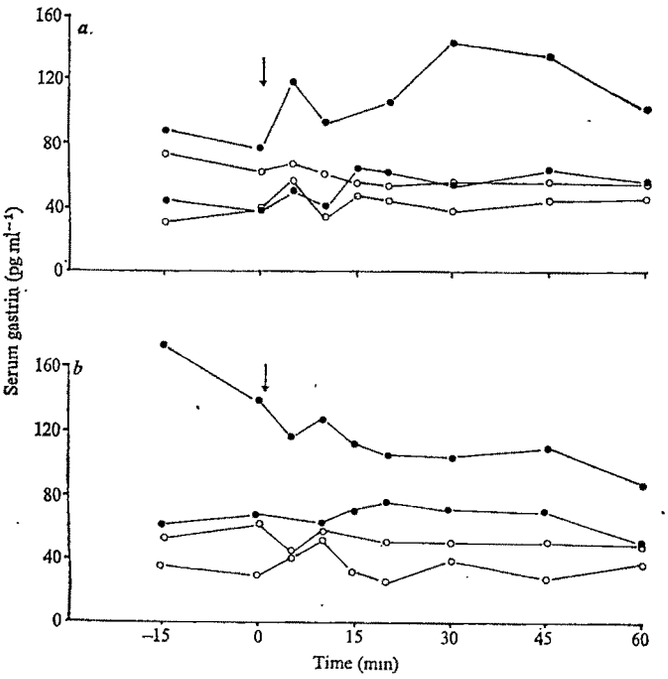


Fig. 1 Serum gastrin concentration in pg ml^{-1}. Burimamide (*a*) or saline (*b*) was injected at time 0. Open or closed circles denote studies performed in the same animals.

were followed by retching and restlessness which subsided within 2–5 min. Blood was taken from a separate leg vein 15 min before and 0, 5, 10, 15, 20, 30, 45 and 60 min after test injections. The blood was allowed to clot, and the serum separated and stored at −20° C. Gastrin was estimated later by radioimmunoassay (A. V. and B. G., unpublished). Gastric juice was measured to the nearest 0.5 ml and the acid titrated against 0.2 M NaOH using an automatic titrator (Radiometer, Copenhagen). Two burimamide and two control studies were performed in each dog in random order.

Figure 1 shows the serum gastrin concentrations in the four saline and the four burimamide-treated animals. In three test and four control studies there was minimal variation in serum gastrin concentrations from basal levels. A slight rise in serum gastrin levels occurred in only one test following burimamide. The differences between control and burimamide tests were not significant.

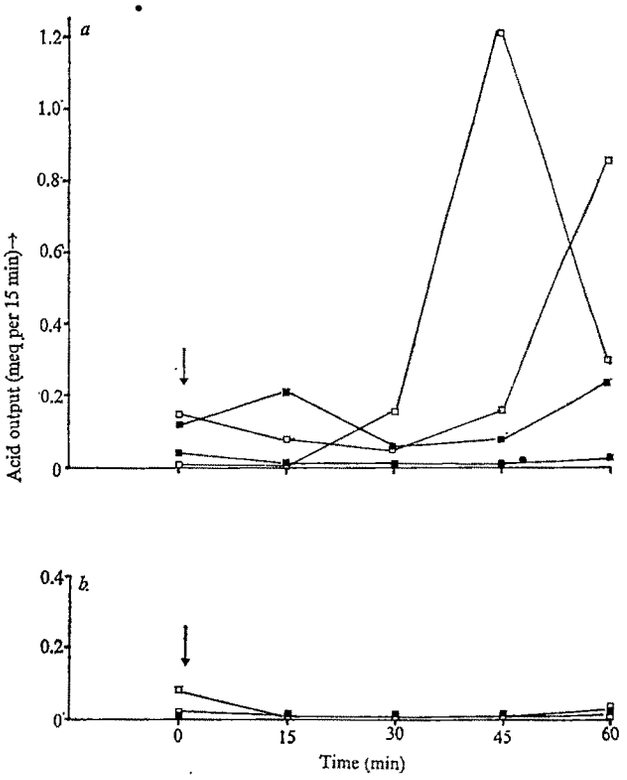


Fig. 2 Acid output from gastric fistulae in meq per 15 min. Burimamide (*a*) or saline (*b*) was injected at time 0. Open or closed squares denote studies performed in the same animal.

Two of the four dogs studied showed a slight rise in gastric acid secretion (Fig. 2) from 30–60 min after burimamide. The rise was, however, small in terms of output of acid, reaching a maximum of 1.20 and 0.72 meq per 15 min over basal levels in the two dogs. Dogs receiving control saline injections showed no increase in acid output.

The results of this study show that serum gastrin levels are not altered by burimamide given intravenously to fasting dogs. The reduction in acid secretion observed with burimamide is unlikely to be a consequence of lower levels of circulating gastrin. The slight rise in serum gastrin after burimamide in one study may be a secondary phenomenon following a slight reduction in gastric acidity and deinhibition of the antrum.

In searching for a unifying mechanism linking gastric acid secretion and gastrin release, earlier studies demonstrated that secretin[7] and glucagon[8] both inhibit gastric acid secretion, and they both depress fasting serum gastrin levels[4–6]. In this respect they differ from H_2-receptor antagonists. Differences between the action of secretin and burimamide are amplified by studies on histidine decarboxylase in rat gastric parietal cells. Whereas secretin does not stimulate histidine decarboxylase in the rat[9] recent studies have shown that both burimamide and metiamide produce a 3–5-fold rise in histidine decarboxylase levels in rat glandular stomach[10]. The compound SC 15396, an antigastrin, also produces a rise in histidine decarboxylase in the rat[11,12] while inhibiting gastric acid secretion stimulated by both histamine and gastrin[12]. It is thought that this antigastrin acts at the gastrin receptor site on the parietal cell[11]. There is no evidence to support the concept that H_2-receptor antagonists inhibit gastric acid secretion by acting outside the parietal cell. It is, however, possible that histamine and gastrin act on different receptors on the same cell as postulated by Grossman and Konturek.

The late rise in gastric acid secretion following burimamide in two studies, although small in terms of milliequivalents of acid, was clearly not seen after the control

injections. Since the duration of action of burimamide given intravenously is short in both dogs (M. I. Grossman and S. J. Konturek, unpublished) and pig (G.O.B., M. W. Waterworth, M.D., and S.B., unpublished), the late rise in acid release may be the result of 'rebound' following withdrawal of the inhibitor. This phenomenon is known after certain calcium containing antacids[13], and it would be clinically relevant if this phenomenon occurs when H_2-receptor antagonists are used therapeutically in man.

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1 Ash, A. S. F., and Schild, H. O., *Br. J. Pharmac. Chemother.*, **27**, 427 (1966).
2 Black, J. W., Duncan, W. A. M., Durant, C. J., Ganellin, C. R., and Parsons, E. M., *Nature*, **236**, 385 (1972).
3 Wyllie, J. H., Hesselbo, T., and Black, J. W., *Lancet*, **ii**, 1117 (1972).
4 Hansky, J., Soveny, C., and Korman, M. G., *Gastroenterology*, **61**, 62 (1971).
5 Hansky, J., Soveny, C., and Korman, M. G., *Gut*, **14**, 457 (1973).
6 Becker, H. D., Reeder, D. D., and Thompson, J. C., *Gastroenterology*, **65**, 28 (1973).
7 Gillespie, I. E., and Grossman, M. I., *Gut*, **5**, 342 (1964).
8 Lin, T. M., and Warrick, M. W., *Gastroenterology*, **61**, 328 (1971).
9 Johnson, L. R., and Aures, D., *Proc. Soc. exp. Biol. Med.*, **134**, 880 (1970).
10 Maudsley, D. V., Kobayashi, Y., Williamson, E., and Bouvaird, L., *Nature new Biol.*, **245**, 148 (1973).
11 Kobayashi, Y., and Maudsley, D. V., *Br. J. Pharmac. Chemother*, **32**, 428P (1968).
12 Kahlson, G., Rosengren, E., and Svenson, S. E., *Br. J. Pharmac. Chemother.*, **33**, 493 (1968).
13 Harvey, S. C., in *The Pharmacological Basis of Therapeutics* fourth ed. (edit. by Goodman, L. S., and Gilman, A.), 1103 (Macmillan Co., London and Toronto, 1970).

Cone mechanisms initiating response of on–off goldfish optic fibres

On–off ganglion cells are common in a wide variety of vertebrate retinae[1], including those of primates (F. M. de Monasterio and P. Gouras, personal communication). These units respond with a short, high-frequency burst of action potentials when a stationary light is turned on in the receptive field and also when the light is turned off. Because a dark or light spot moving across the receptive field elicits a vigorous response, Maturana *et al.*[2] called on–off units in the frog "moving contrast detectors".

I have explored the properties of on–off fibres in the goldfish optic nerve. The generation of the on–off response in goldfish is of special interest because the goldfish retina has three classes of cones with peak sensitivity at 620, 530 and 455 nm (ref. 3), and the majority of ganglion cells give a colour-coded response[4]. I shall show that on–off fibres are a separate class of fibres with properties quite different from those of colour-coded units. I have determined the contribution of each of the three cone mechanisms in initiating the on and off components of the on–off response.

Common goldfish, *Carassius auratus*, were anaesthetised with tricaine during surgical exposure of the optic nerve, and were then immobilised with curare for the duration of the experiment. Tungsten microelectrodes coated with Insl-x were inserted into the optic nerve and the activity picked up from single fibres was amplified, displayed and tape recorded. The optical stimulation of the eye has been described in detail elsewhere[6]. The left eye of the fish was under water and at the centre of a 24-inch diameter plastic hemisphere filled with water. Stimuli were presented on a back-projection screen taped to the surface of the hemisphere being observed by the fish. The stimuli and coloured backgrounds which were projected on to the back-projection screen originated in high intensity Prado projectors fitted with electronic shutters and frames for holding neutral density filters, narrow band interference filters and interference cutoff filters.

Results were obtained for 16 on–off fibres in a total sample of 73 fibres. This is a higher percentage (22%) of on–off units than

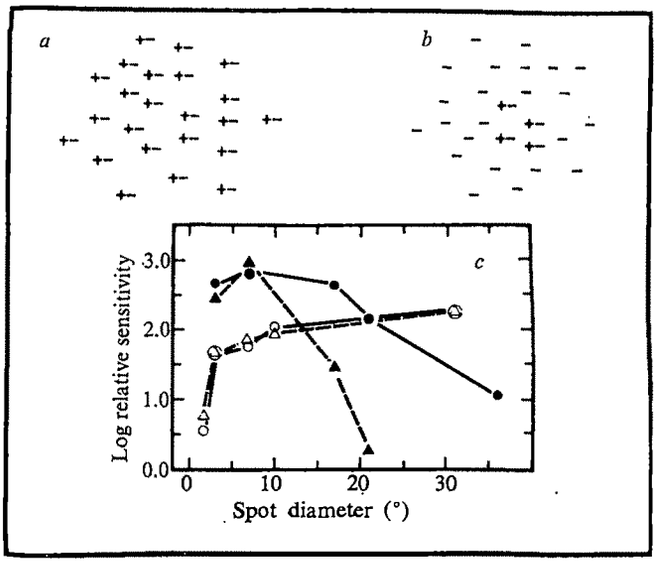


Fig. 1 Receptive field characteristics of on–off fibres. *a*, On–off receptive field centre having both on and off responses across the centre. The centre, about 20° in diameter, was mapped with a 1° spot 2.4 log foot-lambert in brightness. *b*, On–off receptive field centre having both on and off components at the midpoint, but only the off component towards the periphery. The width of the receptive field was about 10° mapped with a 2°, 1.8 log foot-lambert spot. *c*, Influence of spot size on sensitivity of the on (●○) and off (▲, △) components of the response. ▲, ●, An on–off fibre having an inhibitory surround which, for spot sizes above 10° in diameter depressed the sensitivity of both on (●) and off (▲) centre components. ○, △, An on–off unit which showed no reduction in sensitivity of the on (○) or off (△) centre response with larger spot sizes.

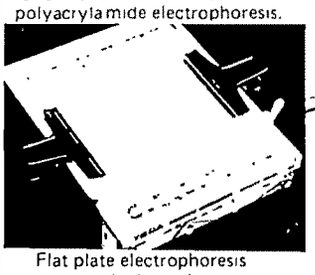
has been reported in a different preparation, the isolated goldfish retina (6%) (ref. 4). I established that both the on and off responses originated in a single optic fibre by showing that photographed action potentials in the on and off bursts had the same height and waveform.

Since stimulation of colour-coded receptive fields can result in an on–off response under certain conditions, I explored the receptive field of on–off units with stationary small spots of white light 1° or less in diameter. Neither the on nor the off components of the response were isolated in a central region of the receptive field. Either both on and off responses were present across the receptive field (Fig. 1*a*), or one or the other of the components dropped out at more peripheral receptive field positions (Fig. 1*b*). Both types of receptive field were quite different from the centre-surround, double-opponent organisation of colour-coded units[4]. The units described here were not directionally sensitive, a feature often associated with the on–off response in other animals. In goldfish optic nerve directionally sensitive fibres are present in small numbers[5].

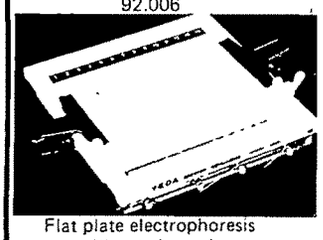
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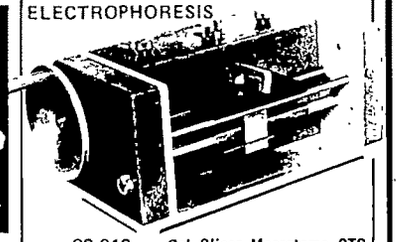


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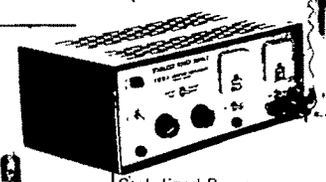
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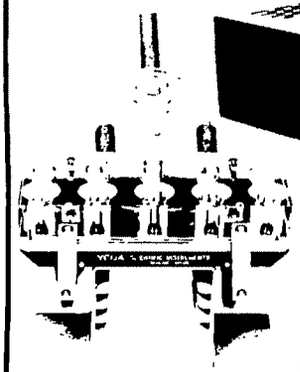
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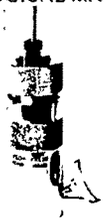
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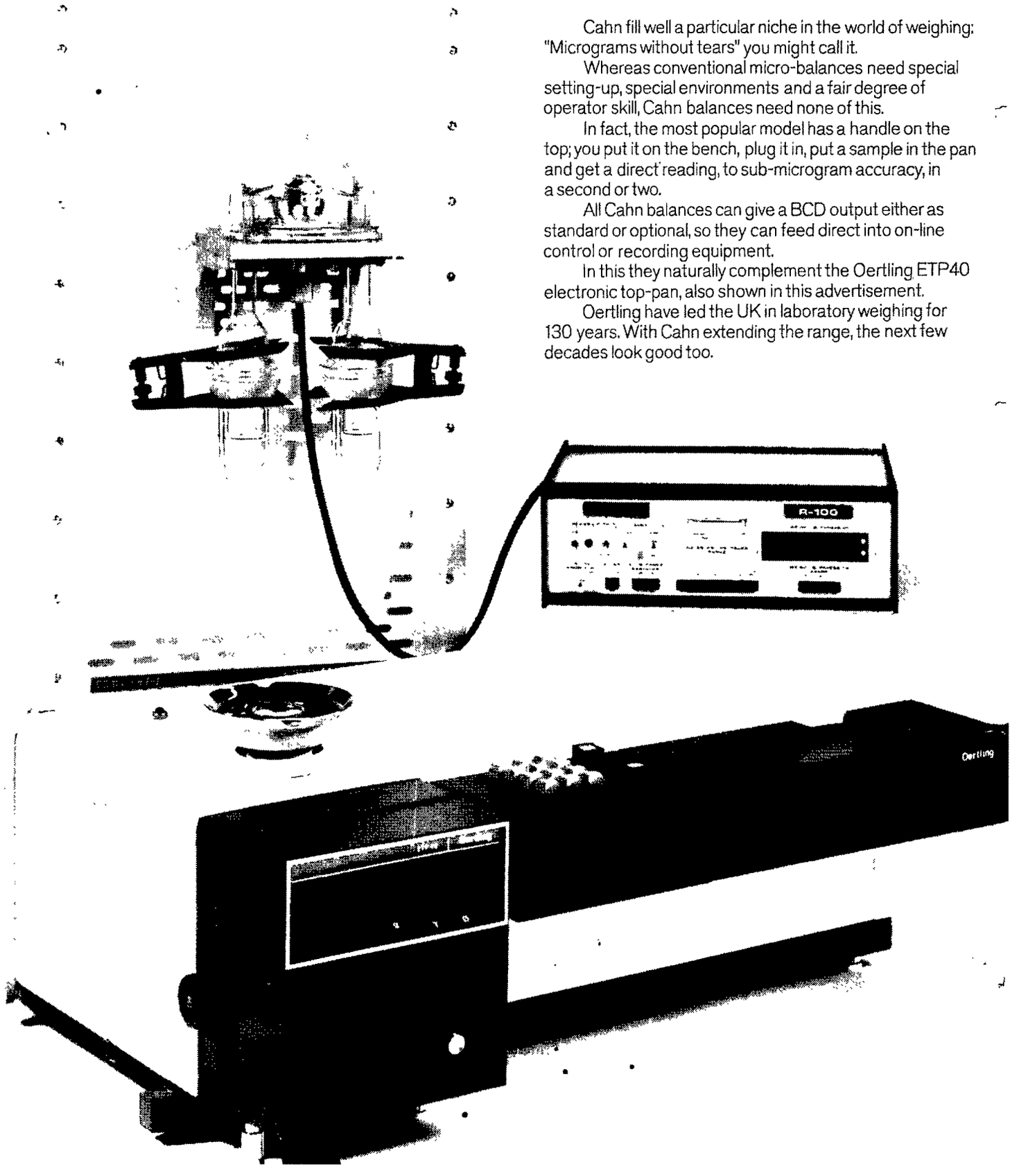
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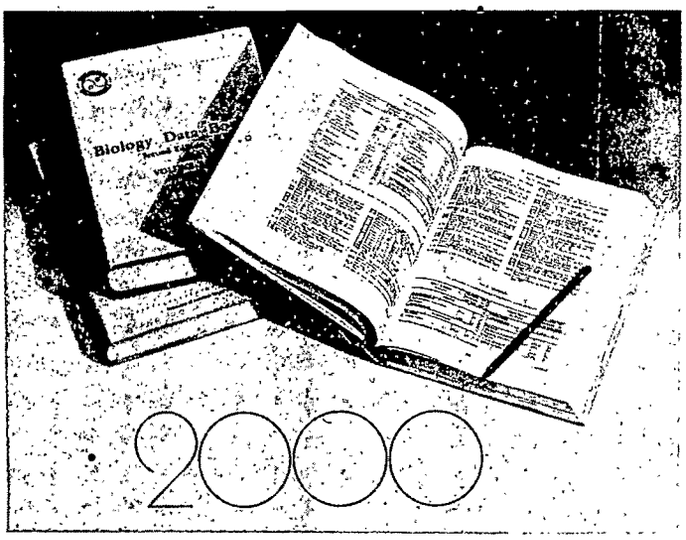
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Area-threshold curves confirmed a previous finding in the frog[6] that the excitatory on–off centre may have around it an inhibitory surround which raises the threshold for on and off components of the centre response (Fig. 1*c*, closed circles). In some cases, an inhibitory surround was not present (Fig. 1*c*, open circles). No response has been noted following stimulation of the surround alone. Within the on–off central region, there was an increase in sensitivity with larger stimulus sizes.

The cone mechanisms responsible for the on and off components were identified by making spectral sensitivity measurements. An example of the results is shown in Fig. 2. On–off responses were obtained to all monochromatic stimuli of 1 s duration at wavelengths from 400–720 nm. The thresholds for the on and off components were similar in this unit, although this was not always the case. Against a white background (Fig. 2*a*), both the on and off thresholds for responses above 500 nm fitted the retinene-2 nomogram of the red-absorbing goldfish pigment peaking at 620 nm. For 500 nm stimuli and below, the thresholds fell on the retinene-2 nomogram for the blue-absorbing cone pigment with peak sensitivity at 455 nm. A green mechanism on–off response was not evident against a white background but became apparent against a magenta background which reduced the red and blue mechanism sensitivities (Fig. 2*b*). The on and off thresholds against the magenta background for wavelengths between 500 and 620 nm fell on the retinene-2 nomogram for the green-absorbing cone pigment peaking at 530 nm.

Figure 2 shows that both the on and off response at a given wavelength are elicited by the same set of cone receptors, sensitive to red, green or blue. In the goldfish this was by no means a foregone conclusion, since on and off could have been elicited by different cone receptor types. As is true with colour-coded goldfish optic fibres[6], a single on–off optic fibre may receive connections from all three receptor systems. Only a few examples have been noted where one of the three cone systems could not be identified with the use of coloured adapting backgrounds. The strong blue mechanism response illustrated in Fig. 2 is characteristic of many optic fibres recorded from the intact, curarised goldfish[6]. The finding is somewhat puzzling in view of the failure of experiments using the isolated goldfish retina to see a blue mechanism response[4,7] and of Mark's report[3] that less than 7% of a sample of 113 goldfish cones contained blue-absorbing pigment. The spectral sensitivity curves of on–off fibres differ from those of colour-coded units in that there is no evidence of opponent interaction between red and green mechanisms. In colour-coded units this interaction tends to narrow opposing shoulders of the red and green mechanism spectral sensitivity curves at intermediate wavelengths where both red green mechanisms are stimulated simultaneously[4].

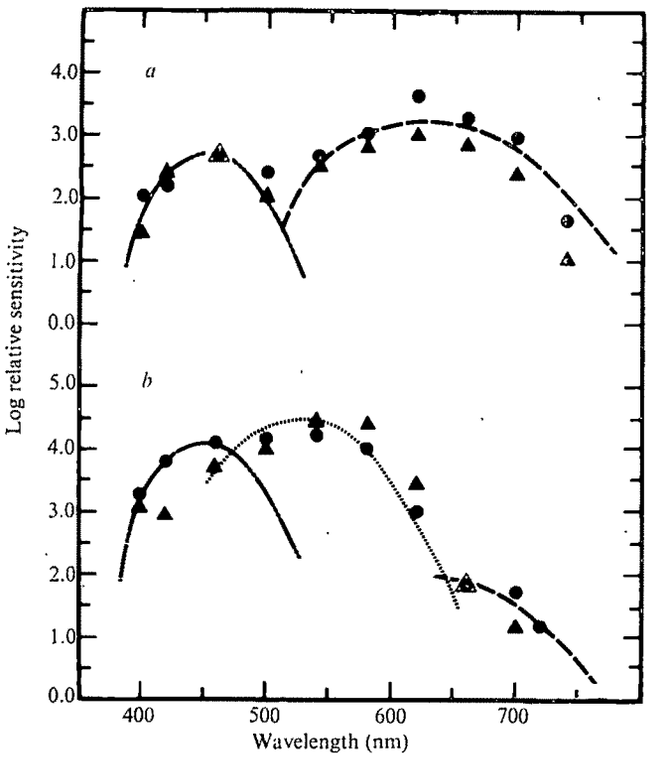


Fig. 2 Spectral sensitivity curves for an on–off unit: on response sensitivity (●) and off response sensitivity (▲). *a*, Curve measured against a 0.1 log foot-lambert white background. *b*, Curve measured against a magenta background made by superimposing a red-adapting background from a 650 longwave pass filter in a 1.7 log foot-lambert beam and a blue-adapting background from a 450 shortpass filter in a 0.6 log foot-lambert beam. The lines are retinene-2 nomograms for goldfish cone pigments having peak sensitivities at 620 nm (— — —), 530 nm (. . . . . .) and 455 nm (—. .—. .—. .—). The spectral sensitivity curves indicate that both on and off responses are given by all three cone mechanisms.

I examined the latency and number of spikes for the on and off components of the responses as a function of intensity. For all wavelengths, and hence for all three cone mechanisms, there was a decrease in the latency of both on and off responses as intensity increased in 0.5 log unit steps above threshold. Occasionally, at the highest intensities, the off threshold would show a slight increase in latency. In general, the number of spikes in the on and off burst increased only slightly or not at all with increase in the stimulus intensity. This feature of the response indicates that on–off units would not have the dynamic frequency range necessary of a separate brightness channel. This in turn suggests that colour-coded fibres must carry both hue and intensity information in the same channel or that a separate class of brightness fibres, not yet encountered, exists in the goldfish.

The retinal pathways which synthesise the optic fibre on–off response are incompletely understood. Stimulation of horizontal cells in turtle retina can lead to an on–off ganglion cell response[1]. But no on–off response from cells clearly identified as being bipolar has been reported. Two types of bipolar response, on-centre and off-centre, have been noted in goldfish[9] and carp[10]. Hence the ganglion cell on–off centre response must originate in one of two ways: (1) the convergence of on- and off-centre bipolars at the ganglion cell or (2) ganglion cell connections from on–off amacrine cells. Both bipolar-ganglion cell and amacrine cell-ganglion cell connections have been noted in the inner plexiform layer of carp[11]. If the first alternative is correct, then both the on- and off-centre bipolar types probably make excitatory connection with the on–off ganglion cell. In the tetrodotoxin-treated frog retina, the on–off ganglion cell response results from two depolarisations of the ganglion cell at onset and termination of light stimulation, with no intervening hyperpolarisation[12]. The second alternative origin of the ganglion cell on–off response is attractive because on–off amacrine cells have been identified in the carp. Toyoda *et al.*[13] classified the response of 178 carp amacrine cells and found 42 or 24% which were on–off, giving a depolarisation at both the onset and termination of stimulation. This is close to the proportion of optic fibres I found to be on–off. The on–off amacrine cell is a logical candidate to provide the major input to on–off ganglion cells. This is especially true in goldfish retina where the remaining ganglion cells have colour coded, double opponent receptive fields in which a role for on–off amacrine cells is difficult to envisage.

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1 Schwartz, E. A., *J. Physiol., Lond.*, **230**, 1-14 (1973).
2 Maturana, H. R., Lettvin, J. Y., McCulloch, W. S., and Pitts, W. H., *J. gen. Physiol.*, **43**, 129-175S (1960).
3 Marks, W. B., *J. Physiol. Lond.*, **178**, 14-32 (1965).
4 Daw, N. W., *J. Physiol. Lond.*, **197**, 567-592 (1968).
5 Daw, N. W. and Beauchamp, R. B., *Vision Res.*, **12**, 1849-1856 (1972).
6 Beauchamp, R. D. and Lovasik, J. V., *J. Neurophysiol.*, **36**, 925-939 (1973).
7 Kaneko, A., *J. Physiol. Lond.*, **235**, 133-153 (1973).
8 Barlow, H. B., *J. Physiol. Lond.*, **119**, 69-88 (1953).
9 Kaneko, A., *J. Physiol. Lond.*, **207**, 623-633 (1970).
10 Toyoda, J., *Vision Res.*, **12**, 283-294 (1972).
11 Witkovsky, P. and Dowling, J. E., *Z. Zellforsch. mikrosk. Anat.*, **100**, 60-82 (1969).
12 Murakami, M., and Shigematsu, Y., *Vision Res.*, **10**, 1-10 (1970)
13 Toyoda, J., Hashimoto, H., and Ohtsu, K., *Vision Res.*, **13**, 295-307 (1973).

Genetic variation in marine fishes as a test of the niche-variation hypothesis

SINCE the discovery of large and variable amounts of protein polymorphism in natural populations[1], biologists have attempted to determine what, if any, biological significance is associated with this particular form of phenotypic variation. Many population geneticists and ecologists[2-5] have adopted a selectionist point of view which states that allelic diversity is a strategy for increasing population fitness in a temporally and spatially heterogeneous habitat. This hypothesis, known as the "niche-variation hypothesis", predicts a positive correlation between the protein polymorphism and morphological variation in a population and the relative heterogeneity of the most relevant physical (for example, temperature) and biological (for example, food and competition) variables of the habitat. The basic assumption underlying this hypothesis is that the presence of more than one variant of a given protein broadens the tolerance limits or the optimal functioning range of the structures and/or reactions associated with the protein. For example, the presence of multiple forms of an enzyme, each with a different thermal optimum, may broaden the thermal tolerance range of an ectothermic (poikilothermic; cold blooded) organism[6].

Though here we are primarily concerned with testing the niche-variation hypothesis, it must be kept in mind that there are other selectionist explanations for the observed patterns of protein polymorphisms. One of these hypotheses is the "time-divergence hypothesis" (discussed below), which makes quite different predictions concerning the factors which are apt to increase or reduce levels of genetic polymorphism[7-9].

Satisfactory testing of the niche-variation hypothesis needs several precautions. First, one must be certain that the proteins being studied 'feel' the environmental parameter(s) of interest; that is, the proteins must exist at an organism–environment interface. The temperature–protein interface in ectothermic organisms represents an especially broad interface for examining environment–protein interactions since, in these temperature-conforming species, a change in habitat temperature will lead to rapid and equivalent changes in cell temperature. Changes in habitat (cell) temperatures will lead to alterations in the velocities of enzymic reactions, changes in the higher orders of protein structure, and altered interactions between proteins and their substrates, cofactors and modulators[6]. If temperature is the environmental parameter being studied, one can therefore select any set of enzymes for electrophoretic study, knowing that all of these enzymes will be affected by variation in this environmental parameter.

Two additional precautions must be taken in designing experiments to test the niche-variation hypothesis. First, the species or populations to be compared must be phylogenetically similar, since different groups of organisms may differ in their characteristic levels of protein polymorphism[4]. Second, one must examine the same enzymes in all populations since different classes of enzymes may have characteristically different levels of polymorphism[4,10-12].

With these considerations in mind, we designed a test of the following prediction based on the niche-variation hypothesis: since temperature changes will affect all proteins in an ectotherm, species living in extremely stable thermal habitats would be expected to have less protein polymorphism (fewer isozyme forms, fewer allozyme forms, smaller percentages of loci which are polymorphic, and lower average heterozygosities) than ectotherms living in habitats where temperature varies widely with a periodicity of less than the generation time. To test this prediction we examined 13 species of marine teleost fishes which live under markedly different temperature conditions. We studied the same enzyme systems in all species and, in most cases, the electrophoretic resolution we obtained allowed us to compare analogous, if not homologous, loci among all 13 species. Since the fishes studied are native to such diverse habitats as McMurdo Sound, Antarctica, where temperature variation is but a few tenths of a ° C throughout the year, and south temperate zone estuarine and intertidal regions, where temperature variations may be of the order of 20° C seasonally, we feel that our experiments allow a powerful test to be made of the niche-variation hypothesis.

Fish specimens were obtained by collecting with quinaldine (*Mugil cephalus*, *Gibbonsia metzi*, *Bathygobius ramosus*, *Abudefduf troschelli*), by hand (grunion: *Leuresthes tenius*), with traps (*Trematomus borchgrevinki*, *T. hansoni*, *T. bernacchii*), with deep, free vehicle set-lines (*Coryphaenoides acrolepis*), or by purchase from bait shops (*Gillichthys mirabilis*) or tropical fish dealers (*Dascyllus reticulatus*, *Amphiprion clarkii*, *Halichoeres* sp.). Except for the *Trematomus* species and *C. acrolepis*, which were stored in conventional deep freezes (approximately −20° C), all specimens were killed and stored in a −76° C freezer. Samples of heart, liver and skeletal muscle, plus one or both eyes, were pooled and homogenised in two volumes of buffer (0.10 M Tris HCl, *p*H 7.0, containing 0.001 M EDTA and 0.0005 M NADP). 0.5 ml of toluene was added to each homogenate before centrifugation (12,000*g* for 30 min). The supernatants were removed and stored at −76° C.

Horizontal starch gel electrophoresis was carried out essentially as described by McKinney *et al.*[13]. The buffer systems used were (1) Poulik, *p*H 8.6 (for glutamic-oxaloacetic transaminase, acid phosphatase, phosphohexose isomerase, lactate dehydrogenase, glucose-6-phosphate dehydrogenase and esterases); (2) Tris-maleic, *p*H 7.4 (for xanthine dehydrogenase, esterases, 6-phosphogluconate dehydrogenase, phosphoglucomutase and peptidases); (3) Tris-citrate, *p*H 8.0 (for fumarase, isocitrate dehydrogenase, malate dehydrogenase, leucine amino peptidase and phosphoglucomutase); (4) Tris-citrate, *p*H 6.7 (for alcohol dehydrogenase, lactate dehydrogenase and 6-phosphogluconate dehydrogenase); (5) Tris-versene-borate, *p*H 8.0 (for α-glycerophosphate dehydrogenase, glutamic-oxaloacetic transaminase, esterases and octanol dehydrogenase); (6) lithium hydroxide, *p*H 8.6 (for leucine amino peptidase, acid phosphatase, fumarase, esterases and general proteins). Staining of enzymic activity was done essentially as described by Shaw and Prasad[14].

Whereas optimal buffer conditions varied somewhat among species, we were able to score at least 75% of the above enzymes in all species, excluding the deep sea fish, *C. acrolepis*. Only a small number of enzymes was detected in the latter species (lactate dehydrogenase, malate dehydrogenase, esterases, α-glycerophosphate dehydrogenase, phosphohexose isomerase and general protein bands), due at least in part to the removal of livers and hearts of most specimens before our acquisition of the fish. Furthermore, enzymes of species adapted to high pressure may be extremely labile[6].

Our findings (Table 1) are not consistent with the predictions

of the niche-variation hypothesis. By no criterion do the proteins of fishes from thermally variable habitats display higher levels of polymorphism relative to proteins from fishes from habitats with stable thermal regimes. Using the estimates of mean individual heterozygosity (these are less dependant on sample size than are estimates of percentage polymorphism), the six species with a thermal range of less than 5° C do not differ significantly in heterozygosity from the six species with a thermal range of 5° C or greater ($P \gg 0.10$; Wilcoxon two-sample test[15]).

What other explanations might account for the patterns of variability observed in our study? One explanation can be based on neutralist arguments. Since, in theory, the number of neutral alleles in a population at equilibrium is proportional to the effective population size[16], the different amounts of polymorphism observed among these fishes may reflect differences in population sizes. Unfortunately, we cannot test this possibility since we know of no population size estimates for any of the species studied.

A selectionist explanation for the different levels of protein polymorphism can be based on the time-divergence hypothesis[7–9] which proposes that the highest levels of polymorphism will be found in populations that have existed for very long time periods in centripetal environments, that is, environments which minimise evolutionary change because of their stability and biotic complexity. Such environments are epitomised by tropical forests, tropical reef assemblages and the deep sea. The assumptions underlying this hypothesis are, first, that heterozygosity is in general advantageous to an organism in all environments because heterosis makes a contribution, however small, to fitness. The second assumption is that directional selection over geological time erodes genetic variation and allelic diversity, whereas stabilising (centripetal) selection allows alleles to accumulate. This assumes that allelic diversity accumulates very slowly in natural populations, Evidence for the latter phenomenon in populations of lizards is discussed by M.S. *et al.* (in preparation).

The prediction based on the time-divergence hypothesis is that tropical and deep sea fishes should be the most variable. Tropical and deep sea ecosystems have probably been perturbed less in climate, latitude, species composition and diversity than other marine ecosystems[17]. Hence species living in these habitats would on the average be exposed to relatively weak directional selection and should be evolving relatively slowly. These conditions would promote the accumulation of allelic diversity.

It is interesting, therefore, that the six tropical species are significantly more heterozygous than the temperate zone and Antarctic species (excluding *C. acrolepis*); $P < 0.05$, (Wilcoxon two-sample test[15]). If the *C. acrolepis* estimate is included with the tropical species estimates, the difference is even greater ($P < 0.025$). In this regard, supporters of the niche-variation hypothesis (see for example, refs 3, 18) have predicted that niche breadth and variation ought to be inversely related to the number of sympatric species, explicitly assuming that species diversity is proportional to competition. Since the richest fish faunas are associated with tropical reefs, the tropical species would, according to this idea, be predicted to have the least variation. They do not.

Other relevant observations include the low mean heterozygosities (0.18, 0.32, 0.38) of three northern Pacific fishes of the genus *Sebastes*[19], collected in localities where the annual temperature range is about 9° C, and the high heterozygosities found in four phyla of deep sea invertebrates[20] and a tropical mollusc[21].

On the basis of our findings and those of other workers, we conclude that no strong inference about the selective control of allozyme variation can at present be made. But within the framework of selectionist arguments, we believe that the available data on polymorphism patterns can in many cases be better explained by a time-divergence model of selection than by the niche-variation hypothesis.

Table 1 Levels of genetic variation in teleost fishes from different thermal regimes

Species (Family)	Collection site (habitat)	Annual thermal range (°C)	n†	L‡	P§	$P_{0.05}$¶	$H \pm$ s.e. ‖
Trematomus borchgrevinki (Nototheniidae)	McMurdo Sound (sub-ice, pelagic)	1	9	21	4.8	0	0.5±0.5
Trematomus hansoni (Nototheniidae)	McMurdo Sound (benthic)	1	26	26	18.5	11.1	2.5±0.4
Trematomus bernacchii (Nototheniidae)	McMurdo Sound (benthic)	1	30	26	42.3	15.4	3.3±0.6
Dascyllus reticulatus (Pomacentridae)	Manila (tropical reef, shallow)	3	10	29	34.5	—**	10.7±1.3
Amphiprion clarkii (Pomacentridae)	Manila (tropical reef, shallow)	3	11	27	22.2	—**	9.1±1.1
Halichores sp. (Labridae)	Manila (tropical reef, shallow)	3	10	28	21.4	—**	5.7±1.5
Coryphaenoides acrolepis (Macruridae)	San Diego Trough (deep benthic)	5	18	6	66.7	—**	11.0±2.8
Abudefduf troschelii (Pomacentridae)	Revillagigedo Islands (tropical, shallow)	7	16	20	35.0	15.0	5.0±1.1
Leuresthes tenuis (Atherinidae)	San Diego (inshore, pelagic)	10	20	33	18.2	15.2	3.6±0.7
Gibbonsia metzi (Clinidae)	San Simeon, California (benthic, intertidal)	10	28	28	28.6	17.9	4.3±0.8
Bathygobius ramosus (Gobiidae)	Revillagigedo Islands (tropical, shallow)	12	16	23	4.3	4.3	0.5±0.4
Mugil cephalus (Mugilidae)	Mission Bay, San Diego (inshore, pelagic, tropical subtropical)	15	20	30	36.7	20.0	7.1±1.1
Gillichthys mirabilis (Gobiidae)	San Diego (benthic, estuarine)	20	30	29	30.0	20.0	4.6±1.3

*The annual thermal range is an approximation of the variation in water (body) temperature a member of the sampled population would experience in its native habitat. Our estimates are based on published water temperature data and personal observations.
†Sample size. ‡Number of loci surveyed. §Percentage of loci polymorphic.
¶Percentage of loci polymorphic when commonest allele has a frequency less than 0.95.
‖ Mean individual heterozygosity determined by actual count of heterozygous genotypes, plus or minus the standard error of the estimate.
**Samples too small to calculate parameter.

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[1] Hubby J. L., and Lewontin, R. C., *Genetics*, **54**, 577 (1966).
[2] Levins, R., *Evolution in Changing Environments* (Princeton University Press, Princeton, 1968).
[3] Johnson, G. B., *Nature*, **243**, 193 (1973).
[4] Selander, R. K., and Kaufman, D. W., *Proc. natn. Acad. Sci., U.S.A.*, **70**, 1875 (1973).
[5] Wills, C. *Am. Naturalist*, **107**, 23 (1973).
[6] Hochachka, P. W., and Somero, G. N., *Strategies of Biochemical Adaptation* (Saunders, Philadelphia, 1973).
[7] Soulé, M., *Am. Naturalist*, **106**, 429 (1972).
[8] Soulé, M., *A. Rev. Ecol. Syst.*, **4**, 165 (1973).
[9] Soulé, M., and Yang, S. Y., *Evolution* (in the press).
[10] Johnson, G. B., *Nature*, **232**, 347 (1971).
[11] Johnson, G. B., *A. Rev. Ecol. Syst.*, **4**, 93 (1973).
[12] Gillespie, J. H., and Kojima, K., *Proc. natn. Acad. Sci., U.S.A.*, **61**, 582 (1968).
[13] McKinney, C. O., Selander, R. K., Johnson, W. E., and Yang, S. Y., *Studies in Genetics*, **7**, 307 (1972).
[14] Shaw, C. R., and Prasad, R., *Biochem. Genet.*, **4**, 297 (1970).
[15] Sokal, R. R., and Rohlf, F. J., *Biometry: the Principles and Practice of Statistics in Biological Research* (Freeman, San Francisco, 1968).
[16] Kimura, M., and Ohta, T., *Nature*, **229**, 467 (1971).
[17] Fischer, A. G., *Evolution*, **14**, 64 (1960).
[18] Rothstein, S. I., *Am. Naturalist*, **107**, 598 (1973).
[19] Johnson, A. G., Utter, F. M., and Hodgins, H. O., *Comp. Biochem. Physiol.*, **44B**, 397 (1973).
[20] Gooch, J. L., and Schopf, T. J. M., *Evolution*, **26**, 545 (1972).
[21] Ayala, F. J., Hedgecock, D., Zumwalt, G. S., and Valentine, J. W., *Evolution*, **27**, 177 (1973).

Nitrosation of phenols in smoked bacon

THE suggestion that nitrosamines could be formed in cured meats[1] has prompted much research into the fate of added nitrite in these foods. Because of their carcinogenicity, the major effort has been directed towards nitrosamine formation *in vitro* in cured meats and *in vivo*, but other nitrosations reported have been those of proteins[2], thiols[3] and creatine and creatinine[4]. We have recently reported our results on the nitrosation of liquid smoke emulsions in model systems[5] and we are prompted to report our studies of phenol nitrosation in smoked bacons by a recent suggestion that competitive nitrosation of phenolic residues may reduce the amount of nitrosamine formation[6].

The smoking of bacon is traditionally accomplished by hanging the meat in a smoke room filled with hardwood smoke, but recently liquid smoke preparations have been incorporated into bacon by either dipping or electrostatic spraying techniques, to produce a 'smoked' bacon. In a comparison of traditionally smoked bacon with one prepared by electrostatic spraying of a liquid smoke (supplied by Hercules Powder Co. Ltd, Erith, Kent) we have examined bacon samples from both processes in the raw and fried state and after simulated gastric digestion[2] of the fried samples; we have also examined the volatiles produced during frying. Both of these processes resulted in the deposition of phenols in the meat matrix (mainly methylphenols (cresols), 4-substituted 2-methoxyphenols (guaiacols) and 4-substituted 2, 6-dimethoxyphenols (syringols)).

The extraction method from the bacons was essentially that described by Issenberg[7] with the modifications that 5 N hydrochloric acid was used as protein precipitant and the nitroso/nitrophenols were separated from the phenols by carbonate extraction; the final concentrate (100 μl) for analysis being in ether solution. Analysis was by gas chromatography on 3% poly-(ethyleneglycol) adipate with a twin FID/thermionic nitrogen detector system. Identifications were made on the basis of the positive nitrogen detector response (a); combined gas chromatography mass spectrometry (GC-MS) (b); high resolution specific ion monitoring of GC-MS (c); and the comparison of relative (t_{phenol}) retention times with authentic compounds, and/or components present in nitrosated liquid smoke emulsion (d) (ref. 5). A control sample of green bacon was treated similarly and on analysis (highest sensitivity) no significant nitrogen containing peaks were observed.

6–Nitro–*m*–cresol ($C_7H_7NO_3$) was found in both bacons, in the fried, spray-smoked bacon and its cooking volatiles, and in raw and fried traditionally smoked bacon. The specific ion scan for *m/e* 153.04258 ($C_7H_7NO_3$) in the spray-smoked bacon volatiles gave two other peaks of t_{phenol} 1.604 and 1.721. The former, is probably the M–O· fragment ion of 6–nitroguaiacol (t_{phenol} 1.607) and the latter corresponds to an as yet unidentified peak (t_{phenol} — 1.735) also present in nitrosated liquid smoke[5].

Both bacons (spray-smoked volatiles and traditional fried) contain a peak at t_{phenol} 1.13 which is also present in nitrosated liquid smoke and has been tentatively identified as 6–nitroso–4–methylguaiacol ($C_8H_9NO_3$). A specific ion scan of the fried traditionally smoked bacon for *m/e* 167.0582 ($C_8H_9NO_3$) produced an intense peak at t_{phenol} 1.137 which suggests that this is identical to the above nitrosophenol from nitrosated liquid smoke. Three other peaks of *m/e* 167.0582 were identified in addition to the above, from this scan, of t_{phenol} 1.310, 1.712 and 1.814 respectively. Again by comparison with nitrosated liquid smoke the first of these (1.310) is probably the M–C_2H_4· fragment ion of 6-nitroso-4-propylguaiacol tentatively identified in the former, the second is probably the M–O· ion from 6-nitro-4-methyl-guaiacol and the last is unknown.

Efforts to improve the quantitative aspects of our extraction and analysis are in progress but not yet complete; estimates of the concentration of the individual nitroso/nitrophenols are at least one order of magnitude greater than the volatile N-nitroso compounds which have been found in bacon.

The identifications made to date are summarised in Table 1; including the compounds listed, a total of 19 significant nitrogen containing compounds were found in extracts from spray-smoked bacon and 12 from traditionally smoked bacon. Several of these have the same relative retention time as compounds identified in nitrosated liquid smokes as nitro- and nitroso-derivatives of cresols, guaiacols and 4-alkylguaiacols. These observations indicate that nitrite interaction with a wide variety of smoke phenols occurs in bacon during production, frying and gastric 'digestion'.

Some comments about the possible biological effects of compounds of these types have been made[6] but these are based mainly on their chemical properties and a proper assessment must await further investigation as must the possible role of phenol nitrosation as a reaction which competes with N-nitrosation for the limited quantity of nitrite available in cured meat. The evidence obtained so far indicates that the formation of nitroso- and nitro-phenols is sufficiently important for it to be

Table 1 Nitroso and nitrophenols in smoked bacons

Spray-smoked bacon		Traditionally smoked bacon	
Fried	Volatiles	Raw	Fried
6-Nitro-*m*-cresol[a,c,d]	6-Nitro-*m*-cresol[a,c,d]	6-Nitro-*m*-cresol[a,c,d]	6-Nitro-*m*-cresol[a,c,d]
	6-Nitroguaiacol[a,b,d]		6-Nitroso-4-methylguaiacol[a,c,d]
	6-Nitro-4-methylguaiacol[a,b,d]		6-Nitroso-4-propylguaiacol[a,c,d]
			6-Nitro-4-methylguaiacol[a,c,d]

[a-d] The methods are for identification (see text).

taken into account when nitrite-balance studies in smoked meats are carried out.

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1 Lijinsky, W., and Epstein, S. S., *Nature*, **225**, 21–23 (1970).
2 Knowles, M. E., McWeeny, D. J., Couchman, L., and Thorogood, M., *Nature*, **247**, 288 (1974).
3 Mirna, A., and Hofmann, K., *Fleischwirtschaft*, **49**, 1361–1366 (1969).
4 Archer, M. C., Clarke, S. D., Thilby, J. E., and Tannenbaum, S. R., *Science*, **174**, 1341–1343 (1971).
5 Knowles, M. E., Gilbert, J., and McWeeny, D. J., *The Potential Formation of C-Nitroso Compounds in Smoked, Cured Meats*, (3rd Meeting on the Analysis and Formation of N-Nitroso Compounds, Lyon, 1973).
6 Challis, B. C., *Nature*, **244**, 466 (1973).
7 Issenberg, P., Kornreich, M. R., and Lustre, A. O., *J. Agric. Fd Chem.*, **36**, 107–109 (1971).

Inhibition of inter-microbial predation by chlorinated hydrocarbons

TIDAL or estuarine regions have traditionally served as sinks for municipal sewage. Some microbial predators in the sea feed on the enteric bacteria contained in such sewage[1]. Recently, the concentration of chlorinated hydrocarbons in the sea, especially in estuarine regions, has increased although it remains considerably below the concentration which directly affects the viability of predators or intestional pathogens. But, estuarine chlorinated hydrocarbon concentrations are within a factor of 20 of those which we have observed to inhibit the chemotactic response of marine bacteria[2]. Because bacterial predators are chemotactic specifically to exudates of their prey[3], inhibition of chemotaxis by chlorinated hydrocarbons may directly affect estuarine self-purification rates. We report here the impact of two chlorinated hydrocarbons, at concentrations observed to inhibit chemotaxis[4], on enteric bacteria predation.

We examined the rate of kill of *Escherichia coli* by marine predators in the presence of 2,4-dichlorophenoxyacetate (2,4–D) and *o,o*-dichlorobiphenyl. The predators were isolated from seawater by repeated subculture in artificial seawater[5] using *E. coli* as the sole carbon source. The resulting predators displayed positive chemotaxis to *E. coli*. The level of kill of *E. coli* in 48 h by the predators in 50 ml portions of artificial seawater was determined by serial dilution and plating in triplicate on EMB agar (Difco) containing lactose. The die-off experiments were run in duplicate with two flasks placed on a rotary shaker (approximately 60 r.p.m.) and two on the laboratory bench at room temperature. Initial predator populations were estimated by five tube most-probable-number counts using the clearing of a tube containing 10 ml of a 10^8 ml^{-1} solution of *E. coli* in artificial seawater as a positive result.

We made several attempts to isolate a single predator species from the mixed predator culture. Methods used included: colony isolation on a broad spectrum of enriched artificial seawater-agar or gelatin solid media; isolation by serial dilution; or isolation by filtration through a series of Millipore filters. In every case a mixed culture resulted when the isolates were reinoculated into artificial seawater with *E. coli* as the sole carbon source. The predator culture consisted of at least two distinct *Spirillum* species as well as one motile pseudomonad. The dominant predator species (90% by visual count in liquid culture) was a *Spirillum* (approximately 1 μm diameter; two spiral turns). The presence of this predator mixture of visable organisms was necessary for *E. coli* lysis. The predator culture filtrate, when passed through a 0.45 μm Millipore filter, did not contain these organisms and also did not produce lysis of *E. coli*. There was no evidence of Bdellovibrios or bacteriophages in the filtrate.

The mechanism of *E. coli* lysis by the dominant *Spirillum* does not seem to be direct contact. The lysis mechanism seems to be based on the maintenance of a critical concentration of lytic enzymes although we were unable to obtain culture extracts which would lyse *E. coli* cultures in the absence of living predator cells. The predacious *Spirilla* utilised materials available in cell walls in that they could be subcultured in artificial seawater containing washed *E. coli* cell walls as the sole carbon source.

Inhibition of kill of *E. coli* by marine predators in the presence of chlorinated hydrocarbons is shown in Table 1. A decrease in the rate of kill was generally observed in the shaken flasks containing the chlorinated hydrocarbons and the percentage kill was no longer directly related to the initial population of the predators. In the standing flasks, the percentage kill generally decreased in the presence of the lower concentration of chlorinated hydrocarbons. In the presence of higher concentrations of the chlorinated hydrocarbons, the percentage kill of *E. coli*

Table 1 Percentage kill of *E. coli* in 48 h by motile marine predators in the presence of chlorinated hydrocarbons

Initial predator population no. per ml.	No chlorinated hydrocarbons	2, 4-D 10^{-4} M	2, 4-D 7×10^{-3} M	*o, o*-Dichlorobiphenyl 10^{-5} M	*o, o*-Dichlorobiphenyl 10^{-3} M
		% Kill of *E. coli*			
Standing					
10	0	7	31	0	31
10^3	6	3	32	17	35
10^5	30	10	29	13	39
10^7	72	26	41	41	47
Fully mixed					
10	20	23	25	18	27
10^3	54	29	41	31	44
10^5	61	32	43	30	43
10^7	71	77	44	55	51

The initial *E. coli* population was 10^7 ml^{-1}. The data are corrected for kill observed in the absence of the predators.

in the standing flasks was generally increased in those flasks in which the initial predator population was less than 10^7 ml^{-1}. The viability of the predators and of *E. coli* was not affected by either the low or the high concentrations of the chlorinated hydrocarbons. This was determined by predator cell counts in control flasks lacking *E. coli* and by *E. coli* counts in control flasks lacking the predators. The activity of the predators also did not seem to be affected by the chlorinated hydrocarbons as shown by a glucostat determination[6] in which the rate of consumption of glucose did not decrease even in the presence of the higher concentration of hydrocarbons.

The lower concentrations of chlorinated hydrocarbons, which significantly decreased the percentage kill of *E. coli* by marine predators in shaken culture, are approximately a factor of 20 greater than those observed in natural ecosystems. Waste effluents, which contain intestinal pathogens of which *E. coli* is an indicator, may contain chlorinated hydrocarbons at concentrations ten to a hundred times greater than seawater residual levels. Such chlorinated hydrocarbons, acting either singly or in combination, may decrease the level of predator—prey interactions responsible for self-purification in natural ecosystems in the vicinity of outfalls.

The lower chlorinated hydrocarbon concentrations, which decreased the percentage kill of *E. coli*, are similar to those we previously reported to inhibit chemotaxis in motile marine bacteria[4]. The higher hydrocarbon concentrations, which increased kill rates in the standing culture flasks for lower predator populations, are those which we observed to increase chemotaxis. This correlation suggests that the observed changes in *E. coli* kill rates are the result of inhibition or excitation of the chemotactic response in the marine predators of *E. coli*.

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[1] Mitchell, R., *Nature*, **230**, 257 (1971).
[2] Walsh, F., and Mitchell, R., in *The microbial degradation of oil pollutants* (edit. by Meyers, S. P.) (Center for Wetland Resources, LSU-SG-73-01, 1973).
[3] Chet, I., Fogel, S., and Mitchell, R., *J. Bact.*, **106**, 863 (1971).
[4] Walsh, F., and Mitchell, R., *Bact. Proc.*, G244 (1972).
[5] Lyman, J., and Flemming, R. H., *J. mar Res.*, **3**, 134 (1940).
[6] Washko, M. E., and Rice, E. W., *Clin. Chem.*, **7**, 542 (1961).

Formation of methylmercury in a terrestrial environment

WOOD *et al.*[1] demonstrated that the mercury from inorganic chemicals can be methylated by aquatic organisms and also, enzymatically, by extracts of methanogenic bacteria. Since the organic forms of mercury are reported to be more biologically available, these methylation processes in aquatic or sedimentary environments, together with the tendency of methylated mercury to accumulate in biota, are a major reason for bioconcentration of mercury in fish and other organisms found in lakes, streams and bodies of seawater that are contaminated with mercury. As methanogenic bacteria also occur in terrestrial environments, we have investigated possible organomercury compound formation in soils contaminated with an inorganic mercury compound.

In a preliminary laboratory study, several 100 g soil samples were spiked with ^{203}Hg as mercuric nitrate, and incubated. Extraction of these samples with hydrochloric acid, followed by benzene extraction of the aqueous phase resulted in increased activity in the benzene fraction compared with reagent blanks. This indicated some conversion of the mercuric nitrate to a chemical form more soluble in benzene, presumably some organic form.

These results prompted us to spike small agricultural plots, at the Environmental Protection Agency's experimental farm at the Nevada Test Site, with radioactively labelled mercuric nitrate. Approximately 1 μCi ^{203}Hg m^{-2} (specific activity 2–20m Ci g^{-1} according to the supplier's specification) was applied in the form of an aqueous solution to the bare sandy loam soil (soil *p*H about 8.5). Plots were watered daily, and starting at the time of application, soil samples were collected periodically and extracted with 2.2 N HCl; organic mercury compounds contained in the filtered aqueous solutions were extracted into benzene. The benzene extracts were concentrated by column distillation; the concentrate was washed with 2.2 N HCl, and the mercury compounds extracted into 1% cysteine acetate solution as described by G. Westöö[2]. The mercury compounds in the cysteine acetate solution were then extracted into a small volume of a benzene solution of dithizone (0.4% w/v). Excess dithizone was removed by treatment with H_2SO_4 and NH_4OH (ref. 3); no attempt was made to achieve a quantitative recovery.

Thin-layer chromatography (TLC) of a portion of the dithizonate solution was performed on Gelman silica gel instant TLC sheets in a Gelman chromatography chamber using a carbon tetrachloride:benzene mixture (75:5 v/v) as the mobile phase. Isolated spots consisting of a mixture of stable mercuric dithizonate, phenylmercuric dithizonate and methylmercuric dithizonate were applied on each side and in the centre of the same TLC sheet and developed together with the radioactive dithizonate extract, to serve as indicators. In control experiments carried out under identical conditions the R_F values for mercuric dithizonate, phenylmercuric dithizonate and methylmercuric dithizonate were determined as 0.19, 0.52 and 0.63, respectively.

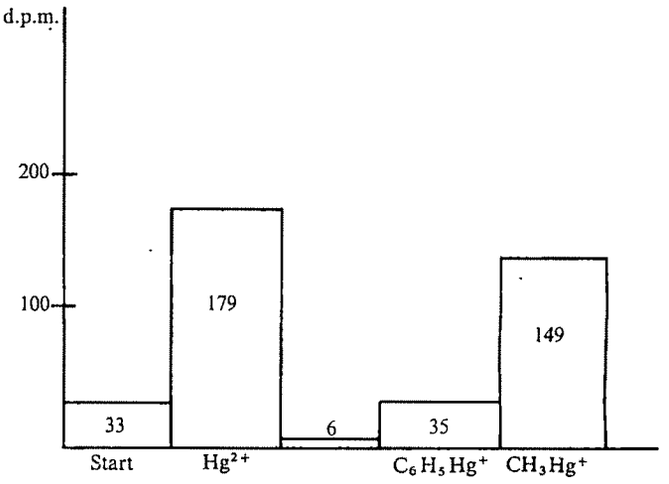


Fig. 1 Distribution of ^{203}Hg on thin-layer chromatography sheet.

The developed, air-dried TLC sheets were cut immediately into horizontal segments using the coloured indicator spots of the above-mentioned stable mercury compounds as reference points, and the individual segments were analysed for radioactivity content by gamma-counting. Figure 1 shows that more than one-third of the total activity of the dithizonates was concentrated in the range of the methylmercuric dithizonate.

It is not known whether the activity found in the range of the phenylmercuric dithizonate was due to phenylmercuric dithizonate originating from mercuration of benzene during extraction, tailing of the alkylmercuric dithizonate, or to some as yet unidentified mercury compound with an R_F similar to that of phenylmercuric dithizonate. A control experiment using a heat-sterilised soil sample which was spiked with $^{203}Hg(NO_3)_2$ solution and analysed as described above showed no activity in the methlymercuric dithizonate range but some activity (about 2%) in the phenylmercuric dithizonate range of the TLC sheet. A portion (2–10%) of the total ^{203}Hg contained in the soil samples was not soluble in 2.2 N HCl after repeated extractions. It could, however, be extracted readily into cold 2.2 N HNO_3, indicating some conversion to elemental mercury. This conversion is not surprising as certain bacteria can convert virtually all mercury compounds into elemental mercury[4–6].

These results show that mercuric salts can, under agricultural conditions, be transformed into methylmercury. We could not differentiate between methyl and dimethylmercury because during extraction, dimethylmercury would have been converted to the monomethyl compound, according to $(CH_3)_2Hg + HCl \rightarrow CH_4 + CH_3HgCl$. The conversion in a terrestrial environment of inorganic mercury compounds to methylmercury implies that recycling of mercury from soil to air and back to soil or water could occur faster than presently assumed on the basis of the volatility of metallic mercury and inorganic mercury compounds alone. Due to the formation of methylmercury compounds and their high volatility,

mercury compounds deposited in soil are more readily available to humans and animals than previously anticipated, the consequent health hazard being magnified by the higher toxicity of methylmercury.

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1 Wood, J. M., Kennedy, F. Scott, and Rosen, C. G., *Nature*, **220**, 173 (1968).
2 Westöö, G., *Acta Chem. Scand.*, **21**, 1790 (1967).
3 Takeshita, R., Akagi, H., Fujita, M., and Sakagami, Y., *J. Chromatogr.*, **51**, 284 (1970).
4 Suzuki, T., Furukawa, K., and Tonomura, K., *J. ferment. Technol.*, **46**, 1048 (1968).
5 Furukawa, K., Suzuki, T., and Tonomura, K., *Agr. biol. Chem.*, **33**, 128 (1969).
6 Nelson, J. D., Blair, W., Brinckman, F. E., Colwell, R. R., and Iverson, W. P., *Appl. Microbiol.*, **26**, 321 (1973).

New finding of the ancient primitive mollusc *Neopilina* in the Atlantic part of the Antarctic

The mollusc *Neopilina* (Monoplacophora) was first found 20 yr ago in the Peru-Chile Trench of the Pacific Ocean[1]. *Neopilina* has since been found in other places[2–4] including various areas of the Pacific, the northern Indian Ocean and the Atlantic part of the Antarctic. So far, no *Neopilina* has been encountered in the Atlantic Ocean proper, though it probably occurs there.

The first *Neopilina* found in Antarctic waters was an immature specimen of *N.(Neopilina)*. It was 2.3 mm long and 2.0 mm wide, with five pairs of gills[3]. It was taken from depths of 1,647 m to 2,044 m at the eastern slope of Burdwood Bank (54°43′ S, 55°30′ W).

Neopilina was found for the second time in the Antarctic during the eleventh biological cruise of the vessel Akademik Kurchatov (1971–1972). The bottom fauna was sampled by this expedition in the northern parts of the South Sandwich and South Orkney Trenches. One of the sections runs across the South Antilles basin (Scotia Sea) and the eastern slope of the Falkland shallow. Two deep sea trawlings were made in the small trench situated in the north-western part of the Scotia Sea. One living adult specimen of *Neopilina* was found on December 14, 1971, 56°29′ S, 50°51′ W. The depth was 4,664–5,630 m, and the bottom consisted of mud with sand and pebbles.

This specimen of *Neopilina (Neopilina)* was 19 mm long, 15 mm wide and 8.5 mm high. The mollusc was broken during trawling and the edges of its shell overlapped slightly behind, so its width is in fact a little greater (by 1–1.5 mm). The apex is shifted (as usually) to the front margin and hangs over it. The valve surface contains very fine and delicate but pronounced concentric folds. The radial striation is the finest and becomes visible only under magnification; five pairs of gills are well developed; the labial palpi have short marginal bordering.

It is quite possible that the small *Neopilina (Neopilina)* specimen found previously on Burdwood Bank[3] was a young form of the *Neopilina* we describe. The taxonomic position of the small mollusc is being investigated.

The bottom fauna found with our specimen was very rich and diverse, containing more than 20 groups of bottom invertebrates. There were several hundred holothurians (including *Elpidia*—the leading form of bottom fauna here), numerous Pourtalesiidae (irregular deep sea urchins) and Ophiuroidea, rich population of Polychaeta (Aphroditidae, Flabelligeridae and others), various Spongia, Actinia, Pogonophora, Amphipoda and Isopoda. Molluscs (apart from *Neopilina*) were represented by Gastropoda, Prosobranchia and Opisthobranchia and Bivalvia (*Cyclopecten* and *Neilo*).

It therefore seems that the food and conditions are quite adequate for recent *Neopilina* and other detritus-feeding bottom invertebrates in the South Atlantic eutrophic regions. The hard substratum to which *Neopilina* adheres (stones, manganese nodules or pebbles) is dispersed in good quantity in the Antarctic region by the action of floating ice. The mucus bacterial film or organic detritus in various forms usually existing on the surface of such hard substratum is used by *Neopilina* as food. They can easily scrape it off with the long thin denticles of brush-like parts of their radula. Therefore the subantarctic regions of the Atlantic Ocean are very favourable for them, especially the areas influenced by the temperate warm waters of the Falkland current washing the slopes of Falkland shallow water zone.

The existence of well-dispersed hard substratum, usually covered with bacterial film can explain the existence of *Neopilina* forms even in parts of the ocean very poor in food and bottom fauna; for example *Neopilina oligotropha* in the North Pacific[4].

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1 Lemche, H., *Nature*, **179**, 413–416 (1957).
2 Filatova, Z. A., and Zenkevitch, L. A., *Oceanology*, **9**, 162–171 (1969).
3 Rosewater, J., *Science*, **167**, 1485–1486 (1970).
4 Rokop, F. Y., *Veliger*, **15**, 91–95 (1972).

Effects of stable chlorine-containing organics on aquatic environments

During 1962, approximately 60,000 tons of chlorine were added to the effluents of sewage treatment plants in the United States[1] and subsequently released to surface waters. By 1970, it was estimated that 100,000 tons of chlorine were added annually, and the quantity will continue to increase as municipalities are required[2] to provide at least secondary treatment for sewage by July 1, 1977 (ref. 3). Principal reactions of chlorine in natural waters, besides hydrolysis, are with ammonia and organic amines[4,5]. Reactive chlorine residuals, for example, hypochlorites, inorganic and organic chloramines, are characterised by reactive chlorine which would decompose or be consumed in various chemical reactions[5]. Jolley[3] has identified seventeen stable chlorine-containing organic compounds at low $\mu g\ l^{-1}$ concentrations in chlorine-treated sewage effluent. The persistent nature of these compounds, which are characterised by chemically stable or inert C–Cl bonds, suggests potential for their accumulation in receiving surface waters[6,7].

Research on the toxicity of reactive chlorine residuals to aquatic life has been extensive[8]. The identification of stable chlorine-containing organics has revealed a group of reaction products which arise from chlorination. Not only are the toxicities of these compounds unknown, but analytical methods used for measuring concentrations of reactive chlorine residuals fail to account for their presence. The identification of stable chlorine-containing organics indicates the need for further research in order to evaluate the total toxicity of all reaction products formed from chlorine released into natural waters.

We report here data on the toxic effects of two species of stable chlorine-containing organic compounds, 4-chlororesorcinol and 5-chlorouracil, on the hatchability of carp (*Cyprinus carpio*) eggs.

Gravid carp were collected and spawned artificially in the laboratory as described before[9]. Tests were run on eggs fertilised and left undisturbed for 30 min before addition of test solutions (water-hardened eggs) and on eggs fertilised immediately in the test solutions (non-water-hardened eggs). The latter were subjected to the toxicant during the hardening procedure. After 30 min the aqueous solutions were decanted and fresh solutions of the appropriate chemical concentrations were added. For each chemical, tests were run at two temperatures (21° and 26°C) with nine concentrations (ranging from 0.001 to 10 mg l^{-1}) plus controls. Two replicates (200–400 eggs each) were performed for each concentration. After the water-hardening period the containers were kept in controlled environment rooms at 21°C or 26°C until the eggs hatched (about 3 d at 26°C and 7 d at 21°C). Twice daily during this period solutions were changed and dead eggs and those infected with fungus were recorded and removed.

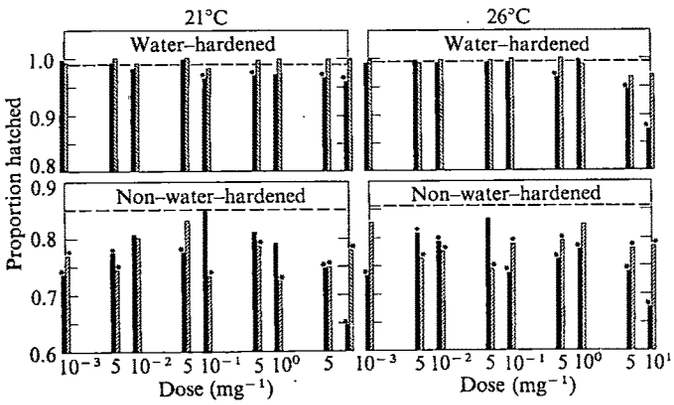


Fig. 1 Response (hatching success) of carp eggs. *Response significantly below ($\alpha<0.05$) controls. Black columns, 4-chlororesorcinol; hatched columns, 5-chlorouracil; ---, control response.

All concentrations were based on serial dilutions prepared in the laboratory rather than *in vivo* measurements. Although the latter is desirable, sensitive analytical procedures are not yet ready for use. These chemicals are relatively stable and the total test period for a run was always less than a week; therefore, qualitative and (or) quantitative changes in the test medium would not be expected.

Results of the tests were scored on the basis of the percentage of the eggs initially placed in a container that hatched. The responses from the replicates at each dose were tested and not found to be significantly different $\alpha=0.05$, assuming a normal approximation to the binomial distribution); therefore the replicates were pooled to give the percentage responses shown in Fig. 1. The mean of the two replicates at each concentration was then compared with the mean of the controls (Fisher's exact test)[10] to determine if a significant decrease in hatchability occurred.

Comparison of the relative toxicity of the two chemicals on non-water-hardened eggs at the two temperatures (Fig. 1) indicates similar toxicity with concentrations of 0.001 mg l^{-1} significantly lowering hatchability in all but the 26°C 5-chlorouracil test medium. This level (0.001 mg l^{-1}) is below the estimated concentrations of stable chlorine-containing organics present in effluents of waste water treatment plants[3]. There seems to be an area at the midrange of concentrations (about 0.05 mg l^{-1}) where no significant effect on hatchability was seen. Whether this resulted from some physical or physiological relationship between toxicant levels and eggs, or possibly from some fungicidal activity of the chemical, is not known and deserves further study. This phenomenon does not detract from the results, however, since the 0.001 mg l^{-1} concentrations exhibited a significant reduction of hatchability. This level (0.001 mg l^{-1}) cannot be taken as the lower incipient lethal level since lower concentrations were not tested.

Eggs that were water-hardened before exposure to the toxicants were more resistant to the toxicants than were non-water-hardened eggs (Fig. 1). Hatching success of water-hardened eggs was similar to or greater than the controls for most of the dose range of toxicants tested, whereas in the non-water-hardened eggs hatching success was always lower than controls (with the exception of 0.1 mg l^{-1} of 4-chlororesorcinol at 21° C). There were no toxic effects except at concentrations $\geqslant$ 0.1 mg l^{-1} of 4-chlororesorcinol. No toxic effects of 5-chlorouracil were seen in water-hardened eggs at 21°C, whereas hatchability was significantly lowered at concentrations $\geqslant$ 5 mg l^{-1} at 26°C.

The data presented here show that both 5-chlorouracil and 4-chlororesorcinol significantly decreased the hatchability of non-water-hardened carp eggs when tested in concentrations as low as 0.001 mg l^{-1}. Results of our research are important not only as the first source of data on toxicity of specific stable chlorine-containing organics, but as a signal that such compounds (which have now been reported to be formed in reactions of chlorine with organic matter in natural waters)[3] need to be considered in future tests of chlorine toxicity.

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1 Laubusch, E. J., *Chlorine, its manufacture and use* (edit. by Sconce, J. S.) Am. Chem. Soc. Monogr. Series No. 154 (1962).
2 US Government Public Law, 92–500 (1972).
3 Jolley, R. L., *Chlorination effects on organic constituents in effluents from domestic sanitary sewage treatment plants*, ORNL–TM–4290 (Oak Ridge National Laboratory, 1973).
4 Palin, A. T., *Water Engng*, **54**, 248–256 (December) (1950).
5 Draley, J. E., *The treatment of cooling water with chlorine*, ANL/ES–12 (Argonne National Laboratory, 1972).
6 Ingols, R. S., and Stevenson, P. C., *Res. Engng*, **18**, 4–8 (1963).
7 McCallum, G. E., *J. Water Pollut. Control. Fedn*, **35**, 1–10 (1963).
8 Brungs, W. A., *J. Water Pollut. Control. Fedn*, **45**, 2180–2193 (1973).
9 Blaylock, B. G., and Griffith, N. A., *Prog. Fish. Cult.*, **33**, 48–50 (1971).
10 Sokal, R. R., and Rohlf, F. J., *Biometry*, **776** (Freeman, San Francisco, 1969).

book reviews

New philosophy at Leiden

Physics at 17th and 18th Century Leiden: Philosophy and the New Science in the University. By Edward G. Ruestow. Pp. 174. (International Archives of the History of Ideas. Series Minor 11.) (Nijhoff: The Hague, 1973.) 24.50 guilders.

WITH the growing interest in the external relations of science during recent years, increasing attention has been devoted to a study of the development of science within various institutional contexts. While the major thrust of this research has focused upon social factors, there has also been some attention given to the development of ideas within the institutions themselves and, particularly in the wake of Kuhn's seminal book, various attempts to delineate the institutional implementation of new scientific ideas to replace the old ones. E. G. Ruestow's study treats the development of one science at a single institution during a two-century period. As such it is a useful case study of the change in scientific orientation within firmly fixed temporal and spatial limits.

The University of Leiden was founded in 1575 as the first Dutch university. With an amazing rapidity it achieved a position of eminence which it has retained through most of its life. Though it had a small beginning with only ten professors, it gradually expanded, and what it lacked in size it made up in quality. Already in the seventeenth century it had a notable library and numerous distinguished professors, not only in scientific subjects, but perhaps even more preeminently in humanistic disciplines. Ruestow selects one strand of its development during the first two centuries of the university's life and attempts to trace the fortunes of physics teaching. The bulk of his book centres on the period from Burgersdijk to Musschenbroek (about 1610-1760).

Franco Burgersdijk was the first philosophy professor at Leiden to gain international distinction. As Dibon's careful researches have shown, his textbooks went through many printings not only in Holland, but in England as well, Burgersdijk's books on logic and natural philosophy being part of the staple diet of students at seventeenth-century Oxford and Cambridge. The chapter on him is a useful summary, though it does not really penetrate as deeply into the sources and characteristic organisation of Burgersdijk's writings as one might wish. After Burgersdijk's passing, his Aristotelian orientation gave way to the attempts at a Peripatetic-Cartesian synthesis in natural philosophy, particularly on the part of his successors Heereboord and De Raey. After a period of Aristotelian counterattack, experimental philosophy begins at Leiden, the first request being made in 1694 to institute an experimental approach to physics. Naturally enough, the early work was on the beaten path of vacuum experiments and other topics which had become subject to this new approach during the previous half century. Early in the eighteenth century, however, the Newtonian revolution reached Leiden and principally through the efforts of 'sGravesande and Musschenbroek became firmly established. Thus Leiden's university became one of the first to espouse the "new philosophy".

Ruestow's story is an interesting one and it bears comparison with the situation in other universities. Though he does not bring to light any new sources, he makes good use of the available ones. Not only has he read extensively in the writings of the professors themselves, but he makes ample use of the documentary history of the university, especially of the magisterial compilation of sources published by Molhuysen more than a half century ago. Moreover, he brings to general attention and summarises the results of various specialised Dutch studies touching on the topic. Perhaps one could have wanted a more detailed bibliography not only of the publications of the professors, but also the hidden articles on their lives. Too often the *Nieuw Nederlandsch Biografisch Woordenboek* is uniquely relied on. Moreover, it seems to this reader that a slight distortion has been introduced by taking the study of physics too much in isolation from other subjects being taught at Leiden (such as botany, medicine, and chemistry, as well as theology). More could also be learned about outside influences, especially from Italy.

In conclusion, here is a good, solid study of a limited area of investigation. It is generally a reliable guide to the subject and, if not as exciting as it might have been, it should serve to recall to English readers the importance of Leiden as a scientific centre. What is more, it is written in a sober and value-free way, which is important at a time when many historical studies are infected with attempts to show that one or another present-day philosophy of science is the uniquely true one.

C. B. SCHMITT

Tropical diseases

Trypanosomiasis and Leishmaniasis with Special Reference to Chagas' Disease. Pp. xii+353. (Ciba Foundation Symposium 20 (new series) held jointly with the Venezuelan Academy of Sciences and "La Trinidad" Medical Center, Caracas.) (Elsevier/Excerpta Medica/North Holland: Amsterdam, London and New York, 1974.) Dfl.46; $18.40.

AMONG the most important diseases of man in tropical and subtropical countries are Chagas' disease in America, sleeping sickness in Africa and leishmaniasis, in its various forms, in both the Old and New Worlds. These diseases are very different from one another but they have one thing in common; they are all caused by flagellates belonging to the order Kinetoplastida. Clinicians and research workers all over the world are studying isolated aspects of the biology of these flagellates and this symposium represents a commendable attempt to bring some of them together and to pool their findings and thoughts.

The fourteen papers in this collection are all by recognised authorities. Three are devoted to Chagas' disease, three to sleeping sickness and two to leishmaniasis. Six cover all three diseases and the discussions which follow each paper also contribute to this common theme.

The taxonomic framework of the Kinetoplastida is summarised by W. H. R. Lumsden and there are chapters on ultrastructure (K. Vickerman), nutrition and biosynthesis (W. Trager), intermediary metabolism (I. B. R. Bowman), chemotherapy (B. A. Newton) and drug resistance (W. Peters). Sleeping sickness is considered from the points of view of epidemiology (J. R. Baker), pathogenesis (L. G. Goodwin) and immunity and antigenic variation (P. de Raadt). The coverage of leishmaniasis is restricted to its epidemiology (R. S. Bray) and the clinical immunopathological aspects of American cutaneous leishmaniasis (J. E. Convit and M. E. Pinardi). The epidemiology of Chagas' disease is described by R.

Zeledón and there are two chapters on its pathology, one by A. Anselmi and F. Moleiro and the other by F. Köberle.

Most of the chapters are well written and straightforward accounts which reveal both the extent of and gaps in present knowledge. The two chapters on the pathology of Chagas' disease represent alternative views. The section on antigenic variation in trypanosomiasis is probably the most speculative in the symposium and the one least likely to survive the passage of time.

What emerges is an excellent statement of the present situation with regard to these diseases. Much of it is a textbook and will be used as such. It is also a source book for those seeking new (or first) fields to conquer and a reference book for those involved in teaching, research and clinical medicine. Two points, however, may confuse uninitiated readers. *Leishmania aethiopica* is mentioned on pages 102 and 250 but not in the check list in the first chapter. Although described after the meeting, this parasite should have been added in proof. *Schizotrypanum* is given subgeneric status in chapter 1 but occasionally reverts to generic status during subsequent discussions.

One fact comes over above all others from this symposium, the deficiency in understanding of Chagas' disease. Here is a disease that affects 35 million people and in parts of Brazil is responsible for one third of all adult deaths. Yet we do not know how many forms of the causative agent, *Trypanosoma cruzi*, exist nor do we have a single drug that is effective once the parasite reaches its host cell.

F. E. G. Cox

History of salt water

The Development of the Chlorinity/Salinity Concept in Oceanography. By William J. Wallace. Pp. xii+227. Elsevier Oceanography Series, Vol. 7. (Elsevier: Amsterdam, London and New York, 1974.) Dfl.46; $17.70.

THE author starts with a careful summary of Greek and Arabic writings about salt, mineral waters and seawater. Errors that appear in Riley and Skirrow are repeated in a paragraph quoting a letter from Synesius to Hypatia describing a hydrometer in about 410 AD. Boyle's *Observations and Experiments on the Saltness of the Sea* is treated as a landmark, and also Halley's study of evaporation, particularly his work on the Mediterranean Sea, and on condensation at higher levels as he saw it in St Helena. Lavoisier is shown as mainly interested in medicinal waters though adding considerably to what was known about the sea. Bergman's analyses began to hint that the main constituents of sea salt are always present in the same proportions.

The careful work of John Murray, published in 1818, brought reproducibility to sea water analysis. Marcet formulated the principle of constancy of composition in 1819, and Forchhammer, and Dittmar who analysed samples collected during HMS Challenger's circumnavigation, confirmed it. Measurement of total solids by evaporation does not give consistent results, and experience showed that for common use it is better to rely on estimated values based on measurements of chlorine content. Sorensen in Sweden and Knudsen in Denmark put the procedure on a systematic basis by careful work in 1899–1902. They refined the methods, initiated a distribution of Standard Sea Water for everyone to use, and published tables relating chlorinity to salinity and density, so that before long all results could be combined into maps and sections sufficiently detailed and consistent to trace water movements, and to work out pressure patterns. There was no reason other than some self satisfaction or sense of completeness why salinities should have been plotted rather than the chlorinities on which they were based.

The final part of the book deals with changes inspired by more recent improvements. The measurement of electrical conductivity is now an easier and more accurate routine than chlorine determination, and salinity has been newly defined in terms of conductivity. The author reviews the problems that remain. The Standard Sea Water is being used as a reference for conductivity instead of chlorinity, though each main batch is checked. The difficulties of measuring absolute conductivity and density are still not surmounted. So long as careful checks are maintained the standards are adequate, but the author thinks we may lose if unquestioning acceptance of constant relationships between chlorinity, electrical conductivity, density, and other physical properties and chemical constituents, discourages critical study of small exceptions to the rule. Such exceptions may, as refined methods are more widely used, provide new lines of investigation into the origin and history of water mixtures.

The author has done a great service to the history of marine chemistry. Judging from his text and very extensive bibliography his work seems to have been completed in about 1970, or he would have included something about Kremling's (1971) new method of measuring density, and recent reports of the Joint Panel on Oceanographic Tables and Standards, set up by Unesco and the international marine science organisations to take care of the new problems.

G. E. R. Deacon

Abandon dualism

Quantum Mechanics in a New Key. By Alfred Landé. Pp. x+130. (Exposition: New York, 1973) $6.50.

THE author states in the introduction his intention to derive quantum mechanics from three non-quantal postulates. These are: symmetry (the probability $P_{\beta\alpha}$ connecting two states α and β is two-way symmetric, so that $P_{\alpha\beta}=P_{\beta\alpha}$); correspondence (the probabilities connecting various pairs of states are to be interdependent by way of a general theorem which is to yield the ordinary probability addition law $P_{\alpha\gamma} = \Sigma_\beta P_{\alpha\beta}P_{\beta\gamma}$ in the average; and covariance (there are no preferred zero points for energy and momentum, nor for time and space coordinates). In this way the author hopes to demonstrate that quantum mechanics is a logically consistent theory of probability and symmetry, and that the wave–particle dualism which emerged in the early history of the subject may now be abandoned as an unnecessary mystification.

In chapter 1 he discusses several critical experiments, and uses Duane's theory of diffraction, originally put forward to explain the diffraction of photons, to show how wavelike effects such as interference may be explained without assuming the existence of matter waves. In chapters 2 and 3 the first and second postulates are introduced and their consequences are considered; these are further developed in chapter 4. The third postulate and its consequences are discussed in chapter 5. Statistical thermodynamics is briefly considered in chapter 6; the author returns to his main theme of observation and interpretation in chapter 7.

The aims of the book are admirable, and most physicists would probably accept the philosophy which it expounds. The lack of mathematical rigour would not, in itself, be a disadvantage in a book of this kind, but unfortunately the author's conclusions do not always seem to follow from his postulates. For example, the statements $P_{\alpha\beta} = |\Psi_{\alpha\beta}|^2$ where $\Psi_{\alpha\beta}=\Psi^*_{\beta\alpha}$ and $\Psi_{\alpha\gamma}=\Sigma_\beta\Psi_{\alpha\beta}\Psi_{\beta\gamma}$ do not obviously follow from the first two postulates. Rather these should themselves be regarded as the basic postulates, from which the first two postulates are then easily deduced. Symmetry and correspondence provide a guide to the choice of postulates, rather than themselves being postulates. The deduction of the result $\Psi_{pq} = C\exp(2i\pi pq/h)$ in chapter 5 from the postulate of covariance, and the derivation of the Schrodinger wave equation from this result are elegant, but I think that the reader would have been helped by a more detailed explanation of the mathematical reasoning behind the steps of the arguments.

J. E. G. Farina

obituary

B. E. Bykhovskii

ACADEMICIAN BORIS EVSEEVICH BYKHOVSKII, a leading Soviet parasitologist, and Director of the Institute of Zoology of the Academy of Sciences of the USSR, died on January 26, 1974.

Bykhovskii was born in 1908, in St Petersburg, and was educated at Leningrad University, graduating from the Biology Department in 1930. His first scientific post was as a laboratory assistant at the Institute of the Fishery Industry; by 1939, when he left the Institute, he had risen to the rank of senior scientist. In 1939, he moved to the Institute of Zoology of the Academy of Sciences of the USSR, which was from then onwards the centre of his scientific activity. From 1940–44 he worked in Tadjikstan as Deputy Chairman of the Tadzhik branch of the Academy, playing a great part in the transformation of this branch into the new Academy of Sciences of the Tadzhik SSR. From 1942–59 he was Head of the Laboratory of Helminthology of the Institute of Zoology of the Academy of Sciences of the USSR, and, simultaneously and until 1962, Deputy Director of the Institute of Zoology. In 1962 he became director of the Institute, a post which he was to hold for the rest of his life. In 1960 he was elected a Corresponding Member and in 1964 a Full Member of the Academy of Sciences of the USSR.

Bykhovskii's main field of research was the helminths of fish. His publications, however, deal with a considerable range of associated problems of general parasitology, ecology and evolution. He organised over twenty-five scientific research expeditions and was founder and Editor in Chief of the journal *Parasitologiya*.

His best-known papers include *Contributions to Knowledge about Monogeneic Trematodes with a Primitive Fastening Armature* (1955); *Information on Monogeneic Trematodes in the Fishes in Tadjikstan* (1960); *The Monogeneic Trematodes of* Silarus glanis (1966); and *Results and Further Prospects of the Work of Soviet Parasitologists in the Study of Parasites of Fish in the Seas of the USSR*.

In September 1965, at the joint session of the Presidium of the Academy of Sciences of the USSR and other scientific institutions, Bykhovskii was among those who strongly criticised Lysenkoism and its adverse effect on Soviet science. His support for the anti-Lysenkoists was of particular importance, as, unlike several other critics, Bykhovskii was a keen Party member with an active interest in politics, and from time to time held official positions in the Leningrad City and Regional Party organisations.

For his services to science and to the fishing industry, Bykhovskii was awarded a number of Soviet decorations including the Order of Lenin and the Order of the Red Banner of Labour.

P. K. Anokhin

ACADEMICIAN PETR. KUZ'MICH ANOKHIN, the eminent Soviet physiologist and brain specialist, died on March 6, 1974.

Anokhin was born on January 27, 1898, in Tsaritsyn (later Stalingrad and now Volgodrad). The son of a worker, he began his studies at the local agricultural and technical college, but, in 1917, these studies were interrupted by the revolution. Anokhin, whose sympathies were with the revolutionaries, took an active part in the defence of Trasitsyn and in the establishment of Soviet rule in the Don area, becoming, towards the end of the civil war, Commissar of Publications in Novocherkassk, and editor of the newspaper *Krasnyi Don*. In this latter capacity, while browsing for subject matter for popularised science articles, he happened upon a publication of the Leningrad Brain Institute, and became fascinated by the work of Bekhterev and Pavlov on conditioned reflexes.

When the civil war ended, Anokhin was able, through the intervention of A. V. Lunacharskii, People's Commissar for Culture, to transfer his studies to the Leningrad Institute of Medical Science where he graduated in 1926. After graduation, he proceeded to Pavlov's own laboratory, working and studying under Pavlov and Bekhterev until 1930, when he was appointed Professor of the Department of Physiological Medicine at the University of Gor'kii, a post which he held until 1934. Later positions included Head of the Department of General Physiology of the Higher Nervous System at the All-Union Institute of Experimental Medicine (1934–46), Director of the Institute of Physiology of the Academy of Medical Sciences of the USSR (1946–49), Head of the Departments of Physiology and Pathology of the Higher Nervous System at the Central Post-Graduate Medical Institute (1936–49 and 1953–55), and Head of the Department of Normal Physiology of the I. M. Sechenov Moscow Medical Institute. During the Second World War he served as neurosurgeon and assistant to the renowned surgeon Nikolai N. Burdenko.

Throughout his working life, Anokhin maintained the interest in conditioned reflexes and the action of the brain, which had first attracted him to neurophysiology. He extended and amended Pavlov's fundamental ideas in the light of later research and put forward a highly complex picture of brain reaction to stimuli, involving a number of areas of the brain, including subcortical areas. One of his postulates was that some 'intention' to perform an action must emerge between stimulus and reaction. Other fields in which he did notable work included the physiological theory of the neural cicatrix, the theory of pathogenesis of amputation pains and central paralysis, and medical cybernetics.

His numerous publications include *Problem of the Central and Peripheral Nervous systems in the Physiology of Nervous Activity* (1935); *Neuroplasty for Battle Injuries of the Peripheral Nervous System* (1944); *Systemogenesis as a General Rule of the Evolutionary Process* (1948); *General Principles of the Compensation of Functional Perturbances and their Physiological Basis* (1954), *Internal Inhibition as a Problem of Physiology* (1958); and *The Biology and Neurophysiology of the Conditioned Reflex* (1967) for which he was awarded a Lenin Prize in 1972. He also wrote a life of Pavlov (1949), edited a number of collections of essays (the most recent being *Cybernetic Aspects in Studying Brain Action* (1970)), was Physiology Editor for the large and small medical encyclopaedias and contributed a preface to the Russian edition of R. Ashby's *Construction of the Brain*.

He was a member of the Executive Committee of the International Organisation for Brain Research, and represented his country at a number of international conferences, including the

International Congresses of Physiologists in Brussels, 1956), and Buenos Aires (1959), the International Organisation for the Study of the Brain (Paris, 1960) and the International Symposium on the Development of the Brain (USA, 1963). He was elected a member of the Academy of Medical Sciences of the USSR in 1945 and a member of the Academy of Sciences of the USSR in 1966.

Announcements

Appointments

Robert C. Seamans, jr., has been re-elected **President** of the **National Academy of Engineering.**

Awards

J. Erik Jonsson has been awarded the **Founders Medal** of the **National Academy of Engineering.**

Ivar Giaever has been awarded the **Vladimir K. Zworykin Award** of the **National Academy of Engineering.**

Corrigendum

In the article "New species of *Dryopithecus* from Kenya" by P. Andrews (*Nature*, **249**, 188; 1974), in the description of *Rangwapithecus*, a new subgenus of *Dryopithecus*, the type species of the new subgenus is the one first listed, namely *Dryopithecus* (*Rangwapithecus*) *gordoni*.

Erratum

In the article "The neurogenic and myogenic hypotheses in human (Duchenne) muscular dystrophy" by W. H. Thomson, J. C. Sweetin and R. A. Elton, (*Nature*, **249**, 151; 1974) in line 9 of the legend to Fig. 1, 33 should read 23.

Reports and publications

Great Britain

World Health Organization. International Health Regulations (1969). Second annotated edition. Pp. 102. (Geneva: WHO; London: HMSO, 1974.) Sw. fr. 6. [211

Berichte des Deutschen Wetterdienstes. Nr. 132: Uber den Leistungsstand des Baroklinen Modells des Deutchen Wetterdienstes:Ergebnisse der Prufung Numerischer Vorbersagekarten 1970–1972. Von W. Buschner, H. Oeckel, J. U. Schwirner und K. O. Wegner. Pp. 38. Annalen der Meteorologie (Neue Folge) Nr. 8: Die Entwicklung der Meteorologischen Beobachtungen in Kranken und Bayern bis 1700. Von FritzKeluim. Pp. 50. Annalen der Meteorologie, (Neue Folge), Nr. 7: The Planetary Boundary-Layer of the Atmosphere. By F. Wippermann. Pp. x + 346. (Offenbach a.M.: Selbstverlag des Deutschen Wetterdienstes, 1973.) [211

US Department of Health, Education and Welfare. Public Health Service—National Institutes of Health. National Cancer Program. Report of the Director, January 1973. Pp. 42. Report of the National Cancer Advisory Board, January 1973. Pp. 11. The Strategic Plan, January 1973 Edition. Digest of Scientific Recommendations for the National Cancer Program. (Bethesda, Md: US Department of Health Education and Welfare, National Institute of Health, 1973.) [221

CERN—European Organization for Nuclear Research. CERN 73–17: Elements d'Analyse Numerique et leur Programmation en Fortran IV. Premiere partie: Calcul des Derivees Successives d'Une Fonction d'Une Variable Reelle. Applications aux Developpements de Taylor, de Burmann et de Thiele. Par F. Louis. Pp. 61. (Geneve: CERN, 1973.) [231

World Health Organization. Public Health Papers No. 54: Control of Air Pollution in the USSR. By N. F. Izmerov. Pp. 157. (Geneva: WHO; London: HMSO, 1973.) Sw. fr. 9; £1.35. [231

European Space Research Organization. General Report 1972. Pp. 230. (Neuilly-sur-Seine: European Space Research Organization, 1973.) [231

World Health Organization. Technical Report Series, No. 532: Trace Elements in Human Nutrition—Report of a WHO Expert Committee. Pp. 65. (Geneva: WHO; London: HMSO, 1973.) Sw. fr. 5; 75p; $1.75. [281

The Development of Electronic Data Processing in Manpower Areas. Pp. vii + 350. (Paris: OECD, 1973.) [281

World Health Organization. Technical Report Series. No. 533: Postgraduate Education and Training in Public Health—Report of a WHO Expert Committee. Pp. 68. 5 Sw. francs; 75p; $1.75. No. 534: Continuing Education for Physicians—Report of a WHO Expert Committee. Pp. 32. 5 Sw. francs: 75p; $1.75. (Geneva: WHO; London: HMSO, 1973.) [291

Carte de la Végétation du Bassin Genevois. Notice Abrégée de la Carte de la Végétation du Bassin Genevois. Par P. Hainard et G. Tchérémissinoff. Pp. 19 (Genève: Conservatoire et Jardin Botaniques, 1973.) [291

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Paper 74–1, Part A: Report of Activities. Part A: April to October 1973. Pp. 396. (Ottawa: Information Canada, 1974.) $5. [291

Guide to Information Services in Marine Technology. Compiled by Martran Limited. Pp. 80. (Edinburgh: Institute of Offshore Engineering, Heriot-Watt University, Chambers Street, 1973.) £1. [82

Universities Research Reactor. Annual Report, 1972/1973. Pp. iii+37. (Risley, Warrington, Lancs.: Universities Research Reactor, 1974.) [112

Bulletin of the British Museum (Natural History). Geology. Supplement 10: A Revision of the Elopiform Fishes, Fossil and Recent. By P. L. Forey. Pp. 1–222. (London. British Museum (Natural History), 1973.) £9.45. [122

Natural Environment Research Council. Merlewood Research Station—Report for 1970/1972. Pp. 66+9 plates. (Grange-over-Sands, Lancashire: Natural Environment Research Council, Institute of Terrestrial Ecology, Merlewood Research Station, 1973.) 50p. [152

The Commonwealth Forestry Institute, University of Oxford. Forty-ninth Annual Report, 1972/1973. Pp. 32. (Oxford: The Commonwealth Forestry Institute, 1973.) [152

Linear and Multilinear Algebra, Vol. 1, No. 1, 1973. Edited by M. Marcus and R. C. Thompson. Pp. 1–88. (London and New York: Gordon and Breach, 1973.) [152

The Zoological Record. 1970, Volume 107, Section 4: Coelenterata. Compiled by the Staff of the Zoological Society of London. Pp. 55. £5.15. 1970, Volume 107, Section 7: Brachiopoda. Compiled by the Staff of the Zoological Society of London. Pp. 36. £3.65. (London: The Zoological Society of London, 1973.) [182

London School of Hygiene and Tropical Medicine. Report on the work of the School, 1972/1973. Pp. 177. (London: London School of Hygiene and Tropical Medicine, 1974.) [212

Government Statistics: a Brief Guide to Sources. Pp. 16. (London: Central Statistical Office, Great George Street, SW1, 1974.) [252

Philosophical Transactions of the Royal Society of London: B: Biological Sciences. Vol. 267, No. 885; The Central Nervous System of *Loligo*. 1: The Optic Lobe. By J. Z. Young. Pp. 263–302 + plates 8–18. UK £2.65; Overseas £2.75. Vol. 267, No. 886: On the Growth and Form of a Bacterial Cell. By R. H. Pritchard (Review Lecture). Pp. 303–336. UK £1.20; Overseas £1.25. (London: The Royal Society, 1974.) [262

Medical Research Council. National Institute for Medical Research—Report for 1972/3. Pp. 150. (London: National Institute for Medical Research, 1974.) [282

Memoirs of the Royal Astronomical Society, Vol. 78, Part 1: Fine Structure in Radio Sources at 81.5 MHz-III. The Survey. By A. C. S. Readhead and A. Hewish. Pp. 1–49. (Oxford and London: Blackwell Scientific Publications, 1974. Published for the Royal Astronomical Society.) [282

Other countries

Anuario del Observatorio Astronomico de Madrid papa 1974. Pp. 386. (Madrid: Observatorio Astronomico, Alfonso XII, 3, 1973.) [212

Bibliographien des Deutschen Wetterdienstes, Nr 27: Agrarmeteorologische Bibliographie 1972. Bearbeitet von Maximilian Schneider. Pp. xix+225. (Offenbach a.M.: Selbstverlag des Deutschen Wetterdienstes, 1973.) [222

Bulletin of the Fisheries Research Board of Canada, No. 182: Morphology and Sediments of the Gulf of St. Lawrence. By D. H. Loring and D. J. G. Nota. Pp. xiv+147. (Ottawa: Information Canada, 1973.) $5. [252

Le Conseil des Recherches Agricoles du Québec. Recherches Agronomiques –Sommaire des Résultats, 1971/1972, No. 17. Pp. 157. (Quebec: Ministère de l'Agriculture et de la Colonisation, 1974.) [252

Technology of Efficient Energy Utilization. (The Report of a NATO Science Committee Conference held at Les Arcs, France, 8th–12th October, 1973.) Pp. xiv+67. (Brussels: Scientific Affairs Division, North Atlantic Treaty Organization, 1974.) [252

United States Department of the Interior: Geological Survey. Bulletin 1304: Mineral Resources of the Idaho Primitive Area and Vicinity, Idaho. By F. W. Cater *et al.* Pp. xiv+431. (Washington, DC: Government Printing Office, 1973.) $4.65. [252

Office de la Recherche Scientifique et Technique Outre-Mer, Paris. Initiations--Documentations Techniques No. 22: l'Aluminium dans les Sols. Par P. Ségalen. Pp. 279. (Paris et Bondy: Office de la Recherche Scientifique et Technique Outre-Mer, 1973.) 100 francs. [252

European Organization for Nuclear Research--CERN. CERN 74-1: Computer-Aided Control of Separated Bubble Chamber Beams. By P. Lazeyras, I. Lehraus, R. Matthewson and W. Tejessy. Pp. 72. (Geneva: CERN, 1974.) [252

International Atomic Energy Agency. Aspects of the Introduction of Food Irradiation in Developing Countries. (Proceedings of a Panel to Consider the Application of Food Irradiation in Developing Countries, organized by the Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture, and held in Bombay, 18–22 November 1972.) Pp. 113. (Vienna: IAEA; London: HMSO, 1973.) 108 schillings; £2.50; $6. [262

Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem. Heft 154: Festveranstaltung und Internationales Kolloquium zum 75 Jährigen Jubilaum der Biologischen Bundesanstalt für Land- und Forstwirtschaft. Pp. 115. 10 DM. Heft 155: Thesaurus Phytomedizin (Pflanzenkrankheiten und Pflanzenschutz). Von Dr. D. Bulmenbach. Pp. 145. 14.50 DM. (Berlin-Dahlem: Biologischen Bundesanstalt für Land- und Forstwirtschaft, 1973.) [262

The International Nickel Company of Canada, Ltd. 1973 Annual Report. Pp. 28. (Toronto: The International Nickel Company of Canada, Ltd., 1974.) [262

Smithsonian Contributions to Zoology. No. 149: Ecology and Behavior of the Giant Wood Spider *Nephila maculata* (Fabricius) in New Guinea. By Michael H. Robinson and Barbara Robinson. Pp. iv+76. $1.45. No. 159: Clinid Fishes of Chile and Peru, with Description of a New Species, *Myxodes ornatus*, from Chile. By John S. Stephens, Jr. and Victor G. Springer. Pp. 24. 70 cents. (Washington, DC: Smithsonian Institution Press, 1973. For sale by US Government Printing Office.) [272

Annals of the Transvaal Museum. Vol. 29, No. 1: Some Suggested Procedures in the Analysis of Bone Accumulations from Southern African Quaternary Sites. By C. K. Brain. Pp. 1–8. Vol. 29, No. 2: South African Lepidoptera, 6. Notes on Hesperiidae and Descriptions of Two New Species (Lepidoptera: Rhopalocera). By L. Vari. Pp. 9–26 + plate 1. (Pretoria: Transvaal Museum, 1974.) [272

United States Department of the Interior: Geological Survey. Professional Paper 743-C: *Complexiopollis* Pollen Lineage in Mississippi Embayment Rocks. By Robert H. Tschudy. Pp. iii+15 (plates 1–9). (Washington, DC: Government Printing Office, 1973.) 75 cents. [282

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Semi-displayed £3.60 per 10 mm. Minimum £7.20, each additional 2 mm 72p. Full page £230.00. Half page across £125.00. 30p is charged for the re-direction of replies to advertisements with a box number.

ADVERTISEMENTS SHOULD BE ADDRESSED TO: T. G. Scott and Son, Limited, 1 Clement's Inn, London, WC2A 2ED. Telephone: 01-242 6264. Telegrams: Textualist, London, W.C.2.

APPOINTMENTS VACANT

GEOPHYSICIST

The Lamont-Doherty Geological Observatory of Columbia University announces a position with the seismology group as a Research Associate or Senior Research Associate. Candidates must have a strong background in seismic instrumentation and data analysis and will be expected to lead existing and initiate new programs of research involving observations of earth strain, tilt, and long-period seismic waves. Qualified candidates are invited to submit a résumé of their qualifications, publications, names and addresses of three references by August 15, 1974, to Dr. Lynn R. Sykes, Lamont-Doherty Geological Observatory, Palisades, New York 10964. Columbia University is an equal opportunity employer.

(2260)

CAMBRIDGE UNIVERSITY
DEPARTMENT OF GENETICS

Application are invited from microbial or molecular geneticists for a University Demonstratorship that is expected to be available from October 1, 1974, or sooner.

The scale of stipends from October 1, 1974 will be £2,247 a year rising by annual increments to £2,931 or, if the person appointed is resident in College, £2,148 rising to £2,832.

Further particulars may be obtained from the Secretary of the Appointments Committee of the Faculty of Biology 'A', Department of Applied Biology, Downing Street, Cambridge CB2 3DX, to whom applications should be sent before June 21, 1974. (2254)

UNIVERSITY OF GLASGOW
DENTAL HOSPITAL AND SCHOOL
RESEARCH ASSISTANTSHIP IN BIOCHEMISTRY

Applications are invited for a RESEARCH ASSISTANTSHIP in BIOCHEMISTRY, tenable for a period of three years from September or October 1974.

Candidates should have an Honours Degree in Biochemistry or an allied subject. There will be an opportunity to apply to register for a higher degree.

The work will involve a study of salivary proteins with a view to correlating abnormalities with pathological disorders. The project is financed by a grant from the Scottish Home and Health Department.

Salary Scale £1,500 to £1,650 per annum. F.S.S.U.

Applications, including curriculum vitae and names of two referees, should be sent to Dr. J. A. Beeley, Department of Dental Biochemistry, University of Glasgow Dental School, 378 Sauchiehall Street, Glasgow, G2 3JZ, from whom further information may be obtained. (2298)

CSIRO

AUSTRALIA

DIVISION OF MINERAL PHYSICS
NORTH RYDE, N.S.W.

RESEARCH SCIENTIST

The Commonwealth Scientific and Industrial Research Organisation has a broad charter for research into primary and secondary industry areas. The Organisation has approximately 6,500 employees—2,100 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

FIELD: **ELECTROMAGNETIC GEOPHYSICS**

GENERAL: The Division of Mineral Physics, a constituent Division of the CSIRO Minerals Research Laboratories, carries out research directed towards the application of the techniques of physics to mineral exploration and exploitation.

The Division is establishing a rock magnetism laboratory to develop the application of magnetic techniques in mineral exploration and processing, and research into the practical applications of palaeomagnetism. The Division has established a low sensitivity and high field laboratory at North Ryde, and plans are well advanced for the construction of a high sensitivity laboratory in a nearby magnetically quiet environment.

DUTIES: Under the general direction of the leader, to extend research in field and laboratory projects related to: studies of the earth's past magnetic field, interpretation of magnetic anomalies, origin and extensions of ore bodies, anisotropy of remnance and susceptibility, magnetic cleaning techniques and structural analysis using remnance techniques.

QUALIFICATIONS: A Ph.D. degree in Geophysics, Geology or Physics or equivalent qualifications, supported by demonstrated research ability, with a sound knowledge of palaeomagnetic field and laboratory techniques. A good grasp of geological field problems is essential. Experience in opaque mineralogy, or chemical leaching, or magnetic anisotropy or high magnetic fields would be an advantage.

SALARY: The appointment will be made within the salary ranges of Research Scientist or Senior Research Scientist: $A9,380-$A14,290 p.a.

TENURE: An indefinite or a three year fixed term appointment may be negotiated. An indefinite appointment carries Australian Government Superannuation benefits.

Applications stating full personal and professional details, the names of at least two professional referees, and quoting Reference Number 589/27 should reach:—

The Personnel Officer,
Australian Scientific Liaison Office,
64-78, Kingsway,
LONDON,
WC2B 6BD

by the 5th July, 1974.

Applications in U.S.A. and Canada should be sent to:—
The Counsellor (Scientific),
Embassy of Australia, 1601 Massachusetts Avenue, N.W.,
WASHINGTON, D.C. 20036, U.S.A.

(2361)

The University of Birmingham

THE WELLCOME FOUNDATION LIMITED

Wellcome

Structure, Physiology and Biochemistry of Insect Neuromuscular Junctions

Applications are invited for a CASE postgraduate studentship in the above field of work.

The student will work primarily in the Department of Zoology and Comparative Physiology at Birmingham University with some time spent in the Unit of Arthropod Physiology at the Wellcome Research Laboratories, Berkhamsted Hill, where biochemical aspects of work in this field are currently in progress.

The Studentship commences on 1st October, 1974 and is tenable for 3 years. The current value of the Studentship is £695 per annum.

Further details may be obtained from the Academic Supervisor:

Dr. M. P. Osborne,
Dept. of Zoology & Comparative Physiology,
University of Birmingham,
Edgbaston, BIRMINGHAM.

(2362)

LISTER INSTITUTE OF PREVENTIVE MEDICINE

Elstree, Hertfordshire

BLOOD PRODUCTS LABORATORY

Recent graduate in microbiology required for control testing of human blood products and to assist in development work.

Please apply stating qualifications, age and experience to The Secretary, Lister Institute of Preventive Medicine, Elstree, Hertfordshire, from whom further particulars can be obtained.

(2374)

Graduate Required

Physics/Chemistry Graduate required for interesting research post within our Central Laboratories Division. The job is concerned with the creepage along and wetting of metal surfaces by liquids. The position is new and offers excellent scope for originality and innovation.

Undergraduates qualifying this year will be considered.

Research facilities and future prospects are good.

Location : North London.

Hours : 9 am to 5.15 pm (Mon to Fri) 36¼ hr. week.

Apply :

EVER READY®

Administration Manager,
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Central Laboratories,
St. Ann's Road,
London, N15 3TJ. Tel. No. 01-800 1101. (2244)

UNIVERSITY OF SOUTHAMPTON

FACULTY OF MEDICINE—MICROBIOLOGY

Applications are invited from suitably qualified science graduates for the post of LECTURER IN MICROBIOLOGY. The person appointed will be expected to undertake teaching and research duties in the field of microbial infections. Preference will be given to candidates with an interest in immunochemistry, virology or the chemistry of microbial structure.

Salary scale: £2,118 to £4,896. The initial salary will depend on qualifications and experience. Further particulars may be obtained from the Deputy Secretary's section (Ext. 353), The University, Southampton SO9 5NH to whom applications (7 copies from United Kingdom applicants) should sent not later than June 22, 1974, quoting reference be sent not later than June 22, 1974, quoting 58/74/L. (2249)

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF BIOCHEMISTRY

POSTGRADUATE RESEARCH

Research activities open to graduates wishing to work for the M.Phil. or Ph.D. degree include:

Yeast viruses: host-virus relationships.

Mycoviruses: characterisation and biochemistry of virus replication cycle.

Grants will be available to suitable candidates. Enquiries should be made to:

Dr K. W. Buck,
Department of Biochemistry,
Imperial College,
London SW7 2AY. (2262)

Kings

HEALTH DISTRICT (TEACHING)
Lambeth, Southwark and Lewisham Area Health Authority (Teaching)

Research Graduate

Graduate required to work on the Biochemistry of various hormones in women with benign or malignant tumours of the breast.

Application forms obtainable from the Personnel Office, King's College Hospital, Denmark Hill, London, S.E.5. Tel: 01-274 6222 Ext. 2726 (Miss Millwood) should be completed and returned by two weeks after date of insertion. (2251)

OXFORD UNIVERSITY

DEPARTMENT OF ZOOLOGY

Applications are invited for the post of Research Assistant to work under Dr Malcolm Coe on the reproductive biology of Giant Tortoises on Aldabra Island in the Indian Ocean. The appointment will be for a period not exceeding three years, of which two years will be spent carrying out field work on Aldabra and one year in Oxford.

Applicants should be available to take up this post by the beginning of August 1974. Preference will be given to candidates with an interest or experience in the field of Herpetology. Salary will be by arrangement according to age, qualifications and experience.

Applications with a full curriculum vitae and the names of two referees should be sent immediately to the Secretary, Animal Ecology Research Group, Department of Zoology, South Parks Road, Oxford OX1 3PS. (2334)

UNIVERSITY OF LEICESTER

The Department of Botany has a vacancy for a Research Technician. Preference will be given to candidates who have HND or HNC in Applied Biology and some relevant experience. The person appointed will be involved in various researches in plant physiology, particularly those involving the aseptic culture of tissues and cells, and will be required to give general assistance in the Department and in the preparation of some laboratory classes. Salary on scale for Grade 3 Technicians, £1,650 to £1,920 (under review). Applications should be forwarded by July 31, 1974 to the Professor of Botany, The University, Leicester. (2346)

UNIVERSITY OF NOTTINGHAM
School of Biological Sciences
DEPARTMENT OF ZOOLOGY

Applications are invited from candidates with good honours degrees for a RESEARCH ASSISTANTSHIP, tenable for three years from October 1, 1974. An interest in tissue culture or neurobiology would be an advantage but is not essential. The successful candidate will be able to register for a higher degree of the University. Salary will be within the range £1,422 to £1,569.

Further details and forms of application, returnable not later than June 30, 1974, are obtainable from the Staff Appointments Officer, University of Nottingham, University Park, Nottingham. Ref. No. 371. (2345)

RESEARCH IN CHEMISTRY

RESEARCH GRANTS will be available from October 1974, for graduate courses in Chemistry, leading to the degree of MSc or PhD for candidates with a 1st or 2nd class honours degree or equivalent.

Write for further information to the Senior Administrative Assistant, School of Chemical Sciences, University of East Anglia, University Plain, Norwich, NOR 88C. (2342)

UNIVERSITY OF SURREY
DEPARTMENT OF BIOLOGICAL SCIENCES
ASSISTANT EXPERIMENTAL OFFICER

Qualified man or woman required with knowledge of computing and statistical analyses for biological, microbiological or medical research. The successful applicant will participate in project planning and associated experimental work as well as being responsible for program writing and organisation of data (ICL 1905F computer). Salary: £1,929 to £2,223.

Further particulars can be obtained from the Assistant Secretary (Personnel), University of Surrey, Guildford, Surrey, GU2 5XH. Tel: Guildford 71281, Ext. 452, to whom applications in the form of a curriculum vitae, including the names and addresses of three referees should be sent to by June 28. Telephone enquiries will be welcomed by Professor Smith, on Guildford 71281, Ext. 687. (2340)

UNIVERSITY OF NEWCASTLE UPON TYNE
DEPARTMENT OF PLANT BIOLOGY

Applications are invited from graduates or from students expecting to graduate in 1974 with a first or upper second class honours in Plant Biology or a related subject for an S.R.C., C.A.S.E. Studentship to work on the effects of carbon dioxide and oxygen concentration on the metabolism of germinating barley. The project will be carried out in co-operation with Arthur Guinness, Son & Co. (Park Royal) Ltd., Park Royal Brewery, London, N.W.10. Candidates will be expected to register for a higher degree.

Applications (including a curriculum vitae and names of two referees) should be addressed as soon as possible to Professor S. L. Ranson, Department of Plant Biology, Ridley Building, The University, Newcastle upon Tyne NE1 7RU. (2339)

UNIVERSITY OF IBADAN
NIGERIA

Applications are invited for (a) SENIOR LECTURESHIP or (b) LECTURESHIP IN FOREST MENSURATION, INVENTORY AND AERIAL SURVEY in the Department of FORESTRY. Applicants must possess a post-graduate degree and have teaching and research experience in this field. Appointee will be expected to have close liaison with Federal and State Departments of Forestry and to advise governments on the inventory and management of High Forest and Plantations. Salary scales: (a) N5,030 to N5,750 p.a. (b) N2,760 to N4,830 p.a. (£1 sterling = N1.47.) The British Government may supplement salary in range £750 to £1,150 p.a. (sterling) for married appointee or £250 to £650 p.a. (sterling) for single appointee (normally free of all tax) and provide children's education allowances and holiday visit passages. These supplementation rates are currently under review. F.S.S.U. Family passages; various allowances; regular overseas leave. Detailed applications (two copies), including a curriculum vitae and naming three referees, should be sent by air mail, not later than June 27, 1974, to the Registrar, University of Ibadan, Ibadan, Nigeria. Applicants resident in U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars of these appointments may be obtained from either address. (2355)

CSIRO
AUSTRALIA
DIVISION OF MATHEMATICAL STATISTICS

The Commonwealth Scientific and Industrial Research Organisation has a broad charter for research into primary and secondary industry areas. The Organisation has approximately 6,500 employees — 2,100 of whom are research and professional scientists — located in Divisions and Sections throughout Australia.

GENERAL: The Organisation's Division of Mathematical Statistics has approximately 100 staff, including 60 professional scientists, located at centres and laboratories in capital cities and some major country towns. It is to be reconstituted as the Division of Mathematics and Statistics later in 1974 after the new Chief of the Division, Dr. J. Gani, takes up duties in Canberra.

CSIRO research in mathematics and statistics currently includes distribution theory, multivariate analysis, scientific inference and experimental design, and will be extended to include mathematical and stochastic modelling, geostatistics, optimisation, operations research and management science. Application of such research, relevant to agricultural, biological, industrial and physical investigations, currently includes design and analysis of experiments and models for ecological, environmental, agricultural and climatological systems and is being extended to include work on industrial processes and mineral surveys.

Professional scientists of the Division act as consultants in planning research and in the application of mathematics and statistics to investigations and experimental results.

RESEARCH SCIENTIST

POSITION NUMBER 440/270

FIELD: **MATHEMATICAL STATISTICS FOR MULTIVARIATE SURVEYS**

LOCATION: Highett, Victoria.

DUTIES: To conduct research into mathematical statistics related to the design and analysis of multivariate social and economic surveys, and applied research related to the mathematical and statistical aspects of the organisation's building and/or engineering research in the Melbourne region and to pursue individual research interests.

QUALIFICATIONS: Applicants should have a Ph.D. degree or equivalent qualifications with demonstrable research ability. Experience in statistical methodology for sociological, psychological or economic studies and in sample surveys of social attitudes would be an advantage.

SALARY: The appointment will be made within the salary range of Research Scientist: $A9,380-$A11,827 p.a., or Senior Research Scientist $A12,298-$A14,290 p.a. Consideration would be given to appointment of a suitably qualified applicant at a more senior level.

TENURE: This position is available for an indefinite period and Australian Government Superannuation benefits are available.

RESEARCH SCIENTISTS

POSITION NUMBER 440/271

FIELD: **GEOSTATISTICS & OPTIMISATION**

LOCATION: Melbourne, Victoria.

DUTIES: To conduct research into multivariate aspects of geostatistics and optimisation, and applied research in mathematical and statistical aspects of the organisation's minerals and/or geomechanics research in the Melbourne region and to pursue individual research interests.

POSITION NUMBER 440/272

FIELD: **BIOMETRICS AND STOCHASTIC MODELLING**

LOCATION: Prospect, N.S.W.

DUTIES: To conduct research into aspects of biometrics and stochastic modelling and applied research related to statistical and mathematical aspects of the organisation's animal physiology research in the Sydney region and to pursue individual research interests.

QUALIFICATIONS: A Ph.D. degree, or equivalent qualifications, with demonstrable research ability and experience in relevant fields of applied research.

SALARY AND TENURE: Appointments will be made within the salary ranges of Research Scientist: $A9,380-$A11,827 p.a. or Senior Research Scientist: $A12,298-$A14,290 p.a.

Either an indefinite or a three year fixed term appointment may be negotiated. An indefinite appointment carries Australian Government Superannuation benefits.

Applications quoting the appropriate Position Number and stating full personal and professional details, and the names of at least two professional referees should reach:—

The Personnel Officer,
Australian Scientific Liaison Office,
64-78, Kingsway,
LONDON,
WC2B 6BD

by the 12th July, 1974.

Applications in U.S.A. and Canada should be sent to:—
The Counsellor (Scientific),
Embassy of Australia, 1601 Massachusetts Avenue, N.W.,
WASHINGTON, D.C. 20036, U.S.A.

(2383)

Principal Biochemist (Pharmacologist)

Two Positions

National Biological Standards Laboratory

Canberra

Duties: Evaluate the pharmacological and toxicological aspects of new drugs from data submitted by drug companies. Report on animal studies carried out to support the proposed uses of drugs and make recommendations on their suitability and adequacy. Provide advice as required on problems concerning the pharmacology and toxicology of substances and the interpretation of experimental data. Carry out and direct research into the actions of drugs with particular reference to methods of drug evaluation and mechanisms of drug toxicity.

Qualifications: An appropriate degree in Science, Applied Science, or Medical Laboratory Technology; or equivalent qualification. Post graduate qualifications and broad experience in drug related fields are desirable.

Salary: $A12,911 to $A13,796 per annum. NB. exchange rate: £1 = $A1.60 approx.

Conditions: First class passage by air for the successful applicant and his dependants together with generous baggage allowances. Salary will be paid from date of embarkation. Four weeks annual leave, accumulative sick leave provisions and a comprehensive superannuation scheme are available.



Please write giving full details of qualifications and experience together with date and place of birth to:

The Recruitment Officer
Public Service Board
Canberra House
10/16 Maltravers St
London WC2R 3EH

By July 5, 1974 (2358)

THE OPEN UNIVERSITY

BIOLOGY TECHNICIAN (2B)

READVERTISEMENT

A vacancy exists for a BIOLOGY TECHNICIAN (GRADE 2B) in the Faculty of Science commencing as soon as possible. Main duties will include: assistance with course experimental work; the construction and servicing of biology summer school and day school equipment; general maintenance of services and equipment in the biology laboratories.

Applicants should possess O.N.C. or equivalent qualifications. Some workshop experience would be required and in addition, general biological laboratory experience would be useful.

Salary, based on qualifications and experience will be within the Grade 2B scale: £1,524 to £1,794 per annum.

Further details and application forms are available from the Personnel Manager (TS5), The Open University, P.O. Box 75, Walton Hall, Milton Keynes MK7 6AL. (Tel: Milton Keynes 74066, extn. 3068). Closing date for applications: Monday, June 24, 1974. (2416)

UNIVERSITY OF DURHAM

DEPARTMENT OF APPLIED PHYSICS AND ELECTRONICS

SENIOR DEMONSTRATOR

Applications are invited from graduates in electronic engineering or solid state physics for one or more posts of Senior Demonstrator. The duties will include some teaching and the supervision of either a first year Electronics Laboratory or a Physics of Materials Laboratory. Participation in research will be encouraged in either digital electronics or electronic materials science.

The post is tenable from October 1, 1974 for two years in the first instance renewable for a third and final year.

Salary on the scale £1,719 to £2,613 (under review) plus F.S.S.U. benefits.

Applications (three copies) naming three referees should be sent by June 21 to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained. (2338)

UNIVERSITY OF DURHAM

DEPARTMENT OF PHYSICS

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for the post of Postdoctoral Research Assistant from August 1, 1974 (or as soon as possible thereafter) to pursue research in Cosmic Ray Physics (Extensive Air Showers) involving measurements of the night sky Cerenkov radiation at the Haverah Park Air Shower experiment. Short periods of work in the United States may also be involved.

The appointment, which is funded by the Science Research Council, will be for a period of two years in the first instance, renewable for a third and final year.

Salary on the scale £1,866 to £2,586 (under review) with F.S.S.U. benefits.

Applications (three copies) naming three referees should be sent by June 24, 1974 to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE from whom further particulars may be obtained. (2337)

THE UNIVERSITY OF MANCHESTER

LIBRARIAN/CURATOR

Applications are invited for the post of Librarian/Curator in the Department of Geology. The person appointed will look after maps and books in the departmental library, and will also be responsible for the care of the Department's teaching and research collection of rocks, minerals and fossils. Candidates should have, or expect to have shortly, a degree in geology, or a combined degree including geology. Initial appointment within the Graduate Assistant salary range p.a. £1,569 to £1,791 (under review). F.S.S.U. Further particulars and application form (returnable by June 28) from the Registrar, The University, Manchester M13 9PL. Quote ref: 116/74/N. (2351)

UNIVERSITY OF EXETER

DEPARTMENT OF PHYSICS

Applications are invited for a Postdoctoral Research Assistantship, supported by the Science Research Council, for theoretical work on fluid flow through distensible tubes, with a view to physiological applications in the fields of urodynamics and venous haemodynamics. Experience in theoretical elasticity, hydrodynamics or computing would be an advantage.

The appointment is for one year in the first instance, from October 1, 1974, and is renewable for a second year. Starting salary £1,929 (under review) plus F.S.S.U. Applications with the names of two referees, should be sent by July 12, 1974, to Dr. D. J. Griffiths, Department of Physics, University of Exeter, Exeter EX4 4QL (Telephone Exeter 77911, ext. 625), from whom further information may be obtained. Please quote 1/12/7072 in all correspondence. (2350)

UNIVERSITY OF NOTTINGHAM

CANCER RESEARCH CAMPAIGN LABORATORIES

IMMUNOLOGY OF CANCER

Applications are invited for postgraduate and postdoctorate posts to participate in research on immunological aspects of cancer under the direction of Professor R. W. Baldwin. Applicants for the postgraduate posts should have a good honours degree and there will be the opportunity to read for a higher degree. The postdoctorate appointment will be on the scale commencing at £2,118 per annum, and the postgraduate appointment will be on a scale £1,422 to £1,569.

Further details and application forms from the Staff Appointments Officer, University of Nottingham, University Park, Nottingham. Closing date, June 30. Ref. No. 372. (2347)

THAMES POLYTECHNIC

SCHOOL OF ARCHITECTURE

LECTURER II IN BUILDING ACOUSTICS AND ENVIRONMENTAL SCIENCE

The School of Architecture invites applications from suitably qualified graduates to teach building acoustics and the application of environmental science to building. Applications from recently qualified graduates who have an interest in these fields will be welcomed.

The successful candidate will be a member of the teaching Division of Construction Technology, which is part of the Faculty of Architecture and Surveying, located at Hammersmith, London, W.6 and will also be associated with teachers of Physics and Materials Science at the Woolwich site of the Polytechnic. Personal interests and research will be encouraged. Duties will include teaching students on full-time diploma courses in Architecture (including acoustics to the final year) and C.N.A.A. degree courses in Surveying.

Salary scale: £2,700 to £3,474 plus £118 London allowance.

Further particulars and form of application may be obtained from the Secretary, Thames Polytechnic, Wellington Street, London SE18 6PF to whom completed applications should be returned by June 25, 1974. (2363)

INSTITUTE OF CANCER RESEARCH

ROYAL MARSDEN HOSPITAL, S.W.3

TECHNICIAN IN CYTOGENETICS

Required to work in a group studying chromosomes in malignant diseases of man. Previous experience in this or related field desirable. Qualifications required: University Degree in relevant subject, H.N.C., or AIMLT. Salary scale £1,440 to £2,292 plus London Weighting.

Apply in duplicate to the Secretary, 34 Sumner Place, SW7 3NU, with names of two referees and quoting ref. 301/B/522. (2349)

BIOCHEMIST

A position at the rank of Assistant Professor is available in the Department of Biochemistry, College of Medicine, University of Saskatchewan. Applicants must have a Ph.D. and/or M.D. degree and have training and interests in the area of nutritional biochemistry. Duties will include research and teaching of biochemistry and nutrition to undergraduate and graduate students. Effective date of appointment is negotiable. Salary will be commensurate with experience and training. Send curriculum vitae and names of three referees to Dr. J. D. Wood, Chairman, Department of Biochemistry, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N OWO. (2365)

THE UNIVERSITY OF LEEDS

DEPARTMENT OF EARTH SCIENCES

CHAIR OF GEOPHYSICS

Applications are invited for a Chair of Geophysics to fill the vacancy which will arise on the retirement of Professor R. M. Shackleton from the Chair of Geology on September 30, 1975.

The salary will be not less than £5,973 a year. The University reserves the right to consider for appointment persons other than those who submit formal applications.

Applications (two copies) stating age, qualifications and experience and naming three referees should reach the Registrar, The University, Leeds LS2 9JT, from whom further particulars may be obtained (quoting reference 29/32) not later than August 31, 1974. Applicants from overseas may apply in the first instance by cable naming three referees who should preferably be in the United Kingdom. (2357)

CSIRO

AUSTRALIA

DIVISION OF ANIMAL PHYSIOLOGY, PROSPECT, N.S.W.

CHIEF OF DIVISION

The Commonwealth Scientific and Industrial Research Organisation has a broad charter for research into primary and secondary industry areas. The Organisation has approximately 6,500 employees—2,100 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

GENERAL: Applications are invited from scientists who have an established record of personal research achievement and leadership for the position of Chief, Division of Animal Physiology. The vacancy will arise following the retirement of Dr I. W. McDonald later this year. The division has its headquarters at Prospect, approximately 20 miles from the city of Sydney. The Pastoral Research Laboratory at Armidale, N.S.W., the Beef Cattle Research Unit, at Townsville, Qld., and the Bloat Research Unit at Melbourne are regional units of the Division. The total divisional staff is approximately 150, including 55 professional staff.

The work of the Division currently includes research on ruminant nutrition, endocrinology, wool growth, climate physiology, energy metabolism, reproduction, and the pasture ecosystem. The primary objective of the Division is to provide knowledge that will improve productivity and efficiency of Australia's animal industries.

DUTIES: The Chief is responsible to the Executive for the conduct and direction of the research activities of the Division, for its general administration and for the control of funds available to it. The Chief is expected to formulate proposals for new research programmes in association with other scientists in Universities and State Departments and rural industry leaders.

The Chief of the Division of Animal Physiology is also a member of the Animal Research Laboratories Committee, of which Dr K. A. Ferguson is Chairman. The Committee has overall responsibility for the co-ordination of the research effort of the CSIRO Animal Research Laboratories, comprising the Divisions of Animal Health, Animal Physiology, Animal Genetics and Nutritional Biochemistry.

SALARY: Salary for the appointment will be the subject of negotiation but will be not less than $A22,875 p.a.

TENURE: Appointment to the Organisation will be for an indefinite period, and Australian Government Superannuation benefits are available. The tenure of Appointment as Chief could be subject to negotiation, but a fixed-term (of the order of 7 years) would be envisaged initially. Such an agreement could be renegotiated for a further period, or arrangements made for a transfer to a senior research position in the Organisation.

FURTHER INFORMATION: Dr A. E. Pierce, a Member of the CSIRO Executive, will be visiting Washington, U.S.A., from August 5 to 9, 1974 and London, England, from August 12 to 16, 1974, and will be available for discussions concerning the appointment with applicants and those interested in hearing more about the position. Those wishing to discuss the appointment should contact either the Chief Scientific Liaison Officer, Australian Scientific Liaison Office, 64 Kingsway, London, WC2B 6BD, England or the Counsellor (Scientific), Embassy of Australia, 1601 Massachusetts Avenue, N.W., Washington, D.C. 20036, U.S.A. Scientists interested in discussing the appointment should ensure that the appropriate oversea office is contacted in ample time to enable suitable arrangements to be made and supply a brief curriculum vitae.

Further information on the Division's activities can be obtained by writing to the Secretary (Administration), CSIRO, P.O. Box 225, Dickson, A.C.T. 2602, Australia.

APPLICATIONS: Applications stating full personal and professional details, the names of at least two professional referees, and quoting Reference Number 245/599 should reach:—

The Secretary (Administration),
Head Office, CSIRO,
P.O. Box 225,
DICKSON, A.C.T. 2602
AUSTRALIA.

by the 6th September, 1974

(2395)

Wellcome

Graduate Scientists

• A chance to change course

The Wellcome Foundation is a rapidly growing British owned international pharmaceutical organisation.

A vital link between our R & D activities and the world-wide distribution of the companies of the Wellcome group is the product registration department based at its London headquarters. Most countries of the world require new medical products to be registered for sale before marketing starts and this work is the responsibility of this department. It involves intelligent use of R & D reports and commercial and other information to meet the varied requirements of registration.

We are looking for a graduate chemist, pharmacist or biological scientist, probably less than 30, to join the department. This is a chance to move away from pure technical work and gain experience in an interesting and important sphere of this group's international activities.

In addition to an attractive salary the group offer excellent conditions of employment including assistance with relocation expenses where appropriate.

Please write giving brief career details and quoting reference U.127, to the Personnel Officer, Group Personnel Division, **The Wellcome Foundation Ltd.,** 183, Euston Road, London NW1 2BP.

(2384)

university of wales
university college of swansea

READER IN BIOCHEMICAL ENGINEERING

Applications are invited for the above post tenable in the Department of Chemical Engineering.

The salary will be on the scale £4,368 to £5,406 per annum, together with F.S.S.U. benefits, increasing to £4,707 to £5,844 per annum, on October 1, 1974.

Further particulars may be obtained from the Registrar/Secretary, University College of Swansea, Singleton Park, Swansea SA2 8PP, to whom applications (10 copies) should be returned by **Monday, July 22, 1974.**

(2344)

THE UNIVERSITY OF SHEFFIELD
DEPARTMENT OF BOTANY

Applications are invited for a post of JUNIOR RESEARCH FELLOW in BOTANY, tenable from October 1, 1974, for one year in the first instance renewable for two further years. Salary £1,473 a year. Further particulars from the Registrar and Secretary, The University, Sheffield S10 2TN to whom applications (three copies) should be sent by July 5, 1974. Quote Ref. R.90/G. (2364)

BANTING AND BEST DEPARTMENT OF MEDICAL RESEARCH
UNIVERSITY OF TORONTO

Applications and nominations are invited for a faculty position at the rank of Associate or Full Professor to start on or prior to July 1, 1975. The applicant's area of research should be related to the molecular aspects of animal cell differentiation. Exceptional junior candidates may also be considered. Salary negotiable and commensurate with rank. Send curriculum vitae to :

Dr Alan G. Goodridge,
Search Committee Chairman,
Banting and Best Department of Medical Research,
University of Toronto,
112 College Street,
Toronto, Ontario M5G 1L6,
Canada. (2360)

IMMUNOLOGICAL TECHNICIAN

Required to work on various aspects of cell mediated immunity. Salary on Whitley Council Scale for Medical Laboratory Technicians, i.e. £1,440 to £2,292 p.a. plus £126 L.W. Applications and enquiries to Dr H. E. Amos, Department of Immunology, Institute of Dermatology, St. John's Hospital for Diseases of the Skin, Homerton Grove, London E9 6BX. Telephone: 01-985 7061. (2353)

UNIVERSITY OF NEW SOUTH WALES
PROFESSOR OF PASTORAL SCIENCES

Applications are invited for appointment to the above-mentioned chair in the School of Wool and Pastoral Sciences. The present head of School is Professor P. R. McMahon. Applicants should have experience in the scientific development and technology of the pastoral industry and its products and be capable of providing leadership in their particular fields of interest. Professional qualifications and/or research experience in areas such as animal production, range management, agricultural economics and pastoral agronomy are desirable. The professor will supervise and participate in teaching and examining undergraduate and postgraduate students in the School and engage in and promote research and advanced study in the field of his chair.

Salary $A19,614 per annum. Subject to the consent of the University Council, professors may undertake a limited amount of higher consultative work. The University reserves the right to fill any chair by invitation.

Details of appointment, including superannuation, study leave and housing scheme, may be obtained from the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close in Australia and London on **October 18, 1974.** (2356)

BASIC GRADE PHYSICIST

Required in the Radiation Physics Department of St. Bartholomew's Hospital. Duties include radiotherapy treatment planning, radiation dosimetry and computer programming. The successful candidate will be expected to undertake research and development work. Salary (depending on qualifications and experience) £1,497 to £2,259 plus London Weighting. Applications to Personnel Department quoting reference PTA/4544/N. (2397)

HAILE SELASSIE I UNIVERSITY
ETHIOPIA

Applications are invited for two posts of ASSOCIATE PROFESSOR/ASSISTANT PROFESSOR IN GEOGRAPHY. The first post is in Human Geography with reference to political and economic geography as well as rural development. The second post is in Physical Geography. The appointments are for two years from September 1974. Appointee to the first post will teach junior classes (40 - 50 students) in Location and Interaction Systems and senior classes (30 - 40 students) in Political Geography and Regional Planning for about nine contact hours a week. Appointee to the second post will teach second year classes (40 students) in Physical Geography, third year classes (40 students) in Techniques of Map Making and third year classes (30 students) in Soils and Biogeography for about eight contact hours a week. Salary scales: Associate Professor Eth.$13,200—Eth.$18,000 p.a. Assistant Professor Eth.$9,600—Eth.$14,400 p.a. (normally free of Ethiopian tax). (£1 sterling = Eth. $5.04). There may be supplementation of salary in range £1,100 to £1,275 p.a. (sterling) (normally free of all tax) and provision of children's education allowances and holiday visit passages. This supplementation rate is currently under review. Housing allowance; dependants allowance; family passages; baggage allowance.

Detailed applications (two copies), including a curriculum vitae and naming three referees, should be sent by airmail, not later than June 27, 1974 to the **Dean, Faculty of Arts, Haile Selassie I University, PO Box 1176, Addis Ababa,** send one copy to: **Inter-University Council, 90/91 Tottenham Court Road, London, W1P 0DT. Ethiopia.** Applicants resident in U.K. should also Further particulars may be obtained from either address. (2369)

UNIVERSITY OF SURREY

DEPARTMENT OF CHEMICAL PHYSICS
NEW M.Sc. PROGRAMMES IN CHEMICAL PHYSICS

The Department of Chemical Physics invites applications for enrolment, in October 1974, into programmes in branches of Chemical Physics, leading to the degree of M.Sc. by examination and project.

Four programmes, Applied Crystallography, Crystal and Molecular Structure Determination, Crystallography and Spectroscopy, are offered, both full-time and by a day-release scheme. Applicants need not decide upon their choice of programme before the end of Term 1, as this term is common to all four programmes.

Entrance qualifications include a first degree in one of the physical sciences, or an equivalent qualification, such as Grad.R.I.C., or Grad.Inst.P.

Further details and application forms may be obtained from Dr G. A. Webb, Department of Chemical Physics, University of Surrey, Guildford, Surrey GU2 5XH.

(2341)

UNIVERSITY OF SOUTHAMPTON
DEPARTMENT OF BIOLOGY

Applications are invited for the post of lecturer in the Department of Biology. Candidates should have an appropriate Honours Degree and a research interest preferably in cytogenetics or cell culture. Opportunities exist for collaboration with the work of the Clinical Geneticist in the Faculty of Medicine. The lecturer will be expected to play a major part in teaching genetics to undergraduates in the Department of Biology and the Faculty of Medicine. Salary scale: £2,118 to £4,896. F.S.S.U. Initial salary will depend on qualifications and experience. Further particulars may be obtained from the Deputy Secretary's Section (Ext. 2400), The University, Southampton SO9 5NH to whom applications (seven copies from United Kingdom applicants) should be sent not later than July 12, 1974. Please quote reference number Na236/A. (2378)

THE HANNAH RESEARCH INSTITUTE
BIOCHEMISTRY DEPARTMENT

Scientific Officer required to assist with work on the metabolism of aromatic compounds by ruminant animals which will include the development of techniques for the quantitative assay of phenolic compounds in forage plants. Candidates should have a degree or HNC in chemistry or biochemistry. The appointment will have a starting salary within the range £1,435 to £2,329. Superannuation with a non-pensionable allowance of 5¼% to offset contributions.

Applications giving age, curriculum vitae, and the names of three referees should be forwarded to the Secretary, The Hannah Research Institute, AYR KA6 5HL by June 28, 1974. (2373)

NATIONAL VEGETABLE RESEARCH STATION
PLANT PHYSIOLOGIST

There is a vacancy for a Plant Physiologist in the grade of Scientific Officer to join a team concerned with development of new and improved systems of vegetable crop production. Degree in agricultural science, horticulture or agriculture, will normally be required: exceptionally a candidate with other equivalent qualifications will be considered. Experience in research work or commercial arable crop production is essential. Appointment in grade of Scientific Officer (£1,435 to £2,329 — under review) according to qualifications and experience. Superannuation. Full particulars and application form (to be returned by July 4, 1974) from the Secretary, NVRS, Wellesbourne, Warwick CV35 9EF. (2371)

UNIVERSITY OF THE WEST INDIES
TRINIDAD

Applications are invited for the following posts in the Faculty of Agriculture:—

1 SENIOR LECTURER/LECTURER or LECTURER/ASSISTANT LECTURER IN THE DEPARTMENT OF LIVESTOCK SCIENCE. Applicants should be graduates in Animal Science or Agricultural Science (with a major in Animal Science) with postgraduate qualifications in ruminant nutrition and physiology. Interest in animal production would be an advantage.

2 RESEARCH FELLOW/JUNIOR RESEARCH FELLOW IN THE DEPARTMENT OF AGRICULTURAL EXTENSION. Applicants should hold a postgraduate degree in extension education, sociology of rural sociology and should have training and experience in rural social research.

Salary scales: Senior Lecturer TT$17,304 — TT$25,824 p.a. Lecturer/Research Fellow TT$12,612—TT$20,316 p.a. Assistant Lecturer/Junior Research Fellow TT$10,200—TT$11,232 p.a. (£1 sterling = TT$4.8). F.S.S.U. Unfurnished accommodation for maximum of three years at 10% of salary, thereafter 20% of salary paid in lieu of housing. Family passages. Detailed applications (six copies), including a curriculum vitae and naming three referees should be sent by airmail, as soon as possible to the Secretary, University of the West Indies, St. Augustine, Trinidad. Further particulars will be sent to all applicants. (2370)

BIOCHEMIST

Preferably with experience of cell culture techniques, to join a research team investigating the enzymology of inherited metabolic disorders. Starting salary up to £1,581 plus £126 London Weighting p.a. Apply, enclosing curriculum vitae and two referees to Dr. A. D. Patrick, Reader in Chemical Pathology, Institute of Child Health, 30 Guildford Street, London WC1N 1EH, (2398)

CSIRO
AUSTRALIA

ANIMAL HEALTH LABORATORY, GEELONG
OFFICER-IN-CHARGE

The Commonwealth Scientific and Industrial Research Organisation has a broad charter for research into primary and secondary industry areas. The Organisation has approximately 6,500 employees—2,100 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

LOCATION: The appointee will be located initially at Melbourne and subsequently at Geelong, Victoria.

GENERAL: Applications are invited from scientists who have an established record of personal research achievement and leadership, interested in the position of Officer-in-Charge, Animal Health Laboratory, Geelong. Experience in the specific field of veterinary virology is essential. Some knowledge of the major diseases exotic to Australian livestock and the management and direction of studies on these diseases, under conditions of maximum microbiological security, would be highly desirable.

The decision to establish the laboratory complex which will constitute the Animal Health Laboratory at Geelong was announced recently. The current estimated capital cost of the complex is expected to be $A56 million and spending, to begin in 1976/77, will be spread over five years. At full operation the Animal Health Laboratory, Geelong, will employ a staff of 170 including 25 scientists. Its initial annual operating costs would be approximately $A3 million.

The Laboratory will have a vital role in minimising the impact of the Australian livestock industry of an outbreak of an exotic disease, such as Foot and Mouth Disease, and will provide a facility for the diagnosis of exotic animal diseases. It will carry out special tests on biological material from livestock held in quarantine before entry into Australia. It will also carry out research on those virus diseases endemic in Australian livestock. The Laboratory will provide facilities for testing the safety and potency of vaccines that may be required for the control of exotic diseases and, should the necessity ever arise, for the production of Foot and Mouth vaccine.

The Animal Health Laboratory, Geelong will be administered and operated by CSIRO on behalf of the Australian Government. Following the establishment of the laboratory, a consultative committee will assist in the determination of priorities and will ensure effective liaison on policy matters. This committee will provide an essential link with the Animal Quarantine Branch of the Department of Health for the testing of livestock in the proposed off-shore High Security Animal Quarantine Station. The committee will also provide a liaison channel with the State Departments of Agriculture and for special research, training, diagnosis and epidemiology with the proposed Bureau of Animal Health in the Department of Primary Industry.

DUTIES: The Officer-in-Charge will be responsible to the Chief, CSIRO Division of Animal Health for the direction of the Animal Health Laboratory, Geelong. Initially, he will play a major role in the planning and construction of the complex and will make recommendations, through the Chief of the Division, to the CSIRO Executive concerning its future operation. Subsequently, he will be responsible for the conduct and direction of the work of the Laboratory. He will also be required to play a major role in developing close relationships with Commonwealth and State groups possessing common interests.

SALARY: Appointment will be made within the salary ranges of Senior Principal Research Scientist or Chief Research Scientist, Grade 1. $A18,077—$A20,750 p.a.

TENURE: It is envisaged that an appointment would be made for an indefinite period with Australian Government Superannuation benefits available, but consideration could be given to a fixed-term appointment.

FURTHER INFORMATION: Dr. A. E. Pierce, a Member of the CSIRO Executive, will be visiting Washington, U.S.A. from 5th to 9th August 1974, and London, England, from 12th to 16th August, 1974, and will be available for discussions concerning the appointment with applicants and those interested in hearing more about the position. Those wishing to discuss the appointment should contact either the Chief Scientific Liaison Officer, Australian Scientific Liaison Office, 64 Kingsway, London, WC2B 6BD, England or the Counsellor (Scientific), Embassy of Australia, 1601 Massachusetts Avenue, N.W., Washington, D.C. 20036, U.S.A. Scientists interested in discussing the appointment should ensure that the appropriate oversea office is contacted in ample time to enable suitable arrangements to be made and a brief curriculum vitae should be supplied.

Further information on the Division's activities can be obtained by writing to the Secretary (Administration), CSIRO, P.O. Box 225, Dickson, A.C.T. 2602, Australia.

APPLICATIONS: Applications stating full personal and professional details, the names of at least two professional referees, and quoting Reference Number 201/449 should reach:—

The Secretary (Administration), CSIRO,
P.O. Box 225,
Dickson, A.C.T. 2602,
AUSTRALIA.

by the 6th September, 1974.
(2396)

Department of Agriculture

Applications are invited for a permanent and pensionable post in the Crop and Animal Husbandry Division, Northern Ireland.

The successful applicant will undertake work on pig production with special reference to increased efficiency, and will be required to undertake teaching duties in the Faculty of Agriculture, Queen's University, Belfast.

Appointments to the post may be at Senior Scientific Officer, Higher Scientific Officer or Scientific Officer level, the qualifications for which are:

SSO Over 25 and under 32 years of age with a 1st or 2nd Class Honours Degree in Agriculture and at least 4 years post-graduate experience, preferably in relation to pig production.

HSO Under 30 years of age with an Honours Degree as above and at least 2 years relevant post-graduate experience.

SO Under 27 years of age with an Honours Degree as above.

Grading and commencing salary will be related to qualifications and experience on the following scales:

SSO £2,798 to £3,895
HSO £2,221 to £2,834
SO £1,435 to £2,329

Please write or telephone for an application form and further information, quoting Ref. SB 135/74/N to the Civil Service Commission, Clarendon House, Adelaide Street, Belfast BT2 8ND (telephone 0232 44300, ext. 26). Completed forms must be returned to arrive not later than June 24, 1974.

(2419)

NORTHERN IRELAND CIVIL SERVICE

UNIVERSITY OF THE WEST INDIES
JAMAICA

Applications are invited for LECTURESHIP IN THE DEPARTMENT OF BIOCHEMISTRY. Duties of appointee will include lecturing and demonstrating to classes of undergraduates in both the Medical and Science Faculties. Salary scale: J$6,168 to J$9,768 p.a. (£1 sterling = J$2.20). F.S.S.U. Unfurnished accommodation at rent of 10% of salary, for maximum of three years, thereafter 20% of salary paid in lieu of housing. Family passages; triennial study leave. Detailed applications (6 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail, as soon as possible to the Registrar, University of the West Indies, Mona, Kingston 7, Jamaica. Further particulars are available and should be obtained from the same source before an application is made.

(2409)

MEDICAL RESEARCH COUNCIL

TOXICOLOGY UNIT

Technical staff required for (a) histology laboratory and (b) biochemical laboratories. Candidates should possess A.I.M.L.T. or H.N.C. Med. Lab. Sciences for post (a) or H.N.C. Appl. Biol. for posts in (b). All posts within the categories of Technician or Junior Technical Officer. Salaries according to age and experience. Apply in writing to the Director, Medical Research Council, Toxicology Unit, Woodmansterne Road, Carshalton, Surrey. (2381)

THE UNIVERSITY OF LEEDS

DEPARTMENT OF EARTH SCIENCES

Applications are invited for a post as RESEARCH ASSISTANT to work on the N.E.R.C. supported project "Palaeomagnetic Study of the African Crust" under the supervision of Dr J. Briden.

The main aims of this project are to extend the palaeomagnetic record back to 3300 m.y., and to test whether relative movements between adjacent cratons can be detected.

The appointee will be expected to register for a higher degree and submit a thesis on his contribution to the project. The appointment is for three years commencing as soon as possible and the salary will be equivalent to that of an N.E.R.C. Research Studentship.

Applications should be made to Professor J. G. Ramsay, Department of Earth Sciences, The University, Leeds LS2 9JT, naming two referees. (2386)

GREENWICH & BEXLEY AREA HEALTH AUTHORITY
BROOK GENERAL HOSPITAL
SENIOR TECHNICIAN—
NEUROPATHOLOGY

A vacancy exists in a new department which will deal with routine material from Neurosurgical and Neurological Units and research projects. The person appointed will be jointly responsible for implementing the services of the department.

Applications to :—

Senior Chief Technician,
giving age, experience and two referees
Brook General Hospital,
Shooters Hill Road,
Woolwich,
London, SE18 4LW. (2401)

SUNDERLAND POLYTECHNIC

SCHOOL OF PHARMACY AND BIOLOGY

RESEARCH ASSISTANTS

Applications are invited for Research Assistant posts in the following areas of study:

Biology: Ultrastructural studies of blue-green algae (Rivulariaceae).

Pharmaceutics: (a) Physical Pharmacy—adsorption, solubilisation and monolayer studies; tabletting and drug release.

(b) Microbiology—mode of action of antimicrobial agents; biodeterioration of pharmaceuticals.

Pharmacognosy: Chemotaxonomic and anatomical studies on medicinal plants;

Pharmacology: Allergy; gastric blood flow.

Successful applicants must have, or be about to obtain, a good honours degree (or the equivalent) and will be registered for M.Phil. and/or Ph.D. degrees of C.N.A.A.

Salary £1,500 by £81 (2) to £1,662 per annum (to be confirmed).

Further details and application forms may be obtained from the Personnel Officer, Sunderland Polytechnic, Chester Road, Sunderland SR1 3SD. (2393)

STANDARDS LABORATORY FOR SEROLOGICAL REAGENTS

Technician AIMLT (Bacteriology or Virology) or HNC in Medical Laboratory Sciences (Microbiology) required to work with Australia Antigen. Graduates may apply for this position.

Written applications with full details of age, experience and qualifications to the Personnel Officer, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT. (2392)

BIRKBECK COLLEGE
(UNIVERSITY OF LONDON)

Applications are invited for the post of LECTURER IN ZOOLOGY with teaching responsibilities at both undergraduate and M.Sc. levels. Salary scale incremental from £2,118 to £4,986 p.a. plus London allowance of £162 p.a. Initial salary on scale will not exceed £2,580 p.a. Further details and forms of application should be obtained from the Secretary, (N) Birkbeck College, Malet Street, London, WC1E 7HX. Closing date: July 1. (2390)

CONSULTOX LABORATORIES LTD.

188 Brent Crescent
London, N.W.10

VACANCY FOR CHIEF TECHNICIAN IN CLINICAL BIOCHEMISTRY/ HAEMATOLOGY DEPT.

Experience in both of the above fields essential, preferably in the field of animal toxicology. Experience in bacteriology also preferred, qualified up to H.N.C./B.Sc. with at least 5 years post-degree experience. Attractive hours, salary negotiable.

Apply to Dr B. P. Block, Director, Consultox Laboratories Ltd., 188 Brent Crescent, London, N.W.10. Tel. No.: 01-965 1157.

(2375)

UNIVERSITY OF DUBLIN
Trinity College

Lecturer in Clinical Microbiology

Re-Advertisement

Applications are invited for the above post. Candidates should have several years experience in Clinical Microbiology, but need not hold a medical qualification.

The post will entail duties in the teaching and research aspects of the subject and in the provision of a Microbiology service to the Federal Dublin Voluntary Hospitals.

It would be of particular interest to a person who is considering a career in Clinical Microbiology.

Salary scale: £2,867 by £162 to £4,001 (bar); £4,163 by £162 to £4,649; plus marriage and children's allowances and a non-contributory Pension.

Further particulars and application forms may be obtained from:

The Staff Secretary,
West Theatre,
Trinity College,
Dublin 2.

Closing date for completed applications is Friday, July 12, 1974. (2391)

For starting new research work the Laboratories of the "Institute for the Research on Alternative Methods to Animal Experiments" need, in Vienna or Munich, MEDICAL AND TECHNICAL SPECIALISTS **with very good knowledge of Tissue Culture.** Some knowledge of German required.

Applicants please write, with references to studies, present activities and salary, to: International Society for the Research on Alternative Methods to Animal Experiments, Austrian Section, A-1060, Vienna, Webgasse 42/3-. (2399)

THE CITY UNIVERSITY
LONDON
DEPARTMENT OF CHEMISTRY

Applications are invited for the

CHAIR OF ORGANIC CHEMISTRY

The salary will be in the range for professorial appointments (minimum salary, £5,973 p.a. on scales operative from October 1, 1974) and there will be a London allowance of £162 p.a. Superannuation will be in accordance with the provisions of the F.S.S.U.

Further particulars may be obtained from the Academic Registrar, The City University, St. John Street, London, EC1V 4PB.

The closing date for applications is July 31, 1974.

Please quote reference number 142/C/N. (AK2394)

THE ROYAL VETERINARY COLLEGE
(UNIVERSITY OF LONDON)

LECTURER IN PHYSIOLOGY

Applications are invited from veterinary, medical and science graduates for the post of Lecturer in Physiology. The main teaching responsibility is Veterinary Physiology for the B.Vet.Med. degree, but the appointee will also be expected to assist with the Honours B.Sc. course and to pursue research. Prospective applicants are encouraged to contact the Head of Department of Physiology, Professor M. G. M. Jukes.

Salary scale £1,929 to £4,548 p.a. (£2,118 to £4,896 from October 1, 1974) plus London Allowance £162. Initial salary determined by experience and qualifications. Superannuation benefits under FSSU.

Application forms and further particulars from the Assistant Secretary (Personnel), (N) The Royal Veterinary College, Royal College Street, London NW1 0TU. The appointment is intended to become effective as near the beginning of October 1974 as can be arranged.

Closing date for applications July 5, 1974. (2388)

BP

Research in Offshore Exploration

With expansion of our offshore exploration activity, the following vacancies have arisen within the Exploration and Production Research Division at our Sunbury Research Centre.

Oceanic Geophysicist/Geologist

to study offshore seismic and other data from deep water areas. He will be aged under 35, have a good honours degree in Physics, Geology or Geophysics, and have at least five years' experience in marine geological and/or geophysical studies, preferably in deep water. He should be familiar both with the equipment used for reflection, refraction, gravity and magnetic surveys and in the processing and interpretation of results. Interest in recent developments in crustal research and/or sedimentary processes on the continental margins would be an advantage.

Marine Geophysicists

to work on surveys in connection with the engineering characteristics of the sea (currents and waves) and the sea bed (sediment type, thickness, etc.). A considerable amount of operational survey work is involved but the development of improved techniques will be a significant element.

Candidates aged under 35, should preferably hold an M.Sc. in Applied Geophysics or Marine Geotechnics. One post requires at least 5 years' experience in the supervision and interpretation of sea bed surveys using sparker, boomer and side-scan sonar equipment. The other requires less experience but a specific interest in equipment development.

Starting salaries will be competitive and will depend on age and experience. First class fringe benefits include a non-contributory pension scheme, subsidised lunches and full sports and social facilities.

■ Please write for an application form, quoting reference U.819, to: The Manager, Central Recruitment, the British Petroleum Company Limited, Britannic House, Moor Lane, London EC2Y 9BU. (2423)

RESEARCH OFFICER IN MATERIALS SCIENCE

Research Officer S.R.C. sponsored required to study solid state electrochemical systems involving oxide bronzes and fast ion conductors. There will be a strong emphasis on practical applications. Candidates with training in Physical Chemistry, or Solid state device fabrication would be especially suitable.

The appointment is tenable for three years from October 1, 1974. Salary will depend upon age and qualifications of the candidate up to £2,715 per annum, with two increments to £3,045 (London allowance included), scale under review.

Applications with curriculum vitae and names of two referees should be sent by July 1, 1974 to Dr Mino Green, Electrical Engineering Department, Materials Section, Imperial College, London SW7 2BT.

Enquiries please phone 589-5111, Ext. 2025.

(2402)

CHELSEA COLLEGE
UNIVERSITY OF LONDON

The Biophysics Laboratory has a vacancy for a LABORATORY TECHNICIAN GRADE 2B. The appointment of a trainee technician would be considered. The work involves research into electrical properties of biological materials and general teaching laboratory duties. Qualifications required are O.N.C. or equivalent, but two A-levels in appropriate Science subjects would be preferable; encouragement will be given for further study. Salary Scale: £1,699 to £1,969 per annum (including London Allowance) or for a trainee £973 to £1,426 per annum (including London Allowance). Please write, giving personal details, to Dr. D. Rosen, Biophysics Laboratory, (N) Chelsea College, Manresa Road, London, SW3 6LX. (2389)

UNIVERSITY OF THE WEST INDIES
JAMAICA

Applications are invited for CHAIR IN THE DEPARTMENT OF MATHEMATICS. This is one of two Chairs. Appointee should have broad interests and considerable research experience in Pure or Applied Mathematics and will be expected to participate in the direction of teaching and research carried out in the Department. Salary scale: J$12,336 to J$15,768 p.a. (£1 sterling = J$2.20). F.S.S.U. Unfurnished accommodation at rent of 10% of salary for maximum of three years, thereafter 20% of salary paid in lieu of housing. Family passages; triennial study leave. Detailed applications (10 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail, as soon as possible to the Registrar, University of the West Indies, Mona, Kingston 7, Jamaica. Detailed particulars are available and should be obtained from the same source before an application is made. (2408)

BIOMETRICIAN

Applications are invited for a permanent and pensionable post in the Biometrics Division of the Department of Agriculture, Northern Ireland. The successful candidate will be concerned mainly with the following;

(1) design and analysis of laboratory and field experiments and surveys;
(2) the statistical assessment of genetical studies on poultry, cattle and herbage plants;
(3) the development and application of computer based systems for handling scientific and other data associated with research and development in Agriculture.

The successful candidate may be required to undertake teaching duties in the Faculty of Agriculture, The Queen's University, Belfast.

Appointment to the post may be at Senior Scientific Officer, Higher Scientific Officer or Scientific Officer level, the qualifications for which are:

SSO Over 25 and under 32 years of age with a first or second class Honours Degree or equivalent in mathematics or biology with specialisation in and/or experience of statistics with special reference to the design and analysis of experiments and surveys. Candidates at this level should have at least 4 years relevant post-graduate experience.

HSO Under 30 years of age with an Honours Degree as above and at least 2 years relevant post-graduate experience.

SO Under 27 years of age with an Honours Degree as above.

Grading and commencing salary will be related to qualifications and experience on the following scales:

SSO £2,798 to £3,895 HSO £2,221 to £2,854 SO £1,435 to £2329

Please write or telephone for an application form, quoting Ref. SB 136/74/N to the Secretary, Civil Service Commission, Clarendon House, Adelaide Street, Belfast BT2 8ND (telephone 0232-44300, ext. 26). Completed forms must be returned to arrive not later than June 28, 1974.

NORTHERN IRELAND CIVIL SERVICE (2420)

DUNSTAFFNAGE MARINE RESEARCH LABORATORY

OBAN, ARGYLL

SCIENTIFIC OFFICER

Applications are invited for the post of Scientific Officer to join a multidisciplinary group working on the breakdown of cellulose in marine sediments. The applicant would be expected to have a University degree or equivalent in Microbiology or related science. Post would be for initial period of two years; starting annual salary around £1,435.

Application forms can be obtained from the Director, Dunstaffnage Marine Research Laboratory, P.O. Box 3, Oban, Argyll PA34 4AD. (2387)

CHAIR OF PHARMACOLOGY

Medical college and large teaching hospital in major medical centre is considering applications for the Chair of Pharmacology. Rush-Presbyterian-St. Luke's Medical Center is an equal opportunity/affirmative action employer. Please send curriculum vitae and bibliography to Dr William Knospe, Chairperson of the Pharmacology Search Committee, Rush-Presbyterian-St. Luke's Medical Center, c/o Department of Medicine, 1753 West Congress Parkway, Chicago, Illinois 60612 U.S.A. (2405)

INSTITUTE OF MEDICAL LABORATORY TECHNOLOGY

Applications are invited for a vacancy in the editorial and information office.

Ideally the successful candidate will have had experience in a medical laboratory (and preferably hold medical technology qualifications and/or a science degree) and in addition some editorial or other office experience. Duties are likely to include editorial and advertising work on a monthly and other publications, liaison with printers and providing an information service to enquirers, for example members of the profession and school leavers.

Salary negotiable around £2,000.

Office is situated in central London convenient to the Underground.

Applications, giving brief curriculum vitae, to the General Secretary, Institute of Medical Laboratory Technology, 12 Queen Anne Street, London W1M 0AU, marked CONFIDENTIAL. (2404)

THE UNIVERSITY OF ADELAIDE

invites applications for appointment as

LECTURER

in

ANATOMY AND HISTOLOGY

A medical qualification registrable in South Australia is desirable but not essential. The appointment will be made as soon as the person concerned is able to assume duty.

The Department of Anatomy and Histology teaches University students of Medicine, Dentistry and Science, and in addition Physiotherapy and Occupational Therapy students of the South Australian Institute of Technology (for further particulars see Statement referred to below). Research fields are neuroendocrinology, human and comparative neurology, cell biology, experimental embryology, and clinical anatomy. Persons with an interest in clinical anatomy (medical graduates) or neuroendocrinology are especially invited to apply.

SALARY SCALE: $A8,698 by $A470(1) by $A469(6) to $A11,982 (plus a loading for medical qualifications at the rate of $A833 a year); with superannuation on the F.S.S.U. basis. Initial salary will be fixed within the scale in accordance with qualifications and previous experience.

FURTHER INFORMATION, including list of particulars required in an application, is available from the Registrar of the University or from the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

APPLICATIONS should reach the Registrar, G.P.O. Box 498, Adelaide, South Australia, 5001 not later than **July 31, 1974.** (2407)

THE UNIVERSITY COLLEGE OF WALES

ABERYSTWYTH

DEPARTMENT OF BOTANY AND MICROBIOLOGY

Applications are invited for the post of DEMONSTRATOR, tenable from October 1, 1974. Applicants should have a special interest in Ecology.

Salary range: £1,758 to £1,923 per annum.

Application forms and particulars available from the Registrar. Closing date: June 28, 1974. (2380)

CHARING CROSS HOSPITAL MEDICAL SCHOOL

There is an opening for a research assistant in a new small paediatric research laboratory which will undertake special investigations mainly into intestinal malabsorption.

Salary according to qualifications and experience within the range £1500-£2000 a year.

Applicants who wish to visit should make an appointment with Mrs. Colvin (Paediatric Secretary) 748 2050 Ext. 2034.

Applications in writing with full details of qualifications and experience and names of two referees to The Secretary, Charing Cross Medical School, Fulham Palace Road, London, W6 9HH by 24th June 1974 quoting reference Paed/HB. (2422)

RESEARCH IN MUSCLE DISEASE

Applications from honours graduates in biochemistry or a related discipline are invited for the post of Research Assistant in continuing studies of muscle metabolism in health and disease.

The successful applicant will be expected to register for the degree of Ph.D. at the University of Glasgow. The post carries N.H.S. grading, with appropriate superannuation and salary (£1,797 to £2,259) from October 1, 1974.

Applications naming two referees should be sent as soon as possible to Dr W. H. S. Thomson, Research Laboratory, Knightswood Hospital, Glasgow G13 2XG. (2428)

THE UNIVERSITY OF MANCHESTER

DEPARTMENT OF PHYSICS

RESEARCH ON SUPERFLUID HELIUM 3 BELOW 3 mK.

Applications invited for the post of Postdoctoral Research Assistant to work on the above topic under the direction of Professor H. E. Hall. The post is for two years from October 1st, 1974 or earlier; salary on the Lecturer scale (initially £2,412 p.a. for an applicant of 26 or over). Candidates with experience of experimental work below 100 mKare preferred. Curriculum vitae, publications list, and names of two referees should be sent to Professor H. E. Hall, Schuster Laboratory, The University, Manchester, M13 9PL. (2421)

POSTDOCTORAL RESEARCH ASSISTANT

required in Department of Clinical Pharmacology for work on the effect of drugs on the adrenal cortex. Previous experience with tissue culture and subcellular fractionation techniques would be an advantage. Appointment for up to 3 years. Salary, commensurate with experience, will be in non-clinical Lecturer range. Apply, The Secretary, St Mary's Hospital Medical School, Paddington, London W2 1PG quoting Ref. CPG. (2465)

UNIVERSITY OF AUCKLAND

NEW ZEALAND

LECTURESHIP IN MECHANICAL ENGINEERING

Applications are invited for the above-mentioned post from candidates wth qualifications in the areas of control engineering and dynamics; industrial thermodynamics; production engineering. Previous experience in research, in teaching and professional practice are all desirable but it is not essential that applicants should have experience in all three.

The salary scale for a Lecturer in NZ$6753 rising to $8568 p.a., commencing salary being determined in accordance with qualifications and experience.

Further particulars including details of superannuation, travel expenses, application procedure, are available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close on **8 July 1974.** (2414)

UNIVERSITY OF MALAWI

CHANCELLOR COLLEGE

Applications are invited for LECTURESHIP IN PHYSICS, tenable from 1 September 1974. Applicants should have a higher degree, preferably a Ph.D. with some teaching and research experience. Their theoretical or experimental research interests should include acoustics, solid state and vacuum physics. The appointee will be expected to teach at all levels of a four-year ordinary degree course. Salary scale (including expatriate addition): K2,809-K4,714 p.a. (£1 sterling = K2.01). Salary may be supplemented either by a University addition of K720 p.a. (taxable in Malawi) or the British Government may supplement salary by £600-£850 p.a. (sterling) for married appointee or £300-£550 p.a. (sterling) for single appointee (normally free of all tax) and provide children's education allowances and holiday visit passages. These latter supplementation rates are currently under review. Gratuity of 15%-25%; superannuation scheme transferable with F.S.S.U.; Family passages; various allowances; biennial overseas leave; housing. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be forwarded by airmail not later than 4th July, 1974, to the Registrar, University of Malawi, University Office, P.O Box 278, Zomba, Malawi. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London, W1P 0DT. Further particulars may be obtained from either address. (2418)

BIOCHEMIST

A leading British pharmaceutical Research Organisation has a vacancy for a SENIOR BIOCHEMIST to initiate and direct research projects in the areas of pharmacokinetics, drug metabolism, molecular pharmacology and enzymology in association with the design and development of new medicines. Persons with apropriate experience are invited to apply in writing for further details, to: The Secretary, Biorex Laboratories Ltd., Biorex House, Canonbury Villas, London N1 2HB, marking the envelope CONFIDENTIAL. (2450)

UNIVERSITY OF WESTERN AUSTRALIA

PERTH

ANATOMY

Applications are invited for appointment as SENIOR LECTURER OR LECTURER in the Department of Anatomy. The University wants to appoint an anatomist interested in new educational methods, particularly in the teaching of gross anatomy and in the clinical applications of anatomy. The Department is well equipped for teaching and research, and is housed in a pleasant modern building. There are ten full-time academic staff and active research programmes in tissue regeneration, neurobiology, cytology, embryology and various aspects of human biology. The appointee will be encouraged to develop his research interests and train research students. Further information on the Department may be obtained from Professor D. B. Allbrook.

The salary ranges are: Senior Lecturer—$A12,268 to $A14,308 p.a.; Lecturer—$A8,698 to $A11,982 p.a. Benefits include superannuation similar to F.S.S.U., fares to Perth for appointee and dependent family, removal allowance, study leave and long service leave and housing loan scheme.

Applications in duplicate stating full personal particulars, qualifications and experience should reach the Staffing Officer, University of Western Australia, Nedlands, Western Australia, 6009, by **June 30, 1974.** Candidates should request three referees to write immediately to the Staffing Officer. (2415)

UNIVERSITY OF EAST ANGLIA

SCHOOL OF CHEMICAL SCIENCES

Applications are invited for Science Research Council Advanced Course Studentships to read for the

M.Sc. IN CHEMICAL SPECTROSCOPY

This one-year course, commencing October 1, 1974, covers a wide range of spectroscopic techniques and includes practical research experience. Applicants must possess at least a 2nd class honours degree or the equivalent. Application forms and further information may be obtained from the Senior Administrative Assistant, School of Chemical Sciences, University of East Anglia, Norwich NOR 88C. (2413)

HEDGES AND BUTLER LTD.

Microbiologist

We are seeking a graduate Microbiologist to provide an efficient and complete mycological and bacteriological service to our Central Laboratory situated in our major production and packing complex at Bromley-by-Bow.

Although primarily a quality control function some applied research together with development of existing methods is envisaged. Self motivation and initiative are essential.

Applicants, male or female, will probably be in the age bracket 24-35 and will possibly already have had three or more years industrial experience. An Upper Second in Microbiology is the minimum degree level required and a higher degree would be advantageous.

This position offers an excellent opportunity to a person interested in furthering management skills whilst retaining contact with the latest technical developments in this field.

Good conditions of employment and other benefits including free life assurance and a contributory pension scheme will apply. A good salary will be negotiated which will reflect the importance of this appointment.

Please write with a detailed curriculum vitae, including details of age, experience and current salary to: Miss J. Long, Personnel Department, Hedges and Butler Limited, Three Mill Lane, Bromley-by-Bow, E.3 or alternatively telephone 01-980 7133 for an application form.



(2400)

Section Leader — Quality Control

John Wyeth and Brother Limited, a major pharmaceutical company situated at Havant, Hants, has a vacancy for a Section Leader within their well equipped Quality Control laboratories. The successful applicant will be responsible to the Head of Chemical Control for the efficient operation of the raw materials and intermediates section and preferably will possess H.N.C. in Chemistry, together with at least three years analytical experience in the pharmaceutical or allied industries.

Conditions of employment are excellent. The Company offers a non-contributory Pension and Life Assurance Scheme and assistance with re-location expenses is given where necessary.

Please apply in writing to:—

Cameron Wolstenholme,
Director of Personnel and Industrial Relations,
John Wyeth and Brother Limited,
Huntercombe Lane South,
Taplow,
Maidenhead,
Berks, SL6 0PH.

Wyeth

(2467)

STEROID CHEMIST

The Radiochemical Centre Limited is a leading world supplier of radioactively labelled compounds for research in the life sciences. Expansion of our production work has created a vacancy for an experienced steroid chemist.

Applicants should have an honours degree and relevant experience in synthetic steroid chemistry.

We expect to make the appointment with a starting salary of up to £3,000 according to previous experience. Staff conditions include a contributory superannuation scheme, generous leave allowance, canteen and social club. Assistance with house purchase may be available.

Telephone Little Chalfont 4444 or write to the Personnel Officer for an application form.

The Radiochemical Centre

Amersham Bucks

(2470)

Education Overseas

Sudan

Lecturer/Instructor—Biology Laboratory Techniques

At the Institute of Laboratory Technicians, Khartoum Polytechnic, to teach (Lectures and Practicals) in Biology Laboratory Techniques. Applicants, aged 30-50 should be AIST or equivalent with technical teaching qualifications and experience in the prescribed duties of the post. Appointment for 3 years.

Salary in excess of current UK earnings plus a tax free overseas allowance. Other benefits include free family passages, paid leave, children's education allowances, and free accommodation and medical attention. Superannuation rights may be safeguarded; all emoluments paid by British Government. Applicants should normally be citizens of, and permanently resident in, the United Kingdom.

For full details and an application form please apply stating age, qualifications and experience to:—

Appointments Officer

Overseas Development Administration

Room 317
Eland House
Stag Place
London SW1E 5DH

(2458)

CHARING CROSS HOSPITAL MEDICAL SCHOOL

Research Assistants (2) required for Endocrine Laboratory to join projects studying effects of hormones on the chemistry of cells, and the application of these studies to clinical investigation. Salary according to qualifications and experience, but the posts are junior, and suitable for new graduates. Applications to Dr J. R. Daly, Department of Chemical Pathology, Charing Cross Hospital Medical School, London W6 8RF, from whom further particulars may be obtained. (2426)

GRIFFITH UNIVERSITY

BRISBANE, AUSTRALIA

SCHOOL OF AUSTRALIAN ENVIRONMENTAL STUDIES

Griffith University will admit its first undergraduate students in March 1975. Its organisation is on a broad school of study basis and it has a commitment to interdisciplinary teaching. Applications are now invited from men and women for the following positions:

SENIOR LECTURER/LECTURER IN POLITICAL ECONOMY

Applicants should have a major interest in regional development or regional economics with relevant practical experience. The successful appointee will have qualifications in one or more of the fields of economics, geography, political science or administration. The School would be especially interested in an applicant who has combined some of these disciplines with teaching and research work.

LECTURER IN APPLIED MATHEMATICS

The appointee will be expected to assist in planning and developing a main study area in system science and applicable mathematics. This will include the study of applied mathematics with emphasis on mathematical methods and operations research. Some experience in the presentation of mathematical ideas to students with limited formal mathematical background would be an advantage.

Salaries will be within the range: Senior Lecturer —$A12,268 to $A14,308; Lecturer—$A8,698 to $A11,982. Rates of exchange as at May 1974 are $A1 = 62p; $A1=$US1.48.

Appointments to these positions will be from October 1, 1974, or as soon as possible thereafter.

For further details of these posts and for the method of application, write to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. (2443)

TECHNICIAN required by Department of Experimental Immunology of INSTITUTE OF CANCER RESEARCH, Clifton Avenue, Belmont, Sutton, Surrey. Duties include maintenance and transplantation of animal tumours (training will be given), general animal husbandry and supervision of juniors. Would suit animal technician washing to learn more general laboratory techniques. Applicants with A.I.A.T., A.I.S.T., preferred. Home office licence an advantage. Starting salary in region £1566-£1770 according to qualifications and experience. Apply in duplicate with names of two referees to the Secretary, 34 Sumner Place, London SW7 3NU, quoting ref. 301/B/523. (2435)

UNIVERSITY OF THE WITWATERSRAND,

JOHANNESBURG, SOUTH AFRICA

SENIOR LECTURER IN MATHEMATICS

Applications are invited for appointment to the above post in the Department of Mathematics. Duties are to be assumed on January 1, 1975, or earlier by mutual arrangement. Candidates should have a strong research interest in some field of modern pure mathematics.

Salary will be in the range R7,245 to R9,315, the initial salary to be determined according to qualifications and experience. Benefits include an annual bonus, pension and medical aid facilities, and a housing subsidy, if eligible.

Intending applicants should obtain the information sheet relating to this post from the Registrar, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, South Africa, with whom applications should be lodged not later than June 30, 1974. U.K. applications may obtain the information sheet from the London Representative, University of the Witwatersrand, 278 High Holborn, London, W.C.1, to whom a copy of the application should be sent. (2430)

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL

(UNIVERSITY OF LONDON)
DEPARTMENT OF NUCLEAR MEDICINE

RADIOIMMUNOASSAYIST

An experienced Biochemist (preferably post-doctoral) or medical graduate with a special interest in radioimmunoassay and saturation assay techniques is required to take charge of the service/development group in the Department of Nuclear Medicine. Responsibilities will include the oversight of routine work currently undertaken within the Department and by peripheral research groups, but special emphasis will be placed on the development of new and improved assay techniques. This is a new appointment offering a challenging opportunity to an imaginative and energetic scientist in a group with a long experience of R.I.A. methods and possessing excellent technical facilities. The successful applicant will be expected to participate in the undergraduate and postgraduate teaching. Salary on the Lecturer scale according to age, qualifications and experience £1,929 to £4,548 per annum plus £162 London allowance, rising on October 1, 1974 to £2,118 to £4,896 per annum plus London allowance. Applications including a curriculum vitae and the names and addresses of two referees should be submitted to Professor R. Ekins (N), The Middlesex Hospital Medical School, London W1P 7PN, by July 1, 1974. Further particulars may be obtained similarly by ringing 01-636 8333, Extn. 7289). (2446)

THE POLYTECHNIC OF CENTRAL LONDON SCHOOL OF ENGINEERING AND SCIENCE BIO-ORGANIC GROUP

RESEARCH FELLOW

£2,151 to £2,421

for work on the enzymology of penicillin production in penicillium chrysogenus. Candidates should have, or expect to obtain a Ph.D. in Enzymology or Protein Chemistry.

RESEARCH ASSISTANT (2 posts)
£1,427 to £1,592

(a) for work on the entry of drugs into mammalian cells in culture; (b) for work on hydrolytic enzymes capable of degrading the natural macromolecules present in sewage disposal systems.

Candidates for both posts should have, or expect to obtain a good Honours degree in Biochemistry or related subjects. Successful candidates may be registered for a higher degree of the CNAA.

Details and application form from The Establishment Officer, 309 Regent Street, London W1R 9AL. (2457)

THE MALAYSIAN RUBBER PRODUCERS' RESEARCH ASSOCIATION

requires an experienced ELECTRON MICROSCOPE TECHNICIAN to take charge of the operation and maintenance of its Siemens Elmiskop Mk 1 and ancillary equipment. The position is suitable for candidates aged between 25 and 35 with experience in biological and physical work and with a sound knowledge of replica embedding and sectioning techniques. The starting salary may be between £1,400 and £2,400 per annum depending on age, experience and qualifications. There is a pension scheme.

The Association will be moving in July to an attractive new location on a 30-acre site at Brickendonbury, near Hertford. Transport from Welwyn Garden City and locality may be provided.

Please write giving details of qualifications and experience to:

The Director,
The Malaysian Rubber Producers' Research Association,
56 Tewin Road,
Welwyn Garden City,
Herts. (2439)

Product Registration— Pharmaceuticals

As a major and successful U.K. based pharmaceutical manufacturer with world-wide interests, our activities and markets continue to expand. This expansion, coupled with new legislation, creates the need to appoint an additional graduate to join the Product Registration Department in Nottingham.

Responsibilities will consist of assistance in the preparation of submissions for registration of pharmaceutical products in the U.K. and overseas countries and in the preparation of technical dossiers for use in various departments of the Company. Liaison with marketing and technical specialists is an integral part of the job.

Applications are invited from graduates in pharmacy or a biological science who have worked in the pharmaceutical industry for about two years. Previous experience in product registration would be an advantage but is not essential.

Employment conditions are first class. Some help with relocation expenses will be given if appropriate. Please write for an application form to: J. F. Pattison, Employment Services Dept.

Boots

The Boots Co. Ltd.,
Station Street,
Nottingham NG2 3AA.

(2468)

Pharmacologist/ Neurochemist

We are looking for an experienced Biochemically orientated Pharmacologist or Neurochemist to join a multi-disciplinary team seeking novel drug therapy for mental illness.

He (or she) will probably possess a Ph.D and preferably have post-doctoral experience, but in any event should have substantial research experience concerning the Biochemistry of the central nervous system.

The person appointed will be expected to take responsibility for important areas of the project work and will work in close collaboration with scientists specialising in behavioural and electro-physiological techniques.

Salary will be competitive and there are good fringe benefits. The site is located one mile from Harlow New Town and Development Corporation property may be available to suitable candidates at economical rent.

If you would like to be considered for this post, please write, outlining your career to date, to:

BRL

John Atkinson,
Site Personnel Officer,
Beecham Research Laboratories,
The Pinnacles,
Harlow, Essex.

BRL 1972.

(2427)

Research in Surface Physics

A graduate Physicist or Electronics Engineer is required to join a team working on fundamental investigations of the thermal oxidation of silicon and the electrical properties of the silicon/oxide interface. Modern surface-physics electronic-measurement and computer-analysis techniques are used and the applicant will be expected to make electrical measurements and assist in their interpretation. A sound knowledge of semiconductor physics and electronic systems is essential and experience in electronic design, computer programming or UHV techniques would be an advantage.

We are situated on the attractive Surrey/Sussex border in close proximity to London, the South Downs and the Coast. Working conditions, salaries and conditions of employment are first class.

Mullard Research Laboratories

Application forms may be obtained from M. L. Malpass, Personnel Manager, Mullard Research Laboratories, Cross Oak Lane, Salfords, Nr. Redhill, Surrey. Ref. 358/N

(2438)

AGRICULTURAL RESEARCH INSTITUTE OF NORTHERN IRELAND
Hillsborough, Co. Down

ASSISTANT EXPERIMENTAL OFFICER

Applications are invited for the post of Assistant Experimental Officer to assist with experimental work with beef animals.

Salary scale: £1,435 to £2,329 plus Superannuation Allowance.

Candidates should possess—a pass degree; H.N.D.; or O.N.D. in Agriculture, or equivalent.

Previous experience of supervisory duties and working with animals would be an advantage.

Application forms and further particulars may be obtained from the Secretary to whom applications should be submitted by Monday, July 8, 1974.

Canvassing will disqualify. (2403)

UNIVERSITY OF PAPUA AND NEW GUINEA

Applications are invited for the Post of CHIEF TECHNICIAN, AGRICULTURE, tenable as soon as possible. Appointee should have a degree in Agriculture or equivalent qualification with appropriate research experience. Responsibilities will include supervision and assistance in research work, and the preparation of materials for examinations and practices. Salary: A$9,099 to A$10,432 p.a. plus A$360 p.a. dependant allowance (£1 sterling = A$1.62). Housing allowances. Applications (2 copies) including a curriculum vitae, a recent small photograph, an indication of starting date, and naming three referees should be sent by airmail to arrive as soon as possible to the Secretary, P.O. Box 4820, University, Papua New Guinea, from whom further particulars may be obtained. (2431)

SCIENTIFIC OFFICER/ HIGHER SCIENTIFIC OFFICER

required at the Agricultural Research Council Unit of Statistics, Edinburgh, to advise on application of existing statistical methods to agricultural research and to take part in the development of new methods.

QUALIFICATIONS: A good Honours degree in Mathematics or Statistics together with appropriate postgraduate statistical experience.

SALARY: Scientific Officer scale £1,435 to £2,329. Higher Scientific Officer scale £2,221 to £2,854 depending on qualifications and experience, a minimum of two years postgraduate experience being required for appointment to the HSO grade.

The post is superannuable, with an allowance to offset contributions. Enquiries and applications to the Director, Agricultural Research Council Unit of Statistics, 21 Buccleuch Place, Edinburgh EH8 9LN. (2442)

KINGSTON POLYTECHNIC
SCHOOL OF GEOLOGY
SENIOR TECHNICIANS
(1) FOR MINEROLOGY AND PETROLOGY SECTION
(2) FOR STRUCTURAL GEOLOGY SECTION

A degree in Geology or Advanced Certificate for Science Lab technicians or several years' appropriate experience desirable. Salary grade 3/4 £1,521 to £2,031.

Further details and application forms from Assistant Registrar, Kingston Polytechnic, Penrhyn Road, Kingston upon Thames KT1 2EE. 01-549 1366. (2424)

UNIVERSITY OF THE WEST INDIES
JAMAICA

Applications are invited for (a) LECTURESHIP or (b) ASSISTANT LECTURESHIP IN THE DEPARTMENT OF PHARMACOLOGY. Duties of the post will be to instruct students in Pharmacology reading for the MB, BS degree of the University, and to do research in Pharmacology. Preference will be given to candidates with medical qualifications. Salary scales: (a) (Medical) J$7,860 to J$10,752 p.a. (Non-medical) J$6,168 to J$9,768 p.a.; (b) (Medical) J$6,300 to J$6,900 p.a. (Non-medical) J$5,006 to J$5,486 p.a. (£1 sterling=J$2.20). F.S.S.U. Unfurnished accommodation at rent of 10% of salary, for maximum of 3 years, thereafter 20% of salary paid in lieu of housing. Family passages; triennial study leave. Detailed applications (6 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail, as soon as possible, to the Registrar, University of the West Indies, Mona, Kingston 7, Jamaica. Further particulars are available and should be obtained from the same source before an application is made. (2434)

MANCHESTER AREA HEALTH AUTHORITY (TEACHING)
SOUTH DISTRICT
WITHINGTON HOSPITAL
MANCHESTER M20 8LR
GRADUATE

required with a good honours degree in biochemistry. The appointee will take part in research into thrombosis and development work in the National Reference Laboratory for Anticoagulant Control Reagents at Withington Hospital.

Further information can be obtained from Dr. L. Poller, Consultant Haematologist, Withington Hospital.

Applications with the names of two referees to the Hospital Secretary, quoting ref. B22. (51323) (2453)

UNIVERSITY OF THE WEST INDIES
JAMAICA

Applications are invited for (a) LECTURESHIP or (b) ASSISTANT LECTURESHIP IN THE DEPARTMENT OF ZOOLOGY, tenable as soon as possible. Preference will be given to persons with an interest in Marine Ecology. Salary scale: (a) J$6,168 to J$9,768 p.a.; (b) J$5,006 to J$5,486 p.a. (£1 sterling=J$2.20). F.S.S.U. Unfurnished accommodation at rent of 10% of salary for maximum of three years, thereafter 20% of salary paid in lieu of housing. Family passages; triennial study leave. Detailed applications (6 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail, as soon as possible to the Registrar, University of the West Indies, Mona, Kingston 7, Jamaica. Detailed particulars are available and should be obtained from the same source before an application is made. (2433)

UNIVERSITY OF EXETER
Demonstratorship in Organic Chemistry

Applications are invited for a Demonstratorship in Organic Chemistry, tenable from 1 October 1974 for two years. Applicants should have a Ph.D. degree or equivalent research experience.

Salary within the range £1941-£2247 per annum with initial placement in accordance with qualifications and experience. The post is superannuable (FSSU).

Further particulars may be obtained from: The Secretary of the University, Northcote House, The Queen's Drive, Exeter EX4 4QJ, to whom applications (six copies; overseas applicants one copy), together with the names of two referees, should be sent by 15 July, 1974. Please quote Reference No. 1/12/7074 in all correspondence. (2436)

UNIVERSITY OF THE WITWATERSRAND,
JOHANNESBURG, SOUTH AFRICA

BERNARD PRICE INSTITUTE FOR PALAEONTOLOGICAL RESEARCH

VACANCY: SENIOR RESEARCH OFFICER/RESEARCH OFFICER

Applications for the above position are invited from the persons whose research interests lie in the fields of palaeobotany and/or palynology. Experience in the field of Gondwana palaeobotany is preferred, but will not be an essential condition of appointment. The level of appointment will depend on the appointee's qualifications and experience.

Salary will be in the following ranges:

Senior Research Officer: R7,245 to R9,315.

Research Officer: R5,520 to R7,935;

the initial salary to be determined according to qualifications and experience. Benefits include an annual bonus, pension and medical aid facilities, and a housing subsidy, if eligible.

Intending applicants should obtain the information sheet relating to this post from the Registrar, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, South Africa, with whom applications should be lodged not later than July 31, 1974. U.K. applicants may obtain the information sheet from the London Representative University of the Witwatersrand, 278 High Holborn, London, W.C.1, to whom a copy of the application should be sent. (2429)

UNIVERSITY OF MALAYA
FACULTY OF SCIENCE

Applications are invited for the posts of LECTURER/ASSISTANT LECTURER in the Departments listed below. Subject to academic suitability and experience, preference will be given to candidates who are competent in Bahasa Malaysia (Malay). Candidates should have at least a good honours degree with suitable teaching and research experience in addition to the special qualifications where indicated. For the Lectureships, a doctorate is normally required.

DEPARTMENT OF GEOLOGY (One post). Applicants should have a higher degree in Geology and be qualified in Palaeontology/Stratigraphy.
SCHOOL OF BIOLOGICAL SCIENCES:

One post in the field of LOWER PLANT PHYSIOLOGY. Preference will be given to the candidate who possesses a good knowledge of the Metabolism and Morphogenesis of Lower plants.

One post in the field of QUANTITATIVE ECOLOGY/BIOMETRICS. Candidates should have a sound biomathematical background with suitable teaching and/or research experience in aspects of quantitative ecology such as population estimation and dynamics, distribution patterns, association and community analysis, and systems analysis.

One post in the field of SOIL MICROBIOLOGY. Special interests in one or more of the following areas:- (1) decomposition of organic materials (2) nitrogen fixation (3) bio-degradation of pollutants.

One post of Lecturer/Assistant Lecturer to be in charge of the UNIVERSITY OF MALAYA FIELD STUDIES CENTRE for the School of Biological Sciences. Candidates should have at least a good honours degree with interest and research experience in Field Biology. Administrative experience would be an advantage.

One post in the field of BIOLOGY OF VASCULAR CRYPTOGAMS with special interest in research, particularly in ferns, mosses or other cryptogamic groups of the Malesian flora.

Salary scales (approx. stg. equivs.): Lecturer £1524-2441; Asistant Lecturer £1330-1460 p.a. In addition the folowing allowances are payable: Variable Allowance £243 min. £649 max. p.a. calculated at 35% of basic salary. Supplementary Housing Allowance £503 p.a. and medical benefits.

Further particulars and application forms are obtainable from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

The closing date for the receipt of applications is **15 July, 1974.** (2444)

CHEMISTRY DEPARTMENT
UNIVERSITY COLLEGE LONDON

There is a vacancy for the post of POST-DOCTORAL RESEARCH ASSISTANT sponsored by the S.R.C. to work on the kinetics, stereochemistry and mechanism of oxidatively-induced reactions of organotransition metal complexes. Appointment for one year, salary £1,929 plus £162 London Allowance (under review). Please write to Mr F. Widdas, Chemistry Department, University College, 20 Gordon Street, London WC1H 0AJ. (2447)

Ministry of Agriculture, Fisheries and Food
Plant Pathology Laboratory, Harpenden

Plant Scientist

■ **To work in team engaged on cereal disease surveys** ■ **Research into the epidemiology of plant diseases** ■ **Develop techniques for disease assessment** ■ **Disease forecasting and appraisal of crop losses** ■ **Opportunity for individual investigation.**
□ Degree or equivalent in Plant Science or related subject □ Interest in quantitative aspects of plant pathology essential □ Experience or interest in the use of computers an advantage □ Age under 30 □ Appointment as Higher Scientific Officer (£2283-£2916) or Scientific Officer (£1497-£2391) according to age and experience □ Salaries under review □ Ref: SB/4/AG.

Ministry of Agriculture, Fisheries and Food
Salmon and Freshwater Fisheries Laboratory, London

Zoologist/Physiologist

■ **To initiate and conduct research into fish migration and 'homing' problems** ■ **Study reactions of fish to altered river conditions resulting from large-scale water transfers** ■ **Liaise with other organisations.**
□ 1st/2nd hons degree or equivalent in Zoology or Physiology □ At least 4 years' postgraduate experience □ Background in Zoological/Physiological research in fisheries desirable □ Age under 32 □ Appointment as Senior Scientific Officer (£3088-£4185 under review) □ Ref: SB/2/AK.
□ Application forms (to be returned by 8 July 1974) from Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, telephone Basingstoke 29222 ext. 500 or London 01-839 1992 (24 hour answering service).

Science group
CIVIL SERVICE

(2448)

ROBERT GORDON'S INSTITUTE OF TECHNOLOGY, ABERDEEN

School of Pharmacy

LECTURER
IN PHARMACEUTICAL CHEMISTRY

Relevant academic, industrial or research experience required.

Salary in range £1,733-£4,173 per annum.

Details from Director, Robert Gordon's Institute of Technology, Schoolhill, Aberdeen, AB9 1FR.

(2462)

Glamorgan Polytechnic

POLITECHNIG MORGANNWG

Research

Applications are invited from suitably qualified persons for positions as Research Assistants or Research Students for projects in the following Faculties:—

Engineering (including Mining);

Science;

Social Sciences and Arts (including Management and Business Studies).

Particular vacancies exist for work on

(a) The fracture of concrete;

(b) The electrical properties of carbon impregnated plastics;

(c) The microbial degradation of coal.

The appointments will be for an initial period of two years but will normally be extended for three years. The persons appointed will be required to register for a Higher Degree of the Council for National Academic Awards. They will be expected to hold a good Honours Degree or a similar qualification. Applications from candidates with some experience in employment since graduation will be particularly welcome. The salary will be £1,500 for research assistants. For research students the payment will be similar to SRC scales but some additional income may be available.

Applications, to be returned by 5th July, should be made by letter stating age, qualifications, educational background, experience and specific field of interest to:

The Secretary (Reference VN), Glamorgan Polytechnic, Llantwit Road, Treforest, Pontypridd, CF37 1DL. Telephone: Pontypridd 0443-405133.

(2464)

Reckitt & Colman
PHARMACEUTICAL DIVISION

Mass Spectroscopist

We are seeking the services of a chemist or biochemist to operate a gas chromatograph—mass spectrometer within the Spectroscopy Section of the Analytical Services Department. The successful candidates will be responsible for running and maintaining an LKB 2091 GC-MS instrument and the development of mass spectroscopy for the determination of structures of reaction intermediates, contaminants of drug candidates, and metabolites of potential drugs undergoing toxicological and clinical evaluation.

Preferred candidates will have had experience in GC-MS either in the pharmaceutical or related industry or a university.

Contributory pension and bonus schemes are in operation, and the Company offers 4 weeks annual holiday, together with excellent social and welfare facilities.

Please apply giving details of qualifications, experience and career to date to: Mrs P A Haynes, Staff Personnel Officer, Reckitt & Colman, Dansom Lane, Hull HU8 7DS.

(2437)

OXFORDSHIRE AREA HEALTH AUTHORITY (TEACHING)

RESEARCH INSTITUTE, CHURCHILL HOSPITAL, HEADINGTON, OXFORD OX3 7LJ

RESEARCH TECHNICIAN

required to assist in our investigation of the effects of radiation on the vascular system of the central nervous system. Previous laboratory experience desirable but not essential. Further details from Dr. J. W. Hopewell, Research Institute, Churchill Hospital, Oxford; applications to The Secretary of the Board of Management, Research Institute. (2469)

UNIVERSITY OF GLASGOW LECTURESHIP IN VIROLOGY

Applications are invited for a Lectureship in the Department of Virology. Candidates should have research experience with bacteriophage. The new salary scale for Lecturers is £2,118 to £4,896 per annum, which will be effective from October 1, 1974, initial salary placement will be within the range £2,118 to £3,285 per annum. F.S.S.U.

Applications (eight copies) should be lodged not later than June 28, 1974, with the undersigned, from whom further particulars may be obtained.

In reply please quote Ref. No. 3443/IM.

ROBT. T. HUTCHESON, Secretary of the University Court. (2451)

CENTRAL BIRMINGHAM HEALTH DISTRICT
THE CHILDREN'S HOSPITAL
PRINCIPAL AND SENIOR GRADE BIOCHEMIST

The Clinical Chemistry Department of the Children's Hospital gives a Regional Paediatric service and offers experience in a wide range of Biochemical techniques, including screening and research into inherited metabolic disease. The appointment of the successful candidate will depend on previous hospital experience, qualifications, and ability to deputise for the Consultant Chemical Pathologist. Salary:— Principal £3,969 to £5,175; Senior £2,964 to £3,843.

For further information please contact Dr. D. N. Raine (telephone 021-454 4851), to whom applications giving personal details and the names of two referees should be sent. The Children's Hospital, Ladywood Middleway, Fiveways, Birmingham B16 8ET. (2452)

BROOM'S BARN EXPERIMENTAL STATION

HIGHAM, BURY ST. EDMUNDS, SUFFOLK IP28 6NP

A ZOOLOGIST

is required as SBREC Fellow at Broom's Barn (which is a unit of Bothamsted Experimental Station). The subject of study is bird and small mammal pests of sugar beet.

Qualifications—1st or 2nd class honours degree, but those graduating in 1974 will be considered. The fellowship is for three years and will enable a suitably qualified applicant to register for a higher degree. The salary scale will be related to the first three points of the Scientific Officer scale: £1,435 x £84 to £1,519 x £97 to £1,616 (scale under review). Superannuation under FSSU with a 4½% non-pensionable allowance to offset personal contribution.

Applications should be sent by July 11, 1974, to the Head of the Station, from whom further particulars can be obtained. (2460)

ROTHAMSTED EXPERIMENTAL STATION

HARPENDEN, HERTS AL5 2JQ

ASSISTANT STATISTICIANS

to analyse and interpret data from agricultural and other biological experiments using the Station's computer. Degree, HNC or equivalent qualification in mathematics or statistics. Some knowledge of agriculture or biology an advantage.

Appointment in grade of Scientific Officer (£1,435 to £2,329). Starting salary according to qualifications and experience. Superannuation with non-pensionable allowance to offset contributions.

Apply in writing to the Secretary giving names and addresses of two referees and quoting reference 2262 by July 11, 1974. (2461)

Administrative Assistant

County Environment Department

The South Yorkshire County Council places great emphasis on the importance of the total environment and has established a separate Environment Department, headed by a Chief Officer. The Department is responsible for initiating and carrying into effect, in accordance with the Council's overall corporate policies, active programmes for the improvement of the environment in South Yorkshire.

Applications are invited for the following post in the Assessment Division of the Department

ENVIRONMENTAL SCIENTIST £3,273 to £3,729

The officer appointed will work within a multi-disciplinary team engaged on research and the formulation of the County Council's Environment Policy. The team, in consultation with local and national authorities, will identify the causes and extent of environmental and ecological damage and advise the County Council on policies for remedial action and future protection.

In particular, the officer will be responsible for the assessment of the natural environment of South Yorkshire and will recommend policies for the protection of areas of scientific importance in the County. He, or she, will be involved, within the corporate approach adopted by the Department, as a point of reference on planning application, protection and improvement schemes proposed by the Department.

The post will be attractive to graduates in natural sciences with experience in assessment techniques and the management of the countryside, including areas requiring environmental protection or improvement and sites of scientific interest.

Please write or telephone for an application form, quoting Post Ref: V40 to the Chief Executive (Personnel), South Yorkshire County Council, Regent Street, Barnsley S70 2TN. Telephone Barnsley 86141 Ext. 13.

Closing date for applications will be: 25 June 1974.

South Yorkshire County Council

The post is advertised after consultation with the Local Government Staff Commission and is not restricted to Local Authority staff, although other things being equal, preference will be given to Local Government Officers serving in England (excluding London) and Wales. (2463)

UNIVERSITY OF LEEDS

DEPARTMENT OF GENETICS

A TECHNICIAN (GRADE 3)

is required for assistance in research in the above department. Familiarity with chemical biochemical and/or microbiological techniques would be a decided advantage. Candidates should have at least 2 'A' levels plus some 'O' levels plus 3 years relevant experience, or comparable qualifications. The work would not be inappropriate for graduates. Salary on the scale £1,650 to £1,920 per annum. Replies to Professor J. R. S. Fincham, Department of Genetics, University of Leeds, Leeds LS2 9JT. (2449)

CHEMIST/BIOCHEMIST

To take part in a study of bile Salt Metabolism in Diabetes and Thyroid Diseases.

Project suitable for thesis. Training will be provided for techniques necessary for the project.

Salary £1,900.

Apply in writing to Dr. G. H. Tomkin, M.D., F.R.C.P.I., F.A.C.P., Dept. of Endocrinology, Dudley Road Hospital. Box 293, Birmingham B18 7QH. Quote ref. 549/N. (2459)

FELLOWSHIPS AND STUDENTSHIPS

UNIVERSITY COLLEGE OF NORTH WALES, BANGOR

SCHOOL OF PHYSICAL AND MOLECULAR SCIENCES

POSTDOCTORAL RESEARCH FELLOWSHIP IN CHEMISTRY

Applications are invited for an S.R.C. Postdoctoral Fellowship to work in collaboration with Professor C. J. M. Stirling on mechanistic studies of carbanions in solution.

The salary will be in the range £2,118 to £2,247 per annum plus F.S.S.U., and the Fellowship will be tenable from October 1, 1974, or such other date as may be arranged.

Applications (two copies), giving details of qualifications, research experience, and the names and addresses of two referees, should be sent as soon as possible to the Secretary and Registrar, University College of North Wales, Bangor, from whom further details may be obtained. (2348)

THE UNIVERSITY OF MANCHESTER

C.A.S.E. STUDENTSHIP IN CHEMICAL METALLURGY

Applications are invited for an S.R.C. studentship leading to the Ph.D. degree for work on the recovery and separation of metals by hydrochlorination and hydropyrolysis reactions. The work will be carried out in the Department of Metallurgy and in the Metals Extraction Division, Warren Spring Laboratory, D.T.I. Stevenage. Applicants should possess or expect to obtain a good honours degree or an M.Sc. in Metallurgy or Chemistry and have a strong interest in extractive metallurgy or physical inorganic chemistry. Conditions in accordance with S.R.C. regulations. Applications to Dr. R. A. J. Shelton, Department of Metallurgy, The University, Manchester, M13 9PL (2343)

UNIVERSITY OF BIRMINGHAM

Department of Pharmacology (Preclinical) and MRC Neuropharmacology Unit

POSTGRADUATE RESEARCH STUDENTSHIPS

Applications are invited for 2 research studentships supported by the Medical Research Council, commencing on 1 October 1974. The research work of the Department and Unit covers various aspects of the pharmacology of the central nervous system with special reference to the mode of action of drugs, and there are opportunities for learning new techniques such as microiontophoresis, radiochemical assay and zonal centrifugation.

Applicants should have a 1st or upper 2nd Class Honours degree in pharmacology, biochemistry, physiology or related biological subjects.

Applications, in writing to:

Professor P. B. Bradley,
Dept. of Pharmacology (Preclinical),
Medical School,
Birmingham,
B15 2TJ (2273)

WESTFIELD COLLEGE

(UNIVERSITY OF LONDON)
DEPARTMENT OF BOTANY AND BIOCHEMISTRY

S.R.C. CASE STUDENTSHIP

For work on the effect of phosphorous concentration on competition between planktonic algae and on the interactions between phosphorus and other inorganic nutrients involved in the eutrophication of surface waters. The work will be in collaboration with the Unilever Research Laboratory at Port Sunlight where the student will spend a minimum of one month per year

N.E.R.C. RESEARCH STUDENTSHIP

For work on "Water and ion movement in live **Sphagnum** and peat". About 2% of the earth's surface is covered by peat and the ecology of one of the major peat forming plants is to be studied.

Both studentships are available from October 1974. Applicants should have, or expect to obtain a 1st or upper 2nd class honours degree in a Biological Science, although consideration will be given to graduates in Physical or Chemical Science who have a keen interest in biological systems.

Apply by July 15 to Mrs B. Thake (CASE) or Dr R. S. Clymo (NERC), Westfield College, Kidderpore Avenue, Hampstead, London, NW3 7ST. (2354)

UNIVERSITY OF SOUTHAMPTON ELECTROCHEMICAL TECHNOLOGY

Applications are invited for Postdoctoral Fellowships and Postgraduate Studentships from suitably qualified scientists and engineers who wish to join a multidisciplinary group working on long term studies of energy storage systems, metal recovery, novel chemical syntheses and pollution control.

Further details are obtainable from Dr. R. E. W. Jansson, Department of Chemistry. The University, Southampton SO9 5NH. (2412)

ULSTER: THE NEW UNIVERSITY

SCHOOL OF BIOLOGICAL AND ENVIRONMENTAL STUDIES

RESEARCH STUDENTSHIPS IN LIMNOLOGY

Applications are invited for two research studentships tenable at the Limnology Laboratory of The New University of Ulster.

1. To study the initial stages of colonisation of a large artificial lake to be filled in the near future with storm water discharge from the new city of Craigavon. The study will have a management-orientated bias and will be primarily concerned with aspects of the physics, chemistry and biology of the open water. The grant is for two years and the candidate will be expected to register for the M.Phil. degree.
2. To study the zooplankton of Lough Neagh with particular emphasis on grazing and re-cycling of nutrients. The successful candidate will be expected to contribute to the work of a small group comparing limnological conditions in Lough Neagh with those inside a large Lund-type butylite enclosure set in the lough.

The grant is for three years and the candidate will be expected to register for a D.Phil. degree.

Both grants, financed by the Northern Ireland Department of the Environment, will be at Research Council rates.

Application forms and Further Details should be obtained from The Registrar, The New University of Ulster, Coleraine, Northern Ireland (quoting Ref: 74/82) to whom completed applications, including the names and addresses of two referees, should be returned not later than 5th July, 1974. (2382)

THE UNIVERSITY OF SHEFFIELD
DEPARTMENT OF CHEMISTRY

Applications are invited for the following:

1. POSTDOCTORAL RESEARCH FELLOWSHIP for studies on chemical carcinogenesis. The appointee will join a team working on chemical and biochemical aspects of polycyclic aromatic hydrocarbon binding to DNA, ranging from synthetic and structural organic chemistry to tissue culture. Candidates should hold (or except to gain) a Ph.D. in organic or biological chemistry and experience in nucleic acid chemistry is desirable but not essential. The appointment is from October 1, 1974 or a date to be arranged until September 30, 1975 at a salary not less than £1,923 per annum plus F.S.S.U.

2. AN S.R.C. (C.A.S.E.) STUDENTSHIP for a project involving the synthesis of a novel group of heterocyclic compounds. These are designed to possess controlled chemical reactivity and potential herbicidal character. The work will be carried out in conjunction with I.C.I. Plant Protection Limited and part of the programme will be performed at their Jealott's Hill Research Station. Applicants should have (or expect to obtain this summer) a good Honours degree or G.R.I.C. in chemistry and the successful will be expected to register for a Ph.D.

Applications for either of the above, including a curriculum vitae and names and addresses of two referees to: Dr. G. M. Blackburn, Department of Chemistry, The University, Sheffield S3 7HF (from whom further information is available in respect of the Studentship). (2352)

UNIVERSITY OF SOUTHAMPTON
DEPARTMENT OF AERONAUTICS AND ASTRONAUTICS
COMBUSTION HEAT TRANSFER

Applications are invited for a Research Fellow at post-doctoral level to work on a Science Research Council sponsored continuing programme concerned with the measurement and prediction of heat transfer from simulated combustion systems. The experimental programme utilises the department's shock tube facilities and involves modern techniques for heat transfer measurement.

The appointment, which requires relevant postgraduate experience, is for one year but may be extended until January 31, 1976. Salary will be in the range £1,929 to £3,048 (under review) plus F.S.S.U. benefits.

Applications, giving details of age and relevant background and quoting the names of two referees, should be made to the Deputy Secretary's Section, The University, Southampton SO9 5NH, as soon as possible. Please quote reference number: Na/238/R. (2376)

YORK UNIVERSITY
POSTDOCTORAL FELLOWSHIP

A Fellowship will be available in one of the following areas:

1. Laboratory studies of small particles using laser techniques.
2. Low temperature matrix isolation spectroscopy.

Applicants should have a strong experimental background in aerosol physics or chemistry, low temperature spectroscopy, or laser applications.

Salary to $8,700 p.a. Contact Dr. W. W. Duley, Physics Department, York University, Toronto, Canada. (2367)

UNIVERSITY OF SOUTHAMPTON
RESEARCH STUDENTSHIP IN CRYOGENICS

Applications are invited for a three-year Research Studentship sponsored by A.E.R.E., Harwell, to study the condensation behaviour of cryogenic fluids. A large cryogenic rig has been constructed at A.E.R.E. for this project. Condensation studies will be confined, at first, to nitrogen, but will lead on to binary and multicomponent fluids to model the condensation of LNG in large plant. Successful applicants will be required to register for a higher degree of the University.

Applicants should have a First or Upper Second Class degree in Physics or Chemical Engineering, or equivalent qualification, and should write, together with the names of two referees, to the Academic Registrar, The University, Southampton SO9 5NH. (2379)

RESEARCH STUDENTSHIPS BASIC AND MOLECULAR ANIMAL VIROLOGY
INSTITUTE OF VIROLOGY, THE UNIVERSITY OF GLASGOW, GLASGOW

Two further research studentships for British Nationals will be available from October 1974 for graduates with a first or at least an upper second class honours degree in a biological science who wish to work for a Ph.D. The Institute is well equipped, has a large scientific staff and the current research programme covers the following fields:

•Virus-specified nucleic acids and proteins; genetics and physiology of mammalian viruses; tumour virology; electron microscopy of cells, viruses and nucleic acids; genetics of mammalian cells.

Applicants should send a curriculum vitae and the names and addresses of three academic referees to Professor J. H. Subak-Sharpe at the above address. (2372)

UNIVERSITY OF SOUTHAMPTON
DEPARTMENT OF PHYSICS
RESEARCH FELLOWSHIP

Applications are invited for a Postdoctoral Research Fellowship in Theoretical Physics for an investigation of Gauge Symmetries in High Energy Physics. The precise experience of applicants is not crucial but familiarity with the study of symmetries in Elementary Particle Physics is essential. The Fellowship is tenable for up to two years nine months from October 1, 1974, and the initial salary will be on a range up to £2,223 plus F.S.S.U. benefits. Further particulars may be obtained from Miss J. Hodgson, Department of Physics, and applications should be sent to Mr. D. A. S. Copland, Secretary and Registrar's Department, The University, Southampton SO9 5NH, to arrive by July 5, 1974. Please quote ref: Na237/R. (2377)

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF DENTAL SCIENCES

Applications are invited for a M.R.C. postgraduate studentship for work related to the combined effects of mechanical stress and the physiological environment on the performance of dental and surgical prosthetic materials.

Candidates must possess a First or Upper Second Class Honours Degree in Materials Science or a related subject and the successful applicant will be expected to register for a higher degree.

Applications, together with the names of two academic referees, should be received not later than July 5, 1974, by the Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote reference RV/276064/N. (2385)

THE UNIVERSITY OF MANCHESTER
DEPARTMENT OF BOTANY

Applications are invited for a Postdoctoral Fellowship established under a grant from Country Kitchen Foods Ltd., Churchill, Somerset, for pursuit of research on virus diseases of the cultivated mushroom, as a continuation of work already in progress. The work will be undertaken, in close collaboration with Dr R. R. Frost, in the Cryptogamic Botany Laboratories of the University. Appointment will be for one year from October 1 in the first instance, renewable for two further years. Salary £2,118 to £2,247 to £2,412 p.a. Particulars and application forms (returnable by July 1, 1974) from the Registrar, The University, Manchester M13 9PL. Quote ref: 110/74/N. (2411)

UNIVERSITY OF BRISTOL
ORGANIC GEOCHEMISTRY UNIT
POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited for a Postdoctoral Fellowship to work in collaboration with Professor G. Eglinton and Dr J. R. Maxwell on the lipid-type constituents of recently-deposited aquatic sediments and their contributing organisms, with a view to understanding the distributions which characterise these sediments, the contributions to different sediments and the reactions which alter organic compounds in the sedimentary environment. The research will involve the use of the Unit's computerised gas chromatograph-mass spectrometer system. Previous experience in gas chromatography and mass spectrometry would be useful, though not essential.

The appointment is for one year, and is available from a date to be arranged with the successful applicant, at a salary of £2,118 minimum.

Applications, giving details of age, qualifications and experience, together with the names of three referees, should be sent by July 20, 1974, to Professor G. Eglinton, Organic Geochemistry Unit, School of Chemistry, University of Bristol, Bristol BS8 1TS, from whom further particulars may be obtained. (2425)

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL
DEPARTMENT OF BIOCHEMISTRY
RESEARCH STUDENTSHIP

Applications are invited for a Research Studentship available from October 1, 1974, in one of the following areas: structural studies on cytochrome oxidase; control of ketone body metabolism; effects of drugs and hormones on glyceride biosynthesis. Where appropriate, research will involve collaboration with clinicians.

Candidates should hold, or expect to graduate this summer with, a 1st or upper 2nd class Honours degree in an appropriate discipline.

Applications should be sent as soon as possible to Dr Dils, Department of Biochemistry, The Medical School, University of Nottingham, Nottingham NG7 2RD. For further details telephone 0602 56101, ext. 3229. (2410)

AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited for the following:

RESEARCH SCHOOL OF CHEMISTRY

RESEARCH FELLOWS:

Appointments will be for two or three years beginning in 1975, and may be made in the following areas:

Organic Chemistry: (Professor A. J. Birch, Mr. R. W. Rickards, Dr. J. K. MacLeod): Structure determination and biosynthesis of natural products, including antibiotics and other biologically-active compounds, mould metabolites, and plant and marine products; insect chemistry; total synthesis involving new reactions, organic aspects of organometallic chemistry; mechanisms and results of metal-ammonia and related reductions.

Inorganic Chemistry: (Professor R. L. Martin, Dr. A. M. Sargeson, Dr. D. A. Buckingham, Dr. M. A. Bennett): Synthesis, structure, and reaction mechanisms of a wide range of transition metal complexes. Current interests involve substitution mechanisms at metal ion centres, reactions of coordinated ligands, strategy and stereospecificity in synthesis and quantitative conformational analysis. Interests in inorganic biochemistry include sulphur chelates, iron and ruthenium porphyrins iron transport chelates and the metal promoted synthesis and hydrolysis of small peptides and related substrates. Organometallic complexes, especially those of olefins, carbon monoxide tertiary phosphines and unstable organic molecules such as cyclic acetylenes. Electronic and magnetic properties of paramagnetic materials.

Physical and Theoretical Chemistry: (Professor D. P. Craig, Dr. J. Ferguson, Dr. R. Bramley): Electronic spectroscopy, especially of crystalline solids, U.V. spectroscopy and photochemistry especially of molecular crystals and small aggregates. Transition metal ion spectroscopy, ion pair interactions, exchange effects and magnetic circular dichroism. Intermolecular forces. Electronic properties of molecular crystals. Magnetic resonance spectroscopy, particularly EPR of photo-excited triplets in molecular crystals.

X-Ray Crystallography: (Dr. G. B. Robertson): Structural chemistry of organometallic, inorganic and biologically active organic molecules (with present emphasis on organometallics). Improved methods in X-ray diffraction analysis.

Mass Spectrometry: (Dr. J. K. MacLeod): Isotope labelling and metastable studies of specific fragmentation processes, combined g.c.-m.s. analyses of biological mixtures. Ion Cyclotron Resonance spectrometry involving studies of gas phase ion-molecule reactions to investigate their structures; reactivities and thermodynamic properties.

Analytical Chemistry: (Miss B. J. Stevenson): Micro and submicro metal and anion analyses. The School is non-departmental and is well equipped to contemporary standards, especially in optical, NMR and EPR spectroscopy, mass spectrometry, X-ray, crystallography and cryogenics. There are comprehensive workshop facilities. In computing there is access to the School PDP 11 and the University Univac 1108 systems.

There is no application form. Applicants for these posts should supply a curriculum vitae, list of publications, a statement of research interest, together with two passport-sized photographs and the names and addresses of three academic referees, to the Acting Academic Registrar, Australian National University, P.O. Box 4, Canberra, ACT 2600, Australia.

Closing Date: **15 July 1974.**

SALARIES: Salaries on appointment to the posts will be in accordance with qualifications and experience within the ranges: Research Fellow $A8696-$11901 p.a. Current exchange rates are approximately $A1 : 67NP : $US1.49.

OTHER CONDITIONS: Tenure: Research Fellow normally for three years in the first instance with the possibility of extension to a maximum of five years.

Reasonable travel expenses are paid and assistance with housing is provided for an appointee from outside Canberra. Superannuation (where applicable) is on the FSSU pattern with supplementary benefits.

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants for the post in the Urban Research Unit should write to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF for further particulars before applying. (2445)

POSTDOCTORAL FELLOWSHIP IN MATHEMATICS/ECOLOGY

A Mathematician is required to assist in developing theoretical models of ecological systems and testing them against experimental data. No previous experience in biology is required. The fellowship will be tenable for one year and the initial salary will be £2,280. Enquiries should be addressed to: Dr P. T. Saunders, Department of Mathematics, Queen Elizabeth College (University of London), Campden Hill Road, Kensington, London W8 7AH. (2432)

THE UNIVERSITY OF THE WEST INDIES

TRINIDAD

Applications are invited for the post of RESEARCH FELLOW/JUNIOR RESEARCH FELLOW (ANIMAL NUTRITION CHEMIST), DEPARTMENT OF LIVESTOCK SCIENCE. Applicants should be graduates in Animal Science or Agricultural Science (with a major in Animal Science) with postgraduate qualifications in the nutrition of livestock; an interest in the nutrition of non-ruminant animals is desirable. Salary Scales: Research Fellow: TT$12,612-TT$20,316 p.a. Junior Research Fellow: TT$10,200-TT$11,232 p.a. (£1 sterling = TT$4.8). FSSU Unfurnished accommodation for a maximum of three years will be let by the University at 10% of salary thereafter 20% of salary is payable in lieu of housing. Family passages Triennial Study leave. Detailed applications (six copies) including a curriculum vitae, and naming three referees should be sent to the Secretary, University of the West Indies, St. Augustine, Trinidad. Further particulars for these posts will be sent to all applicants. (2417)

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF GEOLOGY

Applications for a Research Fellowship are invited from candidates with interests in Carbonate Sedimentology and preferably also in Geochemistry and/or ultra-structures. Appointment to date from October 1, 1974.

Salary: £1,929 to £2,223 (under review) F.S.S.U.

Further particulars, including information about advanced research facilities, from Assistant Registrar (S), University of Birmingham, P.O. Box 363, Birmingham B15 2TT, to whom applications (3 copies) with names of three referees should be submitted by July 15, 1974. Please quote reference: NXZ. (2406)

UNIVERSITY OF SUSSEX

SCHOOL OF BIOLOGICAL SCIENCES

BIOCHEMISTRY GROUP

RESEARCH STUDENTSHIP

Applications are invited for an **SRC postgraduate research studentship for studies on the mechanism of hormone action.** Investigations into protein hormone-membrane receptor interaction and adenylate cyclase involvement in hormonal control processes are contemplated.

Applicants are expected to have a first or upper second class honours degree in Biochemistry or a related field.

Applicants with brief Curriculum Vitae and names of two referees to Secretary of Science, Science Office (E), University of Sussex, Falmer, BRIGHTON BN1 9RH (2456)

UNIVERSITY OF ABERDEEN

DEPARTMENT OF ENGINEERING

S.R.C. RESEARCH STUDENTSHIPS

Applications are invited from graduates with good honours degrees in engineering, physics, mathematics or geology for a few Research Studentships. Research groups within the department include those relating to linear electric motors (Professor J. F. Eastham), electronics and control, electro-chemical forming of metal, engineering dynamics, fluid mechanics, geotechnics including ground anchors and mining subsidence and structures. Further particulars may be obtained from the Head of Department (Professor T. M. Charlton, Engineering Department, University of Aberdeen, Marischal College, Aberdeen AB9 1AS), and applications must be complete by mid-July. (2455)

LECTURES AND COURSES

KINGSTON POLYTECHNIC

Graduate Membership of the Royal Institute of Chemistry

A one-year full-time course with next intake in September 1974. Kingston Polytechnic acts on behalf of the R.I.C. as the administrative college for the Part II examination in the Group 10 region.

Enquiries are invited from suitably qualified candidates (Grad. R.I.C. Pt. I or B.Sc.).

MSc (CNAA) In Molecular Spectroscopy

A two-year part-time postgraduate course in chemical spectroscopy with next intake in September 1974.

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One whole day per week.

Further particulars from the Secretary, School of Chemical and Physical Sciences, Kingston Polytechnic, Penrhyn Road, Kingston upon Thames KT1 2EE. 01-549 1366. (2359)

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Topics: Interaction and trends in art and science; Computer in graphics and design; Trends in computer art (audio, visual, tactile); A new language in art; Applicability of theories to computer art; Computer as a creative partner; The sociology of science and art; Aesthetics of the urban environment and problems of civic art.

The seminar is intended for research workers and advanced students.

Early registration (by Sept. 30, 1974) is desirable; applicants will receive background and preparatory material.

Please write, with brief Curriculum Vitae, to: Dr. Vladimir Bonacic, Art and Science, Bezalel Academy of Arts and Design, 10 Sh. Hanagid St. Jerusalem, Israel.

Preliminary information

April 1974 (2440)

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DEPARTMENT OF PHYSIOLOGY

POST-EXPERIENCE COURSE IN IMMUNOLOGY

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For further information and prospectus apply to The Secretary, Department of Physiology, Chelsea College, Manresa Road, London SW3 6LX. (2466)

GRANTS

THE ROYAL SOCIETY GOVERNMENT GRANT FOR SCIENTIFIC INVESTIGATIONS

Applications for grants from the second allotment of the Government Grant for Scientific Investigations for the year 1974 should be made as soon as possible on forms of application to be obtained from the Executive Secretary of the Royal Society, 6 Carlton House Terrace, London, SW1Y 5AG. No application can be considered which is received later than July 31, 1974.

Applicants must be of British nationality, resident in Great Britain or Northern Ireland. Grants may be made for purposes in connexion with the promotion and support of research in pure science other than for personal maintenance or payment of stipends; for the assistance of scientific expeditions and collections; but not in aid of scientific publications. (2366)

THE NATIONAL KIDNEY RESEARCH FUND

invites applications for grants for renal research at units within the United Kingdom. Application forms are available from The Secretary, 184B Station Road, Harrow, Middlesex HA1 2RH (01-863 4460). Completed applications will be required by July 31, 1974. (2454)

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MEETING

NUDE MICE—THEIR HUSBANDRY AND USE

An informal one-day meeting on the husbandry and use of the Nude mouse will be held at the Medical Research Council, Laboratory Animals Centre, Woodmansterne Road, Carshalton, Surrey, SM5 4EF (Tel: 01 643 8000) on Tuesday, July 16, 1974. Applications to attend this meeting (for which there will be no charge) are invited from interested research workers. Numbers will be restricted to a total of 40 people. Copies of the programme are available from Dr. M. Festing at the above address. (2368)

Vth International Biophysics Congress 1975

COPENHAGEN, AUGUST 4-9, 1975

The Vth International Biophysics Congress will be held in Copenhagen from August 4 to 9, 1975. The opening and closing plenary sessions will be concerned with:

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2. Electrical and chemical excitation.

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4. Ionic channels, ionophores, and noise in membranes;
5. The dynamics of macromolecules;
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7. Cell injury and repair processes;
8. Fundamental studies with light and other types of radiation;
9. The assembly and structure of membranes;
10. Bioenergetics;
11. Contractile systems;
12. Structural characteristics and rheology of biological membranes.

On two evenings there will be symposia in memory of Aharon Katchalsky. Contributed communications will be presented at Poster Sessions.

In connection with the Congress an exhibition of scientific instruments will be arranged. Interested manufacturers should apply to the Congress Secretariat.

Those wishing to attend the Congress should apply for pre-registration forms to:
Vth International Biophysics Congress
c/o DIS Congress Service
3 Knabrostraede
DK 1210 Copenhagen K
Denmark

The deadline for pre-registration is August 15, 1974. Since the number of hotel rooms available in Copenhagen is limited, late applicants may have to find their own accommodation.

The number of participants will be limited to 2,300 active participants, 200 students and 500 accompanying family members. Priority will be given to persons who have first sent in the pre-registration form and fee.

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(2335)

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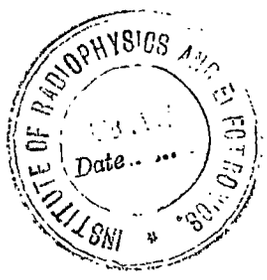
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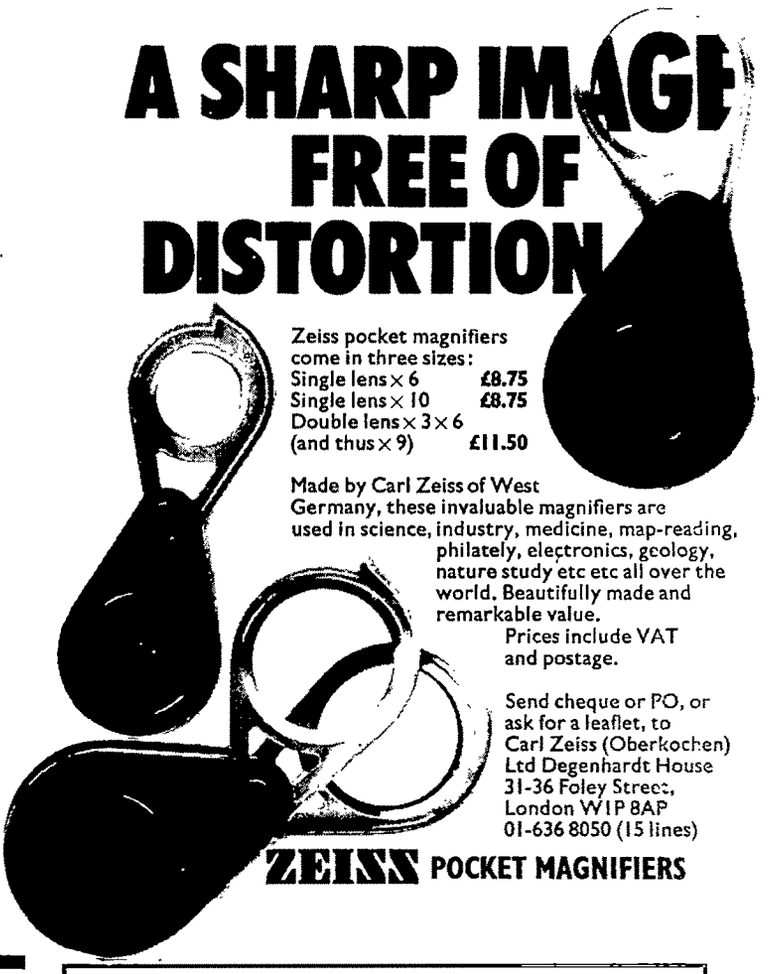
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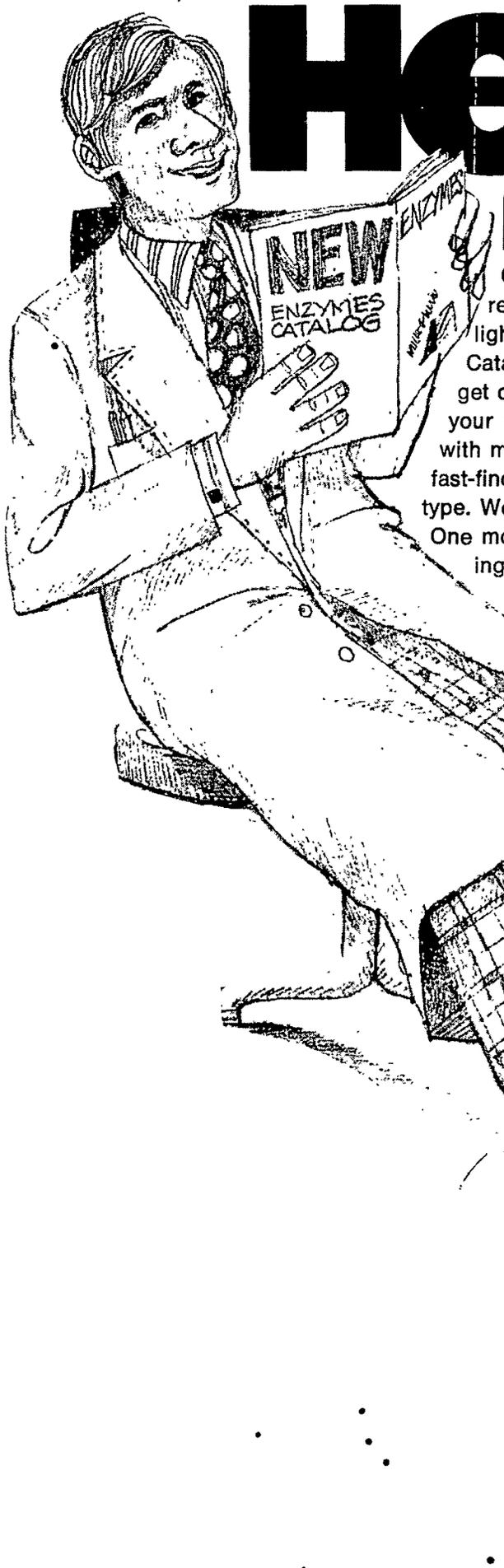
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A fuller guide appeared in *Nature* (**246**, 238; 1973).

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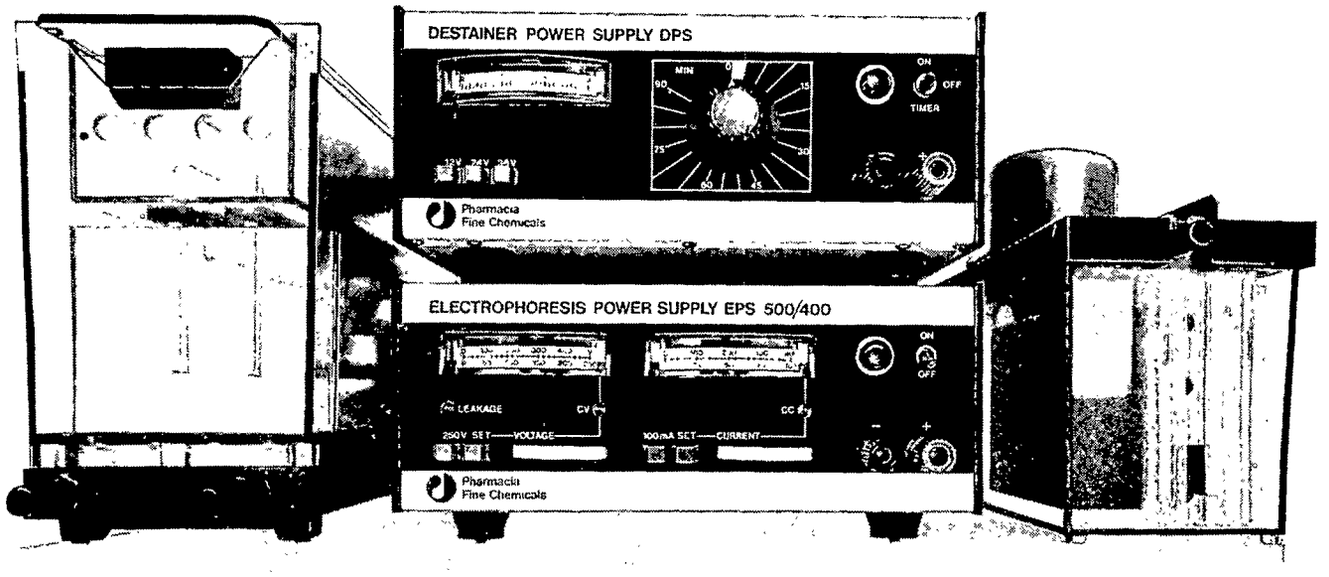
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Rising tide of energy nationalism

THIS issue of *Nature* is devoted largely to energy questions as seen by the scientist, from the whisky distillery in Scotland to the cattle dung plants of agricultural India. We cannot claim to be comprehensive in our coverage, but since it is not entirely unfashionable these days to devote large portions of journals to energy considerations it is unlikely that by half way through 1974 anyone who reads at all could claim that he lacked adequate briefing on almost any aspect of the energy situation. And yet it may be that although one can emerge convinced that there are large numbers of competent people in many countries exploring every avenue and leaving no stone unturned, there is still something naggingly wrong with the utterances of policy makers. "Self sufficiency by the 1980s", "indigenous resources", "national energy research strategies". Is not this talk the distant rumblings of a nationalism in science and technology that could make energy as isolated a field to work in as defence?

For some time, of course, marketing of energy has fostered nationalism through the possibilities of economic warfare, and the events of last winter must presumably be called an energy skirmish if nothing else. It is inevitable that countries which have traditionally disliked each other will now use energy as a weapon with no more hesitation than they have used blockades in the past. That is hardly a matter for debate. What is debatable is whether the remarkable nationalism that the energy crisis has engendered even amongst allies can do anything but harm to world order, and lead to tensions where previously there were none, and unnecessary duplication of activity where previously there was collaboration.

The disturbing thing for a scientist is not just that the possession of energy should be viewed in a national framework but that research itself into all aspects of energy should also be seen as too exclusively a national property. For instance, is it rational to talk of a programme to make the United States independent of the external world in energy matters when it shares thousands of miles of frontier, one common language and many similarities in life-style with Canada? Is the great nuclear reactor debate in Britain one which is too polarised into Britain against the United States. Is it further, one which should have been conducted at an international, or at least, a European level? If brain power as well as energy were regarded as a valuable resource the answer to all these questions would be yes. The giant oil companies have been an interesting example of supranational research and development; it would be unfortunate if with the decline in their influence this mode of operation were to be lost entirely, when it could with profit be used as a model for others.

If narrow interests restricted by geographical frontiers are to be overcome it is urgently necessary that international initiatives be pursued with great vigour. For this reason a proposal of Lord Robens on page 699 is worthy of attention. He suggests the formation of international energy research projects for such matters of common interest as solar energy. It is only around fairly specific objects that international research collaboration is likely to gel, and this as much as anything may be the reason the scientific community has been almost silent on the Kissinger initiative to internationalise energy concerns.

Solar energy would be an ideal project if only because of its world-wide applicability. As Dr Brinkworth points out on page 729, research and development in solar energy could also be seen as a means of giving aid to developing countries. What is more, the equipment need not be so complex that it is impossible to build and operate except in high technology societies. This too is a subtle consequence of too much national research policy; that the device only works when its maker is on hand and consequently it proves impossible to export. Further, if the work is removed to a government laboratory the economic need for an exportable product may be less to the fore and the formal obstacles to international collaboration much more extensive.

The dangers of nationalism in energy research are great. The antidote to them is not to be found in international committees of bureaucrats but in grass roots action by scientists themselves working across frontiers.

100 years ago



Proposed Issue of Daily Weather Charts of Europe and the North Atlantic

I HAVE the honour to inform you that Capt. Hoffmeyer, Director of the Royal Meteorological Institute of Copenhagen, has sent me a circular announcing his intention to publish daily charts of the weather for the district from 60° E. to 60° W. long. and from 30° to 75° N. lat. The charts for the three months—Dec. 1873-Feb. 1874—will be published as an experiment.

The cost will be four francs per month, exclusive of postal charges.

Capt. Hoffmeyer states that he can only deal with central offices, and has requested me to undertake these islands as regards the distribution of the charts. I have therefore to announce that I have been instructed by the committee to subscribe for twenty-five copies of these charts, and I shall be happy to supply copies for the three months to any gentleman, at the cost of 11*s*. to cover carriage from Copenhagen, and postage from London to his address. ROBERT H. SCOTT, *Director*

Meteorological Office, June 22

From *Nature*, **10**, 146, June 25, 1875.

Sealing wax and string at $250 million

Miranda Robertson discusses the contribution that the Fermi National Accelerator Laboratory (pictured at bottom) is making to high energy physics.

THE dedication last month of the National Accelerator Laboratory (FNAL) to Enrico Fermi was a tribute to the man and an acknowledgement of the traditional link between the Chicago laboratory which saw the first nuclear chain reaction and the laboratory at Batavia, 35 miles west of Chicago, which now houses Fermi's original Chicago cyclotron (demoted to muon focussing) as well as the world's most powerful proton accelerator.

But historical links apart, the laboratory must stand as a monument to its director Robert Wilson, one of many ex-colleagues or students of Fermi now working at Batavia. When he agreed in 1967 to undertake the task of constructing the machine, Wilson brought to an already controversial project not only the experience he had gained in building the Cornell accelerator, but his own controversial approach—a latter-day, $250-million version of the sealing-wax-and-string tradition combined with a willingness to take a venture on the chance of greater dividends.

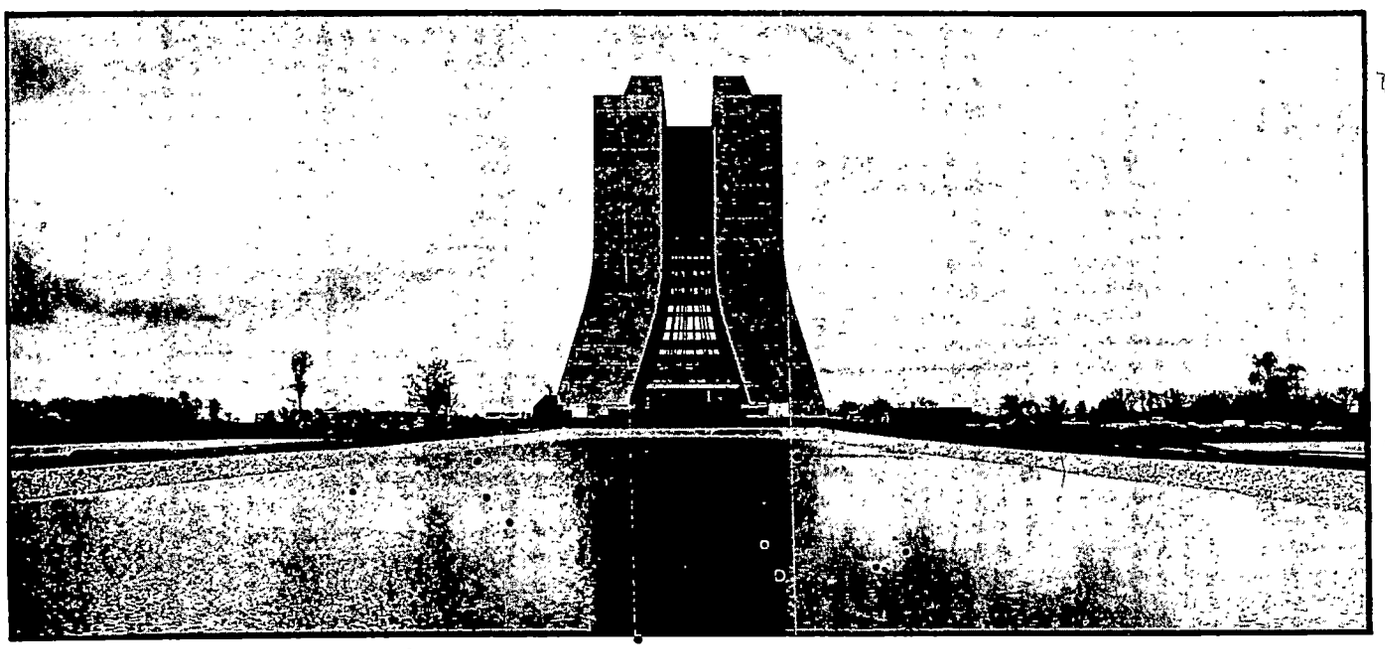


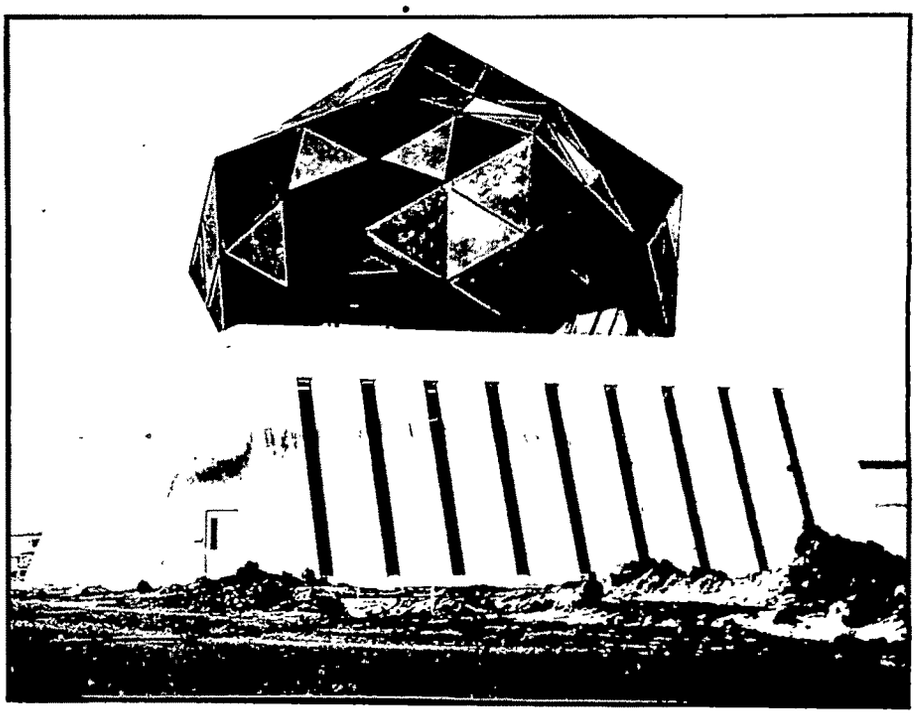
Robert Wilson: controversial

The commitment of funds to so expensive a project was a problem from the start. Wilson's achievement has been to do more than was planned with less than was hoped: to build a 500-GeV accelerator instead of a 200-GeV one with an initial commitment of $250 million instead of $350 million. A second source of early contention was the siting in America's middle West of what was to be an important international research centre. The geodesic dome of the bubble-chamber assembly building, the curved and corrugated roof of the meson area, and the main building with its trapezoid tower and ground floor forest are all part of a conscious attempt to mitigate the otherwise somewhat featureless Illinois landscape and avoid the standard laboratory concrete block.

Wilson's brave innovations have created problems of their own. One of the least of these is the leaky roof of the meson building, actually made of sections of sewer; what is adequate to contain sewage, it turns out, is not adequate to withstand wind and rain. More serious problems connected with the functioning of the machine itself are candidly documented by Wilson in a recent article (*Scient. Amer.*, **230**, 72; 1974) tracing the progress of the project from its inception until pretty well the moment that the article went to press, when the situation was still changing. At that time, infuriatingly for the scientific community, the proton beam was only operational for about half the time. Moreover, operation at more than 300 GeV threatened to become a source of grievance to the local community by causing a 1% voltage drop in the Commonwealth Edison power lines which feed the machine.

By the time of the dedication, however, the voltage drop had been reduced to within an acceptable 0.5% by means of further capacitors and the accelerator was running at 485 GeV. Better still, it is now operational most of the





The geodesic dome at FNAL: red, white and blue fibreglass panels containing 120,000 'beverage cans' donated by the public.

time and the beam intensity has shifted up from 6×10^{12} to 1×10^{13} protons per pulse close to the goal of 5×10^{13}.

Not that either the teething troubles of the laboratory or its tradition of bold innovation are now at an end. Within days of the dedication ceremony, a new transformer inexplicably failed, setting the machine back to an upper energy limit of 300 GeV for most of the summer. On the other hand, a project budgeted at between $4 million and $9 million is under way to test the feasibility of doubling the beam energy yet again. Wilson's path to the tera electron volt would be through superconducting magnets (more new technology), possibly housed within the existing tunnel (more penny saving). So far, because of the technical uncertainties, plans extend only as far as a protodoubler, to be inserted into a small section of the ring through which part of the beam could be run. Experience with the protodoubler should make it possible to gauge the problems of construction, refrigeration and installation of the superconducting magnets, as well as the possible damaging effects of radiation.

Superconducting magnets also enter into plans, still awaiting proton beams and one electron beam approval, for three intersecting storage rings to contain two, and known as POPAE: protons on protons and electrons. But meanwhile, important new phenomena in high energy physics are emerging at an alarming rate from other laboratories, in particular CERN in Geneva and SLAC at Stanford where the last year has seen the first evidence for a unified theory of the weak and electromagnetic interactions (*Nature*, **245**, 119; 1973), and the quark in a curiously paradoxical position—strongly supported by some recent results and equally seriously threatened by others. At this exciting and critical phase in fundamental particle research and with only two years to go before the new CERN accelerator achieves comparable energies, what has FNAL, as the principal sink for high energy funds in the United States, to offer?

The answer lies in the range of high energy secondary particles produced by the collision of the proton beam with its target. FNAL neutrinos and mesons come out at higher energies than those at CERN, and the Batavia synchrotron is the only source of really high energy muons, whose existence as a kind of massive electron is one of the outstanding puzzles of fundamental particle physics. Colliding beams, which have produced all the surprises, produce secondary particles of only relative low energy.

Many of the most recent results promise to support the efforts of theoretical physicists to find a unified explanation of the four physical forces, and to tie together within some fundamental relationship the embarrassing plethora of particles that comes hurtling out of the nucleus when it is hit with particles of energy more than a GeV or so. But some of the very latest threaten to leave them gasping. FNAL should be in a unique position to fill in the emerging picture in three areas.

The first is that opened up by CERN experiments with a neutrino beam on a stationary target of liquid freon in the giant bubble chamber Gargemelle, where the apparent observation of neutral currents represented evidence for the unity of the weak and the electromagnetic force, as proposed by Steven Weinberg and Abdus Salam. FNAL, with its much higher energy neutrino beam, has already corroborated the CERN result (*Nature*, **249**, 211; 1974) and should now be able to contribute a more quantitative understanding of the phenomenon.

Preliminary data should soon be available on a second question, raised this time by CERN's proton-proton colliding beams. Quite unexpectedly, the interaction probability (or cross section) of the protons, instead of remaining constant, turned out to increase with increasing energy. Investigations at FNAL with other kinds of strongly interacting particle, which seem to be subject to the same non linearity, will concentrate on the problematic transition energy between constant and rising cross section.

The third question is that of the structure of the hadron: are all the strongly interacting particles made up of combinations of two or three quarks, according to the theories independently developed by Zweig and Gell-Mann? Both the neutrino scattering experiments and the colliding beams of protons on protons say yes, they could be, although the existence of internal structure in the hadron has to be inferred from the distribution of the emerging particles: no force has yet succeeded in ejecting a quark from its nucleon. Both high energy neutrinos and muons are available at FNAL for probing the interstices of the proton in more detail than before, and at a time when experiments with colliding beams of electrons and positrons at SLAC have produced results which stretch quark theory to the limits of its plausibility. The simplest version of the theory (and the pursuit of simplicity is the vocation of the theoretical physicist) predicts a constant ratio of emerging hadrons to muons as collision energy rises. But the SLAC experiment revealed a hadron/muon ration that increased with energy with no sign of levelling off at the highest reached. Some increase can be accommodated in more complex versions of quark theory, but if the curve goes up any further it will leave even the most elaborate quarks behind.

FNAL should be able to provide the answers to some of the new questions posed by the current spate of exciting and baffling results, and take its place as a focus of scientific, rather than political or administrative, controversy.

international news

Moscow seminar under attack

from Vera Rich

THE latest development in the preparations for the "underground" Moscow seminar on Collective Phenomena and the Applications of Physics to Other Fields of Science' scheduled for July 1–5, 1974, has been threats of prison and (or) exile for the 'host', physicist Aleksandr Voronel, and military conscription for a number of participants.

In a statement made by telephone and addressed to the sponsors of the Seminar, the American Academy of Physics, the Royal Society, the association of scientific workers of France, Italy, Germany and other countries, and to the International Society of Jurists, Voronel said that on the morning of June 8, he was "taken by force" by agents of the KGB (in civilian clothes) from near his home and conveyed to the 43rd department of the militia (civil police), where "two hours later, Lt. Col. Anfisov read out to me a warning in the name of the State Security organs", in terms of the decree of the Supreme Soviet of December 25, 1972. This decree has never been published, nor was the text of it shown to Voronel. The warning, however, consisted of the following three points: the seminar is a "provocative measure, pursuing anti-Soviet aims"; holding it will be "in violation of the order existing in the USSR"; and Voronel's actions in promoting the seminar will fall under statute 74 of the criminal code of the Russian Federative Republic: "incitement of national emnity".

This is the first time that so serious an accusation has been levelled at an individual scientist. Previous harassment of Jewish scientists dismissed from their posts as a result of applying to emigrate to Israel has either consisted of charges of "parasitism" (of not being gainfully employed) or else of offences not directly related to their predicament (as in the case of physicist Viktor Polskii, charged with a serious driving offence).

Although Voronel, as the 'host' for the seminar is obviously the prime target, he is not the only participant personally threatened. Three members of the organising committee: Viktor Brailovskii, Grigorii Rozenshtein and Dmitrii Ram, who are under 40 years of age (the upper limit of liability to conscription) and also two intending participants M. A. Mikulinskii and a certain Khaits (no forename available) have been ordered to report for military service. Although their failure to comply with the order renders them liable to criminal prosecution, the conscriptees (whose academic exemption is assumed to have lapsed when they lost their jobs) are, in the long-standing tradition of those wanted by the KGB or its Imperial predecessors, "hiding in the countryside" until July 1 when (if not apprehended in the meantime) they will return to Moscow for the meeting. Mrs Irina Brailovskaya (herself an intending participant) and Mrs Rozenshtein are being subjected, like Voronel himself, to close surveillance by the KGB, in the hope of discovering the whereabouts of the missing men.



Voronel: threatened

Last Thursday it was reported that another committee member, cybernetici**s**t Aleksandr Lunts, had been arrested. The Scientific Council of the Institute of Solid State Physics of the Soviet Academy of Sciences has officially censured the participation of Professor Venyamin Fain and Professor Yakov Yosilevskii for their membership of the Organising Committee. Yosilevskii, having already resigned from his post at the Institute, could not be threatened with dismissal; Fain, however, after refusing an order to withdraw from the seminar, was formally reprimanded, which is normally the first step to dismissal. In spite of this pressure campaign, which, following his release, Voronel, in a telephone call to a contact in Israel, described as "unprecedentedly rough treatment", the Committee and the participants from the Soviet Union are still prepared to go ahead.

This harassment of the scientists seems to indicate that the preliminary attacks on the seminar (see *Trud* and *Sovetskay Kultura* of May 17, 1974), which attempted to present it as a "provocational attitude" initiated by the University of Tel Aviv have had little impact, except, as Voronel wryly commented, to give it unhoped for publicity in the Soviet press. In fact, the whole concept of the seminar originated in Moscow, as an almost spontaneous by-product of the summary dismissal from their posts of Jewish scientists who wished to emigrate to Israel. In 1973, a number of scientists who had been sacked in this manner were prevented from presenting their papers at an International Physics Conference in Moscow, They succeeded, however, in making the fact known to the foreign

delegates, and an open invitation was issued to attend an unofficial session in Dr Voronel's apartment, to hear and discuss the banned papers. In all, some 40 delegates attended this meeting, sitting tightly packed upon the floor.

Since that time, the dismissed scientists, in order to foster and preserve some sort of scientific environment for themselves, have been holding regular Sunday seminars. Although representing a wide range of disciplines, from Cybernetics to Sinology, the meetings have, in fact, proved a fruitful source of discussion and interdisciplinary understanding (far more so, perhaps, than the official type of conference in the Soviet Union which, after one or two "plenary" sessions, normally fragments into a number of narrow working groups meeting simultaneously so that virtually no interdisciplinary exchange is possible).

Although a second seminar was later instituted, under the leadership of Dr Grigorii Rozenshtein, dealing with the life sciences (Voronel's original seminar contained a preponderance of mathematicians and physicists), the idea of interdisciplinary exchange has remained a cornerstone of the concept of these seminars. Accordingly, the International Seminar planned for July is not, therefore, in any way an outside interference in the activities of Soviet science. Rather is it an invitation from a group of dismissed scientists—who are, incidentally, only exercising the "freedom of speech" and "freedom of assembly" guaranteed to them by articles 125–127 of the Soviet Constitution of 1936—for their colleagues abroad to participate in a special 6-day session of the seminar. The three international secretaries of the meeting, Professor Edward Stern of the University of Washington, Dr Norman Chigier of the University of Sheffield, and Dr Raymond Orbach of the University of Tel Aviv are in no way the organisers—they are, as it were, the intermediaries between the would-be participants abroad and the committee in Moscow. Consequently, in spite of the fears expressed by certain scientists abroad for the safety of their Soviet colleagues, the international secretaries cannot take it upon themselves to cancel the seminar—only Voronel and his colleagues in Moscow could take such an action, and this they are resolutely opposed to doing.

In spite of the increasing pressure, which Voronel himself attributes to the "unfortunate" coincidence of the Nixon visit with the date of the seminar, which, he says, has given the authorities an added incentive and "justification" for taking the meeting as a provocation, at the time of going to press, plans are still going ahead. Certain counter measures to the Soviet pressure are in preparation—the submission of letters signed, respectively, by the international sponsors of the seminar and by leading physicists to Dr Philip Handler of the National Academy of Sciences of the USA and Professor W. K. H. Panofsky of the American Physical Society, urging that the bodies they represent should give appropriate consideration to the situation. A conference on high energy physics, to be held at Imperial College, London, at the same time as the seminar, will be asked to express its solidarity and sympathy. The possibility of formal telegrams to Mstislav V. Keldysh, President of the Soviet Academy of Science, has also been suggested.

Meanwhile, the submission of papers continues: some 120 having been submitted so far, more than 75 of which are from the United States, and including such names as R. A. Dwek (Oxford), D. W. Sciama (Oxford), George Wald (Harvard), John Ziman (Bristol), Sergio Ricossa (Turin), D. Bargeton (Paris) and Peter Hemmerich (Konstanz); the subjects cover a wide range from theoretical cybernetics to medicine, from cytology to the theory of education, and from Marxist sociology to theology.

The intending participants from abroad have made the necessary applications for their visas, and, at the time of going to press, are still awaiting an answer. In the meantime, scientists attending the "official" physics conference in the Soviet Union at the end of June have been invited to stay on for the seminar, as happened last year. In Moscow, the organising committee, under increasing pressure, continue their preparations, and, it is understood, have arranged for alternative venues, should Voronel's apartment become unavailable at the last minute. Although the final outcome remains uncertain, it is clear from the attitude of the organisers, both at home and abroad, that should the International Seminar fail to materialise, it will not be due to any slackening of efforts on their part.

Israelis hope for Mediterranean oil

Nechemia Meyers, Rehovot

DRILLINGS in the Mediterranean may soon bring the promise of oil self-sufficiency to Israel. Preliminary surveys of the sea bed off Israel's coastline—at the edge of, or just beyond, the continental shelf—have turned up geological structures which may yield oil, according to a statement made last week by the head of Israel's Oceanographic and Limnological Research Company, Yohai Ben-Nun. Ben-Nun, former OC of the Israeli Navy, was reporting on surveys made by his company in cooperation with the Marine Geology Unit of the Geological Institute.

Advanced seismic equipment has already been acquired, added the Admiral, for more exact investigation of these structures and for pinpointing possible drilling sites. While not guaranteeing that oil would be found, he described the anticlinal structures as "very promising indeed". Some of the sites are at depths that make exploitation difficult, given present-day technology, but new techniques being developed elsewhere in the world should make drilling economically and technologically feasible in a few years time.

Meanwhile, plans are going forward for drilling on the continental shelf and on the mainland. Dr Zvi Dinstein, who is in charge of Government oil operations, has announced that IL25 million (£2.5 million)—most of it private capital—will shortly be invested in probes along and just off the Coast Plain from Netanya to the Lebanese border. Surveys are also going on further to the south, particularly in the Gaza Strip area.

Considerable differences of opinion exist in regard to the possibility of striking oil around the Dead Sea. A group of foreign and local investors is reportedly willing to invest millions in drillings there, because, they believe, the asphalt found in the area indicates the likely presence of oil. Other observers—and geologists—think the asphalt only shows that oil was there, but that this oil has 'migrated' away.

Israel hoped that it had entered the oil era 20 years ago. For in September 1955, regular broadcasts were interrupted to announce that oil had been struck in the Lower Cretaceous sands at Heletz, 35 miles south of Tel Aviv and seven miles east of the Mediterranean coast. Special songs were composed in honour of the occasion and stocks of local oil companies shot sky-high. But production did not live up to expectations. In 1965, at its peak, Heletz and the surrounding fields yielded 202,000 tons of crude oil. Today production is only one-quarter of that figure.

The Six Day War brought Israel access to the Abu Rodeis oil fields in Sinai, which yielded 4.5 million tons in 1973, as against 5.3 million tons in 1972. A further pull back of Israeli forces could cut off that supply of oil altogether, a fact which lends special urgency to drillings in 'old Israel'.

Dear Russell . . . Dear Dixie . . .

by Colin Norman, Washington

A COUPLE of months ago, the Atomic Energy Commission (AEC) received a rude shock from a sister agency in the federal government when the Environmental Protection Agency published a blistering critique of some of the AEC's key justifications for pushing ahead at breakneck speed with the breeder reactor programme. EPA's temerity in questioning some of the arguments made in support of the most important energy research and development programme in the United States evidently hit a raw nerve in the AEC for it has prompted a frosty letter from AEC chairman Dixie Lee Ray to EPA administrator Russell E. Train, and a polite but equally frosty defence of EPA's actions in a reply from Train to Ray.

EPA's critique, which was published on May 4, tore to pieces many of the arguments put forward by AEC in a draft report on the environmental impact of the breeder reactor programme, and EPA gave the report the lowest possible rating of "inadequate". AEC was under court order to put the draft report into final form by June 14, but following EPA's criticisms, AEC sought and received an extension of the deadline. A final environmental impact statement is now expected sometime late in the summer or early autumn.

The dispute between the two agencies could later play an even more important part in the fierce battle now being waged over the breeder reactor, however, for EPA's criticisms may provide the basis for legal action to delay or block the programme by nuclear critics if AEC's final impact statement fails to respond adequately to the points raised by EPA.

But what seems to have offended AEC officials in particular is the manner in which the criticisms were delivered. According to Dixie Lee Ray's letter, the criticisms came as a shock because the two agencies had previously agreed to cooperate in preparing the draft environmental impact statement, and EPA officials had given "repeated telephonic assurances throughout (the) drafting period that there were no problems" with AEC's analyses. "We cannot understand the way in which EPA handled its recent comments" Ray said, and added that "surely there are more constructive ways of handling this and similar situations".

Train replied that although he shares Ray's concern that the two agencies should work constructively together, effective coordination in producing the statement proved to be impossible, and he said that when differences of opinion arise on impact statements, "our official comments must and will identify these issues and EPA's concerns". In other words Train made it perfectly clear that when his agency believes that another agency in the federal government has produced a poor environmental impact statement, EPA will continue to speak out.

Because of the importance of the fast breeder reactor programme—it is the largest single energy research and development programme in the United States and it will provide the keystone to the entire nuclear power programme in the 1990s and beyond—officials of the AEC, EPA and the White House Council on Environmental Quality met in September last year to try to ensure that the drafting stage of the impact statement would proceed as smoothly as possible. It was agreed at that meeting that AEC would provide EPA with draft copies of chapters of the statement as soon as they were written.

But, according to Train's letter, although AEC supplied EPA with material beginning in December last year, the three most controversial aspects of the statement—those dealing with plutonium toxicity, safety and cost-benefit analysis—were not completed until February. As far as the cost benefit material was concerned, EPA in fact received no draft at all. Thus EPA had little or no input into those parts of the statement, and as it turned out they were the most heavily criticised parts. Train also pointed out that in the September meeting last year EPA officials identified 14 topics which should be discussed in the impact statement, but only two were addressed in full and five were addressed in part.

Although AEC's pique and EPA's defence of its actions represent little more on the surface than a bureaucratic squabble, they do underline a couple of important points. The first is that with the White House beleaguered by calls for President Nixon's impeachment and resignation, federal agencies are taking an idependent line on many issues which would normally be under tight White House control—the EPA, for example, has been taking a stand against several administration policies and several top administration officials have even said in public that they have no fear of White House pressures any more. And the second point is that EPA's independent stand on the breeder reactor statement, which would have been much more difficult in pre-Watergate days, is a vital part of the procedure laid down by the National Environmental Policy Act for ensuring that environmental concerns are taken fully into account in decisions made by the federal government.

Test ban soon?

MR BREZHNEV has now indicated that the Soviet Union is prepared to discuss a ban on underground nuclear weapons tests when Mr Nixon visits Moscow in late June. This together with the continued interest in Washington makes some sort of agreement, even if only an agreement to agree, a near certainty this summer. A treaty is likely to take the form of a threshold agreement in which tests are forbidden only if they produce a seismic signal larger than a specified value. This concept has been strongly criticised in recent weeks; the sort of value being talked about would permit explosions of up to fifty kilotons to be fired if appropriately muffled. A large majority of all tests fired at present are below that level of yield, so the threat to a weapons programme of a threshold treaty could be modest. The speech of Mr Brezhnev does however mention the question of a timetable for phasing out weapons tests entirely, an idea that will be much more difficult to sell to the United States. It will be interesting to see whether the Soviet Union insists on such an addition to any treaty that is signed, as it is potentially embarrassing to the United States and could endanger the talks.

Nuclear safeguards

STUNG by a series of public criticisms of the adequacy of its safeguards against the theft of nuclear materials, the Atomic Energy Commission has elevated nuclear security to a higher position in its bureaucratic hierarchy. The move is, however, unlikely to satisfy the AEC's critics in Congress who are pushing ahead with a bill to force the commission to pay much more attention to nuclear safeguards than it has in the past.

The move consists of amalgamating the present Division of Nuclear Security and the Division of Nuclear Materials Security into a single entity whose job will be to prevent the theft of weapons-grade plutonium and enriched uranium from AEC facilities and in transportation. Critics have long argued that responsibilities for preventing nuclear theft have been fragmented, and so the reorganisation at least helps to answer some of the complaints.

But it falls far short of recommendations contained in two recent studies of the AEC's safeguard regulations, and it also addresses only part of the safeguard problem, for the new division will be responsible only for

Anti-revisionism hits Scientia Sinica

THE attacks on Lin Piao and Confucianism in China which began in August 1973 have now spilled over into the pages of *Scientia Sinica*, the distinguished English-language journal of Chinese science published in Peking. In the most recent issue (Vol. 17, No. 2) alongside papers on a Markov description of stationary turbulence, graptoloidea and graptodendroids and pathogens of rice black streak dwarf disease is a section entitled 'Criticising Lin Piao and Confucius; combating and preventing revisionism'. Three testimonies are presented of the absurdity of Confucius' saying 'only the highest who are wise, and the lowest, who are stupid, cannot be changed'. Lin Piao, at one time Mao's Defence Minister and widely believed to have been killed in a plane crash in Mongolia in September 1971 whilst attempting to flee to the Soviet Union, is accused of following Confucius and 'twaddling that knowledge was endowed by nature' and 'slandering the working people as philistines . . . only caring for oil, salt, sauce, vinegar and firewood'.



"Many hands make mercury vapour light work . . ."

The first paper, uncompromisingly entitled '"The Lowest, who are stupid" are the wisest while "The Highest, who are wise" are the most stupid' is by Tsai Tzu-chuan of the Laboratory for Electric Light Source, Shanghai. He was exercised in the early 1960s by the imperialists who gloated over the Chinese incapacity to make a high-pressure mercury vapour lamp. However his group started from scratch and within half-a-year had delivered the goods. This success greatly heightened the morale of the proletariat and smashed the imperialist slander against China: 'advanced architecture but backward lighting'—which, it must be admitted, isn't heard too often these days. One in the eye for Lin Piao who wanted to restore capitalism by subjugating the working class to his fascist rule. As Chairman Mao put it 'Lifting a rock only to drop it on one's own feet is the behaviour of a reactionary fool'.

Next Yao Shih-chang of the Tuanchieh Production Brigade, Shantung, relates how Mao's saying that 'correct ideas come from the struggle for production, the class struggle and scientific experiment' is illustrated by his quest for growth of the peanut. His first attempt to raise the yield of his own village's crop of peanuts by importing the methods of a neighbouring village actually decreased the yield and he 'got snubbed'. Next year however he studied the cycle of growth more closely—'I was, so to speak, living together with the peanuts'. Eventually by removing earth round the basal parts of peanut plants he found he could raise the yield 25%. This he concludes is the result of not fearing hardships, being bold to practise and ready to get to the bottom of the matter. Lin Piao would not have encouraged this as he thought that the working classes cared only about money, and would have restored landlords. But now millions are clapping hands for joy, so 'let the reactionaries at home and abroad bark what they like.' Finally, Chao Pu-hü, a 'worker-peasant-soldier student' at Kirin Medical College describes his transformation from 'half-baked medical orderly' to 'people's medical officer'. He worked in a deaf-mute clinic and was intrigued by the failure of acupuncture in this field. The books said that there were forbidden zones, and that needles should only be inserted a restricted amount. Chao Pu-hü perceived this as akin to the 'idealist apriorism of Liu Shao-chi, Lin Piao and other swindlers' and proceeded to experiment on himself with prodigious lengths of insertion. This warmed his throat up so he applied the same to a girl Wang Ya-chiu. Three days of the treatment and she called out, for the first time in her life, 'Long live Chairman Mao!' Thus emboldened, he and his colleagues have cured more than 3,000 deaf-mutes in the last five years.

His last colourful words are—'Now those who had tried to check the advance of history as mantis shanks would a carriage were ground to pieces by the wheel of history, yet their reactionary ideology still stinks. We will thoroughly criticise their reactionary world outlook and carry the socialist revolution in the realm of the superstructure through to the end!' And so, on the next page, to a homogeneous denumerable Markov process.

nuclear material contained in AEC facilities, while the AEC's Regulatory division will retain responsibility for safeguarding nuclear material held by the nuclear power industry.

The reorganisation is thus unlikely to disrupt a move, spearheaded by Senator Abraham Ribicoff, to place nuclear security on the same bureaucratic level as the safety of nuclear power plants and to force increased security within the nuclear industry. The move, in the shape of an amendment to a bill designed to split up the AEC into a Nuclear Safety and Licensing Commission and an Energy Research and Development Administration, would create a top-level bureau concerned solely with nuclear safeguards. The bill has now been passed by the Senate Government Operations Committee and it is awaiting full Senate action.

Nature conservation

THE Nature Conservancy Council, having devoted the first months of its existence to internal reorganisation and the task of disentangling itself from the arms of the Natural Environment Research Council, is now ready to turn its attentions outwards again.

At a meeting in London recently, the Director, Mr R. E. Boote, outlined the aims of the 'new' Nature Conservancy. According to the Nature Conservancy Council Act of 1973 the NCC is now required to provide advice to the government on the development and implementation of all policies for or affecting nature conservation in Great Britain. This has enormous implications for the NCC which they intend to take very seriously, now that they are an independent body.

Mr Boote promised that they would not hesitate to make public advice given to the government and disregarded when they felt that circumstances warranted such disclosure.

The NCC continues to manage the 300,000 acres of nature reserves under its control but has relinquished its research stations to NERC. The NCC now commissions the necessary research, mainly from NERC, on the Rothschild customer/contractor principle. When asked whether conflict might arise between the research carried out for the government for NERC and that commissioned by the NCC, Mr Boote replied that as they had complete control over the commissioning of their own research they could always go elsewhere. The NCC is at present financed by a parliamentary grant which this year has been "sufficient".

correspondence

Earth scientists

SIR,—Sir Peter Kent, Sir Kingsley Dunham and Professor Percival Allen charge (*Nature*, June 14) that my article of April 26 was "erroneous and misleading" and "appeared in *Nature* without an attempt to check the facts". They are quite wrong; indeed in their letter they commit the very sins of which they choose to accuse me, not the least of which is to put into quotation marks an inaccurate version of a phrase I actually wrote.

In my original article I claimed that the General Secretary of the European Geophysical Society (EGS) discovered the planning of the Reading meeting of European geological societies "more or less accidentally"—a claim that Sir Peter and his colleagues attempt to refute by pointing out that the EGS General Secretary was specially informed of the meeting by its organisers. Indeed he was; but the fact is that the meeting had first come to his attention (more or less accidentally, as I said) more than two months before the 'official' information was received. Sir Peter and his cosignatories also point out that the EGS President was sent a "special letter" inviting EGS members to participate in the Reading meeting and soliciting topics for discussion. A letter was indeed sent, although in view of the fact that it bears a remarkable resemblance to other letters sent elsewhere, its "special" nature may be doubted.

The most significant aspect of the letter to the EGS President, however, is that the contents of the letter are precisely as Sir Peter and his colleagues describe them—which suggests that the basic point of my article has been missed. No one has suggested that EGS members have not been invited to, or would not be welcome at, the Reading meeting. But what is being suggested is that the organisers of the Reading committee have gone ahead with the first stage of what may well develop into a Geological Society of Europe (GSE) without first consulting the governing bodies of the European earth science societies already in existence, most notably the Council of the EGS.

Sir Peter and his colleagues presumably felt that such consultation was unnecessary. Others, including myself, disagree. For reasons which are too complex to go into here, in both Britain and the United States (and possibly in other countries too) geologists and geophysicists have come to be associated with different organisations. It is perhaps now too late to change that situation; but in my view it would be a tragedy if a similar sort of separation were to be perpetuated on a European-wide basis. This does not necessarily imply the need for an all-embracing European Earth Science Society, for, as I pointed out in my original article, the problems involved in running such a Society would be almost insurmountable. But what it does mean is the need for close coordination. Geologists may deplore it, but in view of the fact that the EGS already exists the onus must be on anyone thinking, however remotely, about a GSE, to take special care to avoid harmful separation. In return, I feel sure that existing organisations will see the need to modify their own programmes accordingly. In any event, true coordination is much more than simply sending letters announcing a conference.

Sincerely yours,

PETER J. SMITH

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Kidney transplants

SIR,—The article "Transplantation: the failing machinery", (*Nature*, April 19, 1974) I thought, represented the kidney donor problem in this country very accurately and unfortunately its comments were confirmed by Dr Dudley's letter (May 31). He was quite right that in the north-west area of London there are no neurosurgical centres, but for years the Atkinson Morley Hospital in the south-west has been the source of many kidneys going to St Mary's. It should be pointed out that head injuries form only a small proportion of the potential donors. In the south-east only 20% of all kidneys come from patients dying from trauma and less than 5% from a recognised neurosurgical unit. The majority of our donors come from general hospitals and they are by no means always on a ventilator. Indeed, in September, 1973 there was a suitable donor at St Mary's Hospital, Harrow Road, which was turned down by their Transplant Unit. This year there was a similar case at St Charles Hospital, W10. These kidneys were in fact taken by clinicians from the London Transplant Group and used in the national pool. It cannot be argued that kidneys from these donors were unsuitable for transplantation. In fact I have sent similar kidneys to St Mary's Hospital and the national pool with good results. Eighty per cent of all kidneys taken in south-east London are from similar donors (60% are not on a ventilator) and 50% function immediately.

As far as potential donors suffering from primary cerebral tumours are concerned, and the fact that they go elsewhere for terminal care, is no reason why those kidneys should be lost to the transplant pool. In Newcastle, all similar patients are put on a donor register and whichever hospital is concerned with their terminal events notifies the transplant team before death.

From my own experience of working in the Casualty Department at St Mary's, Harrow Road, and from conversations with nurses, housemen and students, it is fairly obvious that there is suitable donor material not being collected in at least one of the hospitals in the north-west area of London. I think that this represents "unequivocal evidence" that there is a lack of understanding and cooperation between doctors which is responsible for the lack of kidneys available for transplantation today.

Yours faithfully,

M. BEWICK

Department of Surgery,
Guy's Hospital, London SE1 9RT

SIR,—May I make a few comments on Mr H. A. F. Dudley's criticisms (May 31) of my article on kidney transplants (April 19)?

The figures for the numbers of kidneys received from and contributed to the central pool came from the DHSS Organ Matching Service at Bristol. And having spoken to a number of doctors and surgeons, I was convinced that there was, in some cases, more take than give—and not necessarily because of any variation in the number of cranial trauma patients.

The lack of cooperation between doctors was one of the reasons given by the Royal College of Physicians for the lack of kidneys. I also spoke to a considerable number of hospitals and doctors and found in many cases complete ignorance about the transplant programme and about transplant procedures regarding donor kidneys. And if Mr Dudley wants "unequivocal evidence" surely Mr Barnes' story is it.

Yours faithfully,

ROBIN LAURANCE

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news and views

Collaboration fixes unique QSO spectra

IN these days of proliferating publications, the article which appears on page 743 of this issue of *Nature* is worthy of more than just passing attention. The paper itself is of great significance to astronomers, since it provides some of the first indications of the radio spectra of the QSOs OH471 and OQ172. These are the only radio sources yet definitely identified with optical objects which have redshifts greater than $z=3$, and as such they are, on the cosmological interpretation of those redshifts, the two most distant objects known to man.

The very high redshifts also affect the radio spectra, of course, although the absence of features comparable to the lines in optical spectra makes it impossible to measure the redshift of any QSO by radio observations alone. But in effect by shifting the radio spectra the redshift brings new regions into the band of frequencies which can be covered by radio telescopes based on Earth. Perhaps some of the features seen in these spectra are, then, typical of features which would be discovered at rather shorter wavelengths in the spectra of QSOs with smaller redshifts.

In the excitement caused by the identification of these objects, it would surely have been natural to expect everybody with access to a radiotelescope to make observations and rush them into print. The result would undoubtedly have been a scattering of reports in various journals, causing much adrenalin to be produced by other frustrated radio astronomers desperately trying to keep up with 'the literature' and to find some overall picture of the radio spectra of the two QSOs.

But in fact a different approach has been adopted. Contributions which might have formed the basis of ten separate short notes here, there and everywhere have been combined in the one article we are publishing today. The degree of international cooperation involved is notable even for astronomy; as a result this paper provides in one place just about all that needs to be said (at this stage) about the radio spectra of OH471 and OQ172. *Nature* is always happy to cooperate in ventures like this which both reduce the number of separate papers we have waiting to be published and, we hope, provide a service to the scientific community (see, for example, the Cygnus X-3 issue of *Nature Physical Science*, **239**, 113–136; 1972).

JOHN GRIBBIN

Origin of plasmocytoma cells

THE DNA polymerase activity known as terminal deoxynucleotidyl transferase has been reported in murine plasmocytoma cells, with interesting implications for the study if tissue differentiation (see page 775 of this issue of *Nature*).

Terminal deoxynucleotidyl transferase differs from the other known DNA polymerase activities in mammalian cells in that it catalyses the addition of deoxyribonucleotides to the 3′-OH end of preformed polynucleotide chains in the absence of a DNA template. Although this effect has been useful as a model for the mechanism of polymerisation (*Proc. natn. Acad. Sci. U.S.A.*, **65**, 1041–1048; 1971) and for end group labelling of deoxyoligonucleotides (*Eur. J. Biochem.*, **22**, 271–276; 1971), the biological function of the enzyme is unclear, and it has been suggested that it is a degradation product of DNA polymerase. But its role as a marker for the origin and state of differentiation of certain cells is an interesting possibility.

The terminal transferase activity was first isolated in calf thymus, and has since been demonstrated in the thymus of numerous animals, but in no other tissue. Thus the activity seems to be entirely tissue specific, and also shows a development cycle which arises early in the process of embryonic development and persists in the thymus of young and adolescent animals.

McCaffrey *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 521–515; 1973) have found that cells from a patient with childhood acute lymphoblastic leukaemia (ALL) contain an enzyme with the properties of terminal transerase; this suggests that these tumour cells are thymic cells blocked very early in differentiation. Furthermore, the ALL cells lacked detectable surface immunoglobulin, a chacteristic of thymocytes. No activity was found in normal T-cells (thymus-derived lymphocytes), nor in three cultured human cell lines. This suggests that the enzyme was a marker for true thymocytes and provides hopes that an assay of this DNA polymerase might provide a diagnostic marker for whether a given leukaemia is of thymus or bone-marrow (bursal) origin.

Now Penit, Paraf and Chapeville have found that terminal transferase exists in murine plasmocytoma cells. This finding is surprising since these tissue-cultured cells have been thought of as B-cells (they express the immunological properties of cells derived from bone marrow). If the presence of terminal transferase activity in a cell is truly indicative of a thymic origin, then it would not be unreasonable to suggest that plasmocytoma cells represent an intermediate stage of differentiation between B and T cells. As Penit *et al.* point out, this has a precedent in a cloned line of thymoma (thymic tumour) cells produced by irradiation in mice. The thymoma cells possessed the T-cell characteristics of θ-antigen and anatomically thymic origin, and the B-cell characteristics of readily detectable surface immunoglobulin and immune complex receptor activity.

Unfortunately it is not yet possible to be certain that the expression of terminal transferase is evidence of an intermediate state of differentiation in plasmocytoma cells since immunological data are lacking, especially with regard to thymus antigens. A synthesis of immunological and biochemical approaches may elucidate the origin of cells containing terminal transferase activity and determine whether this will have diagnostic uses.

TONY PAWSON

Minor tyrosine genes of major importance

THE structure and function of transfer RNA are so well understood that it is surprising how little is known of the transcription of this RNA and of the genes from which it is transcribed. This is partly due to the difficulties involved in isolating the tRNA genes which are scattered through the genome in small clusters.

Unless the tDNA is chemically synthesised, it is only possible to obtain DNA enriched in tDNA genes, rather than pure tDNA. For example, using the DNA of a species of mycoplasma enriched so that the tDNA is 10% of the total, it has been possible to show that uridine is converted to pseudouridine post-transcriptionally. Forming hybrids between total cell DNA and tRNA has given some information about the size of the genes and supports the idea that the genes for tRNA are grouped together in twos or threes. But by far the most informative method has made use of the transducing bacteriophage φ80. This phase has an attachment site (in *Escherichia coli*) near the two minor tyrosine genes which together contribute 30% of the total tyrosine tRNA. The two genes are indistinguishable except in su^+_{III} cells where one of them—the suppressor gene—has a single base change in the anticodon region, which allows tyrosine to be read instead of the terminator codon UAG.

When the phage carrying one or other of the two minor tyrosine tRNA genes is transduced into *E. coli*, large amounts of tyr tRNA can be detected in the post-infection phase. In mutants in which this amount of RNA is reduced, it was found that this was because the processing of the RNA was less efficient, leading to an accumulation of the first transcription product. Using φ80 carrying one of the minor *tyr* tRNA genes (the suppressor mutant), the accumulated RNA was examined by Altman and was shown to contain some 40 nucleotides more than the mature tyr tRNA (*Nature new Biol.*, **229**, 19; 1971). Subsequent sequencing of the molecule by Altman and Smith (*Nature new Biol.*, **233**, 35; 1971) revealed several interesting features of this tyr tRNA precursor. It seems that, like the mature tRNA, the precursor also has a 'clover-leaf' secondary structure but differs from it in that bases modified on the active tRNA are unmodified in the precursor. The authors also present evidence that mutations which alter the amount of mature RNA produced are close to the point at which the precursor molecule is cleaved.

Chysen and Celis have used one of these mutations—one which reduces the rate of synthesis of su^+_{III} tyr tRNA—to determine whether the two minor *tyr* tRNA genes are transcribed into one large precursor or two separate ones (their results are described in *Nature*, **249**, 418; 1974). In this case it was necessary to infect *E. coli* with a transducing phage carrying both su^+_{III} and wild type *tyr* genes. They reasoned that if the two genes are jointly transcribed then a mutation which affects the rate of transcription of the su^+_{III} gene should also affect the rate of transcription of the wild type gene.

The experimental approach made use of the separation of tyrosine tRNA from bulk tRNA obtained by polyacrylamide gel electrophoresis. Once eluted from the gel the ^{32}P-labelled tyrosine tRNA was subjected to RNase T_1 digestion. Characteristic T_1 fragments identified tyr_{II} tRNA (the major species) and the two minor species tyr_I su^+_{III} and tyr_I wild type. By comparing the relative amounts of tyr tRNA I and II mutations which reduced the amount of tyr_I tRNA were identified, and those which reduced the level of su^+_{III} tRNA also reduced the level of wild-type tRNA. It seems therefore that the two genes are transscribed into one single precursor tRNA. Confirmatory evidence of this was obtained from an analysis of the RNA synthesised, after infection with φ80 carrying both minor *tyr* genes in cells deficient in RNase P (RNase P is the enzyme which cleaves the precursor to form the mature tRNA). The bands obtained by electrophoresis of the RNA were eluted and digested with RNase T_1. Of the fingerprints obtained there was none characteristic for a 'singlet' precursor of the wild-type tRNA.

Knowing that the two minor tyrosine tRNAs are transcribed together, it becomes particularly interesting to have some information concerning the structure of the genes. This is provided in an article in page 738 of this issue of *Nature* by Landy, Foeller and Ross, although at the time of writing the existence of the 'doublet' precursor was not fully established. Their approach was to compare the DNase digestion pattern of phage φ80 (parental) with φ80 su^+_{III} (singlet—the suppressor gene) and with φ80 su^+_{III} (doublet—the suppressor gene and the wild type). Limited digestion with site-specific nuclease endo R. *Hin*d, followed by electrophesis under different conditions, led to complete resolution of all the fragments. Most of the fragments were the same for all three RNAs of course, but three fragments were unique to the parental φ80; one fragment was unique to the singlet, one to the doublet, and five fragments were found in both singlet and doublet but not in the parental phage. These last fragments did not form hybrids with mature tyr tRNA and so their transcript may be that part of the precursor which is cleaved during tRNA biosynthesis. It seems that the size of one tRNA gene plus any unduplicated intergenic sequence is approximately 200-260 base pairs—about two or three times the size of tyr tRNA. The authors suggest a maximum of 125 base pairs for the intergenic sequence between the duplicated genes. Further sequencing of the DNase fragments will allow these figures to be more accurately assigned and provide a complete analysis of the *tyr* tRNA gene(s).

PAMELA HAMLYN

Alpine tundra change after road building

from Peter D. Moore
Plant Ecology Correspondent

ALPINE tundra habitats are simple in structure, yet often diverse in species. The species which can survive under such rigorous climatic conditions are of considerable biological interest because of their elaborate phenological and physiological adaptations (see Billings and Mooney, *Biol. Rev.*, **43**, 481; 1968). Their extreme adaptation to climatic stress may render them particularly susceptible to hardships of other kinds, such as human interference. A short growing season results in low productivity and this means that recovery following disturbance is bound to be slow.

Bell and Bliss (*Biol. Conserv.*, **5**, 25; 1973) studied regeneration of vegetation on areas laid bare by road construction in Olympic National Park, Washington, and found that after more than 30 years plant cover was still very low. Greller (*ibid.*, **6**, 84; 1974) has now produced similar data from Rocky Mountain National Park, Colorado. In this park two roads provide easy access to an open expanse of alpine tundra; one of the roads was constructed in 1920 and the other in 1932, but recolonisation of the verges, laid bare by construction activities, has been very slow.

Greller found that north-facing slopes had become dominated by *Poa fendleriana*, and south-facing slopes by *Agropyron scribneri*, both of which are tussock-grass species, well adapted to growth on unstable soils. It is a matter for concern that these slope communities are low in diversity and lack many of the more interesting

species of the tundra vegetation, but Greller's conclusion that succession plays only a minor part in these situations cannot be justified from his data. Fifty years is a relatively short time in the history of most successions and under the extreme climatic stress of alpine habitats one might expect even slower developmental rates. The current lack of vegetation can hardly be construed as proving a lack of successional development. Natural periglacial soil processes must surely act in an equivalent fashion to produce similarly denuded slopes.

Greller's suggestion that succession may be curtailed by soil dryness on the artificially steepened slopes of the banks is not likely to be correct. Many alpine species have xeromorphic adaptations, and Teeri (*Science*, **179**, 496; 1973) has found that *Saxifraga oppositifolia* can withstand water deficits as great as −55 bar. Drought is unlikely to prove a serious hindrance to the growth of alpine communities. Soil instability may slow down or even divert the course of succession, but one cannot assume that this is the case after only 50 years.

Nevertheless, one is faced with the fact that habitat disturbance caused by road building leaves scars upon the tundra landscape which may take many generations to heal and which, indeed, may never heal at all. This consideration should occupy a position of importance in the minds of those responsible for the routing of such highways. An additional problem is that of trampling pressures by those who gain access to the tundra along the highways. Alpine vegetation is particularly susceptible to this additional stress, as has been demonstrated in the Cairngorm mountains of Scotland, where the provision of easy access to the public (by roads and a ski lift) has led to ever-widening paths and trampled soils (see Watson in, *The Biotic Effects of Public Pressures on the Environment*, edit. by E. Duffey, Natural Environment Research Council, London; 1967). It may be that the instability of roadside slopes in the tundra areas of Rocky Mountains National Park will be one of the least concerns of its conservators.

Blastokinin induction by steroids

from our Steroid Biochemistry Correspondent

SINCE the identification in rabbit uterine fluid of a specific protein blastokinin or uteroglobin, much research has been carried out to define the precise physiological role of this compound. It appears in uterine fluid after ovulation and its concentration is high at the time of implantation. One of the effects of the protein is to stimulate the transition of the morula to the blastocyst, a transition, however, which will also occur *in vitro* without blastokinin provided some other protein which will bind progesterone is present. Addition of progesterone to culture media containing blastokinin stimulates RNA and protein synthesis by the embryo and blastokinin may function as a binding protein for steroids which may be needed by the embryo. Blastokinin will not only bind progesterone but also seems to be controlled by progesterone.

The control of this protein by progesterone and related compounds has been investigated by Arthur and Chang (*Fert. Steril.*, **25**, 217–221; 1974). Steroids were administered to rabbits daily for 5 d post-oestrus; 24 h later the animals were killed and the uterine fluid was examined by Sephadex column chromatography for the presence of blastokinin. The protein was detected in rabbits treated with progesterone and the 17α-acetoxyprogestogens, chlormadinone acetate and medroxyprogesterone acetate. The amount of blastokinin in uterine fluid was similar after treatment for 5 d with these steroids to that found on the fifth day of pregnancy. These progestogens are known to have a spectrum of biological activity similar to progesterone whereas the other group of orally active progestational compounds related to 19-nortestosterone have a different spectrum of activity; these latter compounds, as well as oestrogens, were ineffective in inducing blastokinin.

The relation between steroid structure and blastokinin induction merits further investigation because the inducing effect of some of these steroids may be related to their contraceptive action. It is now recognised that the hormonal contraceptives owe their high efficacy to effects at a number of sites in the reproductive process and contraceptive efficacy can be obtained with doses of synthetic steroids which do not inhibit ovulation. Arthur and Chang suggest that administration of steroids during the pre-ovulatory phase of the cycle may change the uterine environment by induction of specific uterine proteins so that it will no longer support embryonic development and/or implantation. Furthermore it might be possible to develop compounds which have this effect but which would be without the adverse side effects associated with some of the hormonal contraceptives currently used. The idea is attractive but so far blastokinin has not been demonstrated in human uterine fluid.

The topologist meets the biologist

from a Correspondent

A MEETING on May 21-24 organised by the Mathematics Institute of the University of Warwick and sponsored by the Science Research Council was intended to initiate a dialogue between biologists, mainly in embryology and neurophysiology, and specialists in differential topology and dynamical systems.

The possibility of such a dialogue owes much to the efforts of C. H. Waddington (University of Edinburgh), R. Thom (Institute des Hautes Etudes Scientifiques, Paris) and E. C. Zeeman (University of Warwick). Waddington has for many years stressed that topology is a natural language for developmental biology. Thom sees his catastrophe theory as an essay towards an algebra of morphology, enriching the range of our ways of describing and classifying forms. His talk at the Warwick meeting was euphoric, aphoristic, enthralling and exasperating in turns.

Zeeman has taken on the task of convincing biologists, by means of concrete applications to problems they see as important, that catastrophe theory can be predictive. The reaction to his talk on amphibian gastrulation suggested that a fruitful dialogue has begun. A sequence of talks on development—by P. D. Nieuwkoop (State University of Utrecht), D. Ede (University of Glasgow), J. Cooke (National Institute for Medical Research, Mill Hill), J. Lewis (Middlesex Hospital Medical School, London) and P. Shelton (University of Leicester)—could have left the mathematicians in no doubt about the fascination and complexity of the regions into which they are venturing.

At other points the meeting turned more to debate between biologists, notably in a marathon session on hardware and software in perception, involving principally D. Marr (University of Cambridge), W. R. Adey (University of California, Los Angeles) and D. Sunday (California State University). It seemed that the brain is not ready for the topologists or that they are not ready for the brain.

One session was devoted to problems in which differential equation models have traditionally been used. Handling solutions of coupled ordinary differential equations as trajectories is an essentially topological procedure. So it seems very likely that the power and scope of models for interacting animal species, chemical oscillators and circadian rhythms can be greatly extended. In this session A. Winfree (Purdue University) revealed how illuminating it can be to apply topological reasoning to circadian rhythms and to travelling waves in chemical reactions.

Convection in a layered mantle

from Peter J. Smith
Geomagnetism Correspondent

SEISMIC data have revealed that the Earth's upper mantle contains several discontinuities in density and seismic velocity, the most prominent of which lies at a depth of about 650 km. But what constraints does the existence of such discontinuities place on the form and magnitude of the convention currents which are believed to share the same region of upper mantle space? The full answer to this question would depend on a complete knowledge of the nature of the transitions producing the discontinuities—a matter which is far from being resolved. Insofar as the discontinuous changes in physical properties are due to mineralogical phase transitions they are relatively well understood; and their effect on convection is probably quite small. Richter (*Rev. Geophys. Space Phys.*, **11**, 223; 1973), for example, has shown that the Rayleigh–Benard convection driven solely by unstable vertical temperature gradients, the presence of the olivine–spinel phase transformation does not alter the structure of the flow and produces a roughly two-fold increase in steady amplitude. But if, in addition, chemical changes are involved, the problem changes because the position of the critical boundary will depend not on state parameters but on advection.

The question of whether or not the mantle is chemically inhomogeneous is still a matter of debate, although there must be a strong presumption in its favour. Press (in *The Nature of the Solid Earth*, McGraw-Hill, 1972), for example, investigated bulk sound velocity as a function of density for a set of Earth models obtained using Monte Carlo techniques and concluded that an offset in the velocity–density plot near the 650 km phase transition represents an increase in the Fe/Mg ratio. Several other workers have reached similar conclusions, although such agreement is not necessarily as impressive as it seems because there are known to be certain problems associated with the determination of mean atomic weight using bulk velocity–density data. Moreover Wang (*J. geophys. Res.*, **75**, 3264; 1970) has shown that it is possible to find an Earth model with a chemically homogeneous upper mantle which is quite consistent with free oscillation data. Anderson and Jordan (*Phys. Earth Planet. Interiors*, **3**, 23; 1970), on the other hand, support iron enrichment of the lower mantle using several methods which are apparently not open to the objections directed at bulk velocity–density systematics.

In summary, then, chemical stratification in the upper mantle, though not proven, is highly likely; and so it is important to investigate what effects such layering is likely to have on convection processes. Richter and Johnson (*J. geophys. Res.*, **79**, 1635; 1974) have now attempted to do this by analysing the stability, to heating from below, of a system comprising two immiscible fluid layers of different density separated by a boundary which will be advected by the flow. What emerges from the fluid dynamical analysis is a stability diagram for the system showing the critical Rayleigh number (that is, the critical value of the normal Rayleigh number, R_a) for the most unstable mode as a function of R_ρ, the Rayleigh number based on the density contrast between layers. In other words, the stability diagram is a curve in R_aR_ρ space which separates the stable region below (towards lower R_a, R_ρ values) from the unstable region about (towards higher R_a, R_ρ values). In this context, stability means that at a given point in R_a-R_ρ space all perturbations decay with time.

For R_ρ lower than about 5×10^3, the most unstable mode is a standing oscillation on the interface between the two layers (overstable mode); and once the oscillatory motion begins, the fluid never returns to a state of total immobility. This overstable regime only exists within a very narrow band of R_a values, however, suggesting that its occurrence is not very probable. Below this band (lower R_a) the system is stable, but above it (R_a higher than about 10^4) direct convection throughout the entire depth of fluid (that is, throughout both layers) becomes possible. If such convection were actually to take place it would, of course, destroy the original chemical layering; conversely the persistence of a chemical boundary precludes total-depth convection. For R_ρ higher than about 5×10^3 the situation is quite different. In this region the most unstable mode comprises separate convection within each layer and is thus independent of the density jump across the boundary between the layers. The stability curve separating the stable and unstable regions in R_a-R_ρ space is now parallel to the R_ρ axis, and so the stability or instability of the system depends only on the value of R_a.

These results place important bounds on acceptable Earth models. If the overstable mode is ruled out as improbable, for a model in which R_ρ is lower than 5×10^3 the occurrence of convection is consistent with the continued presence of a chemical boundary because the convection would cross the boundary and chemically homogenise the system. Thus in a model in which both convection and chemical layering are inferred, R_ρ must be higher than 5×10^3—and in this case there will be separate convection within each layer. In the case of the real Earth, much therefore depends on whether the 650 km discontinuity is just a phase change or whether it represents the combined effect of a phase transformation and a chemical change. Assuming that a chemical change is indeed present and that it involves an increase in iron relative to magnesium, several authors have calculated that R_ρ would be of the order of 10^6. This clearly implies that convention may only occur separately above and below a depth of about 650 km.

This result does much to emphasise the importance of resolving the problem of whether or not the upper mantle is chemically inhomogeneous. The convective motions for seafloor spreading are likely to be those in the uppermost layer, and the thickness of this layer will influence the magnitude of the convective cells within it. Richter (*Rev. Geophys. Space Phys.*, **11**, 223; 1973), for example, showed that many of the possible flows in the mantle have horizontal scales comparable with the depth scale. The fact that the depth scale may be governed by the presence of chemical layering clearly makes it necessary to determine whether or not such layering actually exists.

Modeling tide and surge interaction

from J. Darbyshire

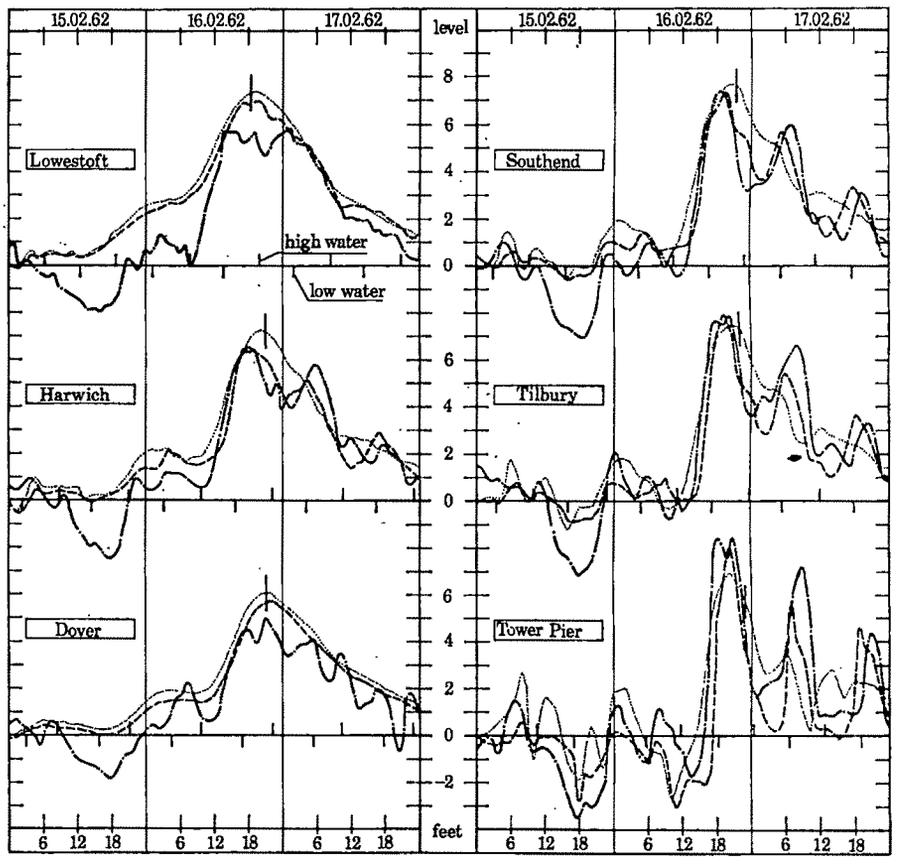
GREAT disasters and natural upheavals often provide an incentive for scientific research and this has been very true in the investigation of storm surges (or the extra tides caused by meteorological effects). The first such stimulus was probably the great Thames flood of 1928 which led to the systematic recording of these effects at Southend and to work on their prediction at the Bidston Observatory near Birkenhead. The methods originally used were largely empirical and the work of Doodson and Corkan was very important during this period.

The next great stimulus was the disastrous flooding of the English east coast and the Dutch coast during January 31–February 1, 1953, when many lives were lost and many of the Dutch dykes were seriously breached. After this there was set up at the British Meteorological Office, a flood warning system which made use of the fact that tidal abnormalities on the English side of the southern North Sea are usually preceded by similar smaller but detectable abnormalities at Aberdeen about 9 h earlier. This would give an even longer warning to the continental

coasts. The 1953 surge also led to a spate of research. Rossiter published an article in the *Philosophical Transactions* which described very accurately the abnormalities at various times at all the ports on the circumference of the North Sea where tidal records were available. These results led to further attempts to improve methods of prediction and I was involved with one of them (J. and M. Darbyshire, *Proc. R. Soc.*, **A235**, 260–274; 1956). Hansen of Hamburg, on the basis of these observations, produced (*Tellus*, **8**, 287–300; 1956) the first mathematical model based on the use of digital computers and achieved a very high measure of success. The storage capacity of computers of this period were, however, rather too limited to cope with the hundreds of equations involved in finite difference methods and Ishiguro of the National Institute of Oceanography devised methods based on an electrical analogue of the North Sea which were to prove very useful (*Storm Surges in the North Sea—An Electronic Model Approach*, report 4, paper 25 (iii), Advisory Committee on Oceanographic and Meteorological Research; 1966).

The research effort in Britain, however, was still largely centred at the Liverpool Tidal Observatory at Bidston (later on to become the Institute of Coastal Oceanography and Tides and now a part of the Institute of Oceanographic Sciences) and the lead of the late J. R. Rossiter in this field will always be remembered. The work was extended to river estuaries, particularly the Thames, but it soon became evident that there was a large interaction effect between tide and surge which could not be ignored. Proudman had already predicted this on theoretical grounds. A computer model of the Thames based on these considerations was produced by Rossiter and subsequently Rossiter and Lennon. Models of the North Sea had ignored this interaction effect, but a new article by Joyce Banks, also from Bidston, extends the river model into the southern North Sea and allows for the effect of tidal interaction (*Phil. Trans. R. Soc.*, **A275**, 567–609; 1974).

Banks confines the investigation to the period of the notorious Hamburg surge of February 1962. In the figure is reproduced part of one of her diagrams which shows a comparison of observed surge effects at various English ports with predictions based on her model both with and without allowance for tidal interaction. An examination of the results shows that the tidal effect is very small in the open sea and the only appreciable difference is at Tower Pier. The initial negative surge is not successfully predicted and this is probably due to its being caused by conditions outside the southern North Sea and not accounted for by the model.



Time variations in wind-induced sea levels of the Hamburg surge at British ports. –.–, Observed residual elevation after removal of the barometric surge; – –, surge level ξ_{5+1} computed on the basis of wind and tide; . . . . , wind surge ξ_5 computed in the absence of a tide. Times of high and low water of the total predicted tide are marked along the abscissae. The vertical line near the peak surge corresponds to the time of high water of the computed lunar tide.

It seems a very far cry from the early empirical days to the modern highly sophisticated computer methods, yet a prediction of the surge at Lowestoft for February 1962, based on my very simple formula published in 1956, compares very favourably with that storm in the figure and as the formula took some account of conditions in the northern North Sea, the results do show a slight initial negative surge. Empirical methods, although very useful from a practical point of view, do not, however, help a great deal to gain a fuller understanding of the fundamental processes involved.

Randomness in evolution

from A. Hallam
Palaeontology Correspondent

HISTORICAL events can be studied in two radically different ways. Each individual event can be considered on its own merits, and interpreted independently, or many such events can be dealt with collectively, with a view to formulating general laws. The first type of approach can be termed idiographic and the second nomothetic. All historical events have both idiographic and nomothetic aspects, but palaeontologists have traditionally focused on the former. With regard to the major question of evolutionary diversification and extinction of particular fossil groups, a wide variety of patterns has been recognised, but little success has been achieved in attributing these to specific causes or in creating general models with predictive power. Consequently several American workers have recently adopted the nomothetic approach, to test the possibility that some aspects of the evolutionary record behave as stochastic or random variables. Each event may depend on the joint occurrence of many underlying genetic, morphological, physiological and ecological causes but the event itself may only be predictable statistically, even though it has a conventional cause. The analogy of the gas laws in physics comes to mind, with each fossil taxon playing the part of individual gas molecules.

Van Valen, in the first number of a new journal called *Evolutionary Theory* (July 1973), tackled the problem of faunal extinctions by applying the

survivorship curve technique of population ecology, which is a simple plot of the proportion of the original sample that survives for various intervals of time. The use of a logarithmic ordinate ensures that the slope of the curve at any given age is proportional to the probability of extinction at that age. Van Valen's analyses of numerous taxa showed almost uniform linearity in the survivorship curves, allowing for sampling error. This is rather surprising, because it requires that the probability of fossil discovery does not decrease appreciably with the age of the strata, as has been argued by Raup in an article in *Science* (**117**, 1065; 1972); also because, for living taxa, both constant origination and extinction rates are implied. Van Valen is led on to propose a new evolutionary 'law', that extinction in any adaptive zone occurs at a stochastically constant rate. Exceptions to the 'law' are considered to be either spurious, rare or doubtful. He also proposes a new unit to measure the rate of extinction, the macarthur, named after a well known American evolutionary ecologist.

Raup, Gould, Schopf and Simberloff adopt a different approach in the *Journal of Geology* (**81**, 525; 1973) to the more general question of diversification and extinction, by using a computer to generate phylogenetic diagrams, or cladograms. The lineage of each clade, or monophyletic subunit, was generated by the computer program and caused to terminate or branch in order to produce a hypothetical phylogeny, with the termination and branching events being stochastically controlled using random numbers. Part of the input dealt with the establishment of an equilibrium diversity, in accord with modern evolutionary theory. The cladograms so produced exhibit a great variety of shapes: some underwent early exponential increase in diversity and then declined gradually to extinction (the trilobites); others showed a gradual diversity increase followed by a rapid extinction (dinosaurs) or passed through a near-extinction phase to develop a new phase of high diversity (ammonites). The average extinct clade increases in diversity up to the mid point of its stratigraphical range and then declines in diversity until extinction. When faced with such variation in pattern many palaeontologists have been inclined to suspect or postulate that the organisms in question were inherently different in evolutionary potential. The observed variation does not exclude, but nor does it demand this. Comparison with a real example, the fossil record of the reptiles, shows good general agreement but also some interesting differences. Thus the late Cretaceous extinctions showed more coincidence between different groups than predicted from the computer model.

It seems therefore that an exceedingly simple stochastic model can produce branching and diversity patterns very similar to those described in the real world; also that the taxonomy utilised by palaeontologists imposes various constraints similar to those used in the logic of the computer program. The question arises, where does one go from here? In the first place palaeontologists should be even more suspicious of any glib deterministic explanation of the diversification and extinction of any particular fossil group. More positively, there is a more sophisticated tool for investigating the extent to which the coincidence in time of extinction of different fossil groups departs from randomness. This should be easy to demonstrate for the striking mass extinctions at the end of the Permian and Cretaceous, but what of the other geological periods?

Two dimensional rotons

from Peter V. E. McClintock
Condensed Matter Correspondent

A ROTON in a two-dimensional assembly of helium atoms has a smaller energy than a roton in three dimensions, according to a calculation by Padmore of Erindale College, University of Toronto (*Phys. Rev. Lett.*, **32**, 826; 1974).

The thermodynamic properties of bulk liquid helium below its superfluid transition temperature can be understood reasonably well on the basis of a model, due to Landau, in which it is assumed that the thermal energy is all in the form of a gas of particle-like excitations moving through the superfluid, which plays the part of an inert background or aether. There are two main types of excitation: phonons, which are quantised sound waves much like those occurring in solids; and rotons, which are peculiar to liquid helium and are believed to correspond to some sort of stirring motions on the atomic scale, such as microscopic vortex rings. There is, as yet, no agreement as to the precise physical nature of rotons, but their detailed energy–momentum relationship, known as the excitation spectrum, has been established through neutron scattering experiments and is consistent to a high precision with the measured heat capacity of the liquid.

Although attempts to deduce the excitation spectrum theoretically from first principles for an assembly of weakly interacting helium atoms have not been very successful, Feynman and Cohen (*Phys. Rev.*, **102**, 1189; 1956) managed to derive a spectrum of the correct general shape and approximately in quantitative agreement with experiment, by using quantum mechanics. Their approach involved to a considerable extent the use of physical intuition to develop a wave function able to describe the superfluid background, and they then used a so-called trial wave function, not specified in detail, in order to describe the elementary excitations. A variational method was used to investigate how the trial wave function should be modified in order to minimise the energy of the whole assembly, and thus discover the true wave function from which the excitation spectrum could then be derived. Padmore has extended the Feynman and Cohen approach in an attempt to derive theoretically the excitation spectrum of a two-dimensional (2D) assembly of helium atoms.

Assemblies which are effectively 2D occur where one of two atomic layers of helium are deposited on a solid surface. The helium atoms are held in place by the attractive Van der Waals force which prevents them from moving in a direction perpendicular to the surface; but they can remain completely free to move parallel to the surface. Under appropriate conditions of density and temperature, such assemblies have been shown to form either 2D crystalline solids, or liquids, or gases. By using, for the substrate, materials such as exfoliated graphite or Vycor glass which have very large surface areas in relation to their volumes, it is possible to measure the heat capacity of 2D liquid helium, and experiments have shown that the heat capacity appears to be anomalously large. A possible explanation lies in the idea that rotons can still exist in a 2D assembly, but with smaller energies than they have in 3D. A neutron scattering experiment to measure the excitation spectrum does not seem possible because of the random orientations of different sections of the substrate, so a calculation of the spectrum is therefore of particular interest.

The most important conclusion which emerges from Padmore's calculation is that the energy of a roton in 2D is indeed considerably less than in 3D. Although the calculated roton energy, is, as in the 3D case, still somewhat larger than would be consistent with the measured heat capacity, the change in energy in going from 3D to 2D seems to be in excellent agreement with the relatively few experimental data at present available. Padmore also reports his calculated characteristics for rotons in 2D helium at a number of different densities, so no doubt there will soon be reports of further experiments aimed at checking these more detailed conclusions.

Accurate spectroscopic factors

from Peter E. Hodgson
Nuclear Theory Correspondent

DURING the past ten years the one-nucleon transfer reactions have been developed into one of the most powerful tools for studying the single-particle aspects of nuclear structure. Among these the (d,p) reaction has been most widely used, but extensive studies have also been made using (d,n), (p,d), (d,t), (d,h) and many similar reactions that either add a proton or neutron to or remove one from the target nucleus.

As a typical example, consider the (d,p) reaction on ^{24}Mg. The incident deuteron interacts with the ^{24}Mg target and deposits a neutron in one of the unoccupied neutron states, and the remaining proton goes on alone. At lower energies this process can take place by absorption of the deuteron to form a compound nucleus followed a long time after by proton emission, but at higher energies the direct process that takes place in a time comparable with the time taken by the deuteron to cross the target nucleus predominates to such an extent that the compound nucleus process is negligible.

The differential cross section for this direct (d,p) reaction can be written as the product of two factors, one depending only on the structure of the nuclei involved and the other only on the reaction mechanism:

$$[d\sigma/d\Omega] = S_{JL} F_{JL}(\theta,E)$$

All the dependence of the cross section on the angle of emission θ of the proton and on the incident deuteron energy E is contained in the reaction factor $F_{JL}(\theta,E)$, and this can be calculated by the distorted wave theory. These angular distributions are in general characteristic of the total quantum number J and orbital angular momentum quantum number L of the state entered by the captured neutron so that comparison between the observed angular distribution and those calculated for various J and L enable these quantum numbers to be determined, and these give the spins of the corresponding states of ^{25}Mg.

The spectroscopic factor S_{JL} is a measure of the single-particle strength of the neutron state and is given by the ratio of the measured cross-sections to the calculated value of the $F_{JL}(\theta,E)$.

The analysis is thus able to give the quantum numbers and the single-particle strengths of all the nuclear states of ^{25}Mg reached by the reaction. Numerous analyses of nuclear states in all stable nuclei have been made with this technique, and it is one of our main sources of information on nuclear structure. The main limitations to this method are, first, that it is not always possible to determine J and L and, second, that the uncertainties in the distorted wave theory make the spectroscopic factors uncertain to about 20%, even in the best circumstances.

A new method of analysing one-nucleon transfer reactions has now been proposed by C. F. Clement of Harwell (*Nucl. Phys.*, **A213**, 469 and 493; 1973) and used to obtain more reliable J,L and more accurate spectroscopic factors. This method depends on the j-dependent sum rules that relate the spectroscopic factors of the reactions that remove a neutron (or proton) from a nucleus to the spectroscopic factors for the corresponding reaction that adds a neutron to the same nucleus. The j is the total angular momentum of the transferred nucleon. These sum rules provide a rigid set of equations that must be satisfied by the spins of the final states and by the spectroscopic factors of the corresponding transitions.

These sum rules must be distinguished from the more familiar J-dependent total sum rules that relate the spectroscopic factors to the total single-particle strengths of the states. The j-dependent sum rules provide additional constraints on the spectroscopic factors only if the spin of the ground state of the target nucleus is different from zero; if it is zero they are the same as the total sum rules. The method is thus applicable only to nuclei with ground state spins different from zero. All these sum rules were derived long ago by J. P. French, but it is only recently that they have been applied to nuclear structure analyses.

Clement and Perez (*Nucl. Phys.*, **A213**, 510; 1973) have now applied this analysis to the reactions (d,t) and (d,p) that remove neutrons from or add them to the states of ^{45}Sc with spin 7/2. The sum rules give a set of eight equations connecting sixteen sums of spectroscopic factors for stripping and pick-up to states of different spin. The measured spectroscopic factors satisfied these equations to an accuracy of 4% in relative magnitude and 10% in absolute magnitude. Several additional spin assignments to states of the residual nuclei ^{46}Sc and ^{44}Sc were made by this analysis.

An important problem in all analyses of single-particle states is the fraction of the strength that is far removed from the main concentration. Clement and Perez find that it would be difficult to maintain their fits to the sum rules if more than 10% of the total is in the continuum. Thus nearly all the f7/2 strength is concentrated in a limited energy region; this is an important conclusion for the basic single-particle model of nuclear structure and supports the validity of the distorted wave theory.

Subsequently Clement and Perez have applied the sum rule analysis to other nuclei and have still further reduced the uncertainties in the absolute spectroscopic factors. This is clearly a technique of great power that can provide valuable information on nuclear structure and stringent tests of the present techniques for obtaining it.

Fitting the flower to the bee

from a Correspondent

WITH the increasing use of F$_1$ hybrids in insect-pollinated agricultural crops and vegetables, special methods have to be devised to get enough effective pollinating insect visitors to achieve the complete cross fertilisation that is necessary. At the Third International Symposium on Pollination organised in Prague from May 15-18 by the International Commission for Bee Botany of IUBS, the Bee Research Association and the Academy of Agricultural Sciences and other bodies in Czechoslovakia, examples discussed included red clover, lucerne, field bean, melon and cucumber. A detailed knowledge of floral biology, pollen and nectar production, and their composition is needed; for instance, Ing. H. Haslbachová (College of Agriculture, Brno) reported success in increasing pollination (and hence seed yield) in tetraploid red clover by applying growth regulators which alter the morphology of the subsequent flowers in ways which make them easier for the bees to work.

Visitation by bees is necessary for good crops of strawberries from some cultivars, and L. J. Connor (Ohio State University) has found that the number of visits a flower receives depends on its structure. Flowers with stamens short in relation to the receptacle are difficult to work and not much visited. Unfortunately the first flower on a stem (giving the earliest, most profitable, fruit crop) has short stamens, even in cultivars whose later flowers have long stamens. Here the most likely solution is breeding plants for floral morphology that suits the pollinating bees.

G. D. Waller (US Department of Agriculture Laboratory, Tucson), in seeking to find out why it is difficult to achieve adequate pollination in onion seed plots (*Allium cepa*), found that honeybees—the major pollinator—are unwilling to forage on the onion flowers. The only difference between these flowers and others available nearby is that the potassium concentration of the onion nectar is about ten times as high as that of other nectars. An interspecific hybrid *Allium cepa* × *A. fistulosum* produces nectar with a normal potassium

content and is very attractive to honeybees. These results provide the first evidence that a minor constituent of nectar can significantly inhibit its collection by honeybees.

Much less is known about pollination in the tropics than in temperate regions, though an even higher proportion of tropical crops probably depend on insect pollination. S. E. McGregor (USDA Laboratory, Tucson) presented a list of 120 such crops, belonging to 39 botanical families. T. Chandler, a Canadian currently working at the Forestry Training Institute, Arusha, Tanzania, has studied pollination of lucerne by the African honeybee *Apis mellifera adansonii*, which has come in for much reprobation recently on account of its aggressive (or defensive) behaviour. This subspecies proves much more effective than the European ones at 'tripping' the flower, and thus pollinating it, largely because lucerne pollen is attractive to them, whereas other pollens are preferred by European honeybees. Also, the African honeybees are efficient at detecting which florets are at the right stage for tripping, and thus do not waste time visiting those that were too immature.

Work by Mackensen and Nye on breeding *Apis mellifera* specifically for collecting pollen from lucerne has now been successfully extended to red clover by H. Nørgaard Holm in Denmark (Højbakkegård, Tåstrup).

Since the second pollination symposium in London in 1964, commercial rearing of certain species of soil-nesting and stem-nesting bees has progressed beyond all expectation. Reports came of work in Canada, United States, France, Hungary, Czechoslovakia and other countries. From Poland L. Bornus and his colleagues (Beekeeping Institute, Puławy) brought news of the highly successful rearing of bumblebees for pollination, achieved largely by careful determination of these bees' preferences at each stage of their seasonal cycle.

In an account of the first 25 years of the Bee Research Association at Chalfont St Peter, Buckinghamshire, Eva Crane reported that a 23-year index to research reported in its journal *Apicultural Abstracts* (1950-1972) has just been completed. The information is stored on magnetic tape, and it is hoped to publish it as author and subject catalogues, linked to abstracts in the journal. The 17,000 publications represented include many giving information on pollination, and a volume *Pollination of Seed Crops*, based on publications reported in *Apicultural Abstracts*, has already appeared. Estimates of the world value of crops resulting from insect pollination vary from £1,000M to £10,000M a year or more.

Gap junction between cells in contact

from a Correspondent

ALL cells in tissues and all but two of twenty-five cultured cell lines form junctions with one another and communicate through these junctions by transfer of electrical signals and chemical substances (the latter was originally termed metabolic cooperation). The junctions form extremely rapidly, within a minute certainly, and presumably break just as rapidly. Cells therefore are constantly forming and breaking contacts for metabolic cooperation and intercytoplasmic exchange. The relevance of such processes to inductive interactions during tissue differentiation and embryogenesis makes this one of the newest and perhaps most interesting areas in biology. The Cell Surfaces Discussion Group held a timely meeting on May 23 at the National Institute for Medical Research, Mill Hill on the structure and function of intercellular junctions.

The particular consideration of the meeting was the gap junction between cells in contact, permitting the flow of matter across the membranes between two cells. E. L. Benedetti (Institute of Molecular Biology, Paris) catalogued interesting similarities and differences between the propagation of an electric current between cells and passage of chemical tracers. Thus both electric coupling and chemical transfer are bidirectional and operate across the same junction. Some separation of these events is implied, however, since the former is present in most embryonic cells, excitable and non-excitable, and there may be some restraint of chemical transfer in embryonic tissues.

The inability to form gap junctions is also seen in some cell lines such as L fibroblasts, for example, and this is recessive in cell hybrids. Thus, heterokaryons with human fibroblasts can electrically and chemically couple and revertants that lack part of the complement of human chromosomes first lose competence for passage of chemical markers across the gap junction and subsequently the capacity for electrical coupling.

The types of chemicals transported across gap junctions is quite wide, but, as was emphasised by J. Pitts (University of Glasgow), there is an upper molecular weight limit of about 1,000. The flux of a metabolite, such as a purine nucleotide, is calculated to be about 10^5–10^6 molecules s^{-1} across the gap junction, that is about equivalent to the *Escherichia coli* chromosome. The capacity of mouse fibroblasts to form gap junctions may not be lost irretrievably and it is possible to select for 'revertant' cells that form junctions, but not as efficiently as say BHK cells.

E. Wright (Institute of Virology, University of Glasgow) described experiments that set out to isolate BHK cell mutants incapable of forming competent junctions. The basis of the selection is to cocultivate BHK wild type cells with a thymidine kinase negative mutant and BUdR and to isolate cells which survive ultraviolet irradiation. Interestingly, although these mutants (*Mec*$^-$) as expected are extremely poor recipients of thymidine from a BHK wild type cell population, metabolic cooperation can be established if a purine marker such as adenine is used so that there seems to be discrimination between the passage of two nucleosides across the gap junction.

As both Pitts and Wright agree, the idea that the many metabolites capable of passage across the gap junction may require independent transport processes is extremely difficult to reconcile with the evidence that purified gap junctions appear to contain relatively few proteins. For example, W. H. Evans (National Institute for Medical Research, Mill Hill) finds one major protein of molecular weight 29,000 in fractions of mouse liver plasma membrances enriched in gap junctions. Similar results were reported for synaptic junctional complexes by I. Morgan (Centre de Neurochemie, CNRS, Strasburg) and A. Matus (University College, London). The most common method of preparation, however, involves detergent solubilisation of the non-junctional areas of membrane and it may be that some dissolution of junctional membrane components may also take place.

The mechanism of formation of gap junctions remains obscure. Since junctions can form in the absence of protein synthesis, pre-existing membrane proteins are utilised. The junctional areas, however, are clearly differentiated from the rest of the membrane by the absence both of typical enzyme markers such as 5′-nucleotidase and leucyl napthylamidase and most of the plasma membrane proteins and glycoproteins. It must be assumed, therefore, that the initial events in junction formation requires the clustering of specific junctional proteins by lateral diffusion in the surface membrane.

The association of cytoplasmic contractile elements with gap junctions now seems established. One supportive result is the probable identity of an actin-like protein in preparations of mouse liver gap junctions. The role of these elements in formation maintenance and function of the gap junction, however, remains to be elucidated.

energy review

How Britain is facing the energy problem

"Our energy strategy must be flexible, balancing between different fuels and different sources, steering between overcaution and recklessness." Mr Eric Varley, Secretary of State for Energy, gives his view of the situation.

THIS edition of *Nature* devoted to energy matters is very timely. The problems which we face in this field are complex and far-reaching and, as Secretary of State for Energy, I am concerned that they should be as widely understood and thought about as possible. My department was set up in the midst of crises and its first six months have inevitably been preoccupied with the transition to greater stability. But we are now tackling the problems—many of them pressing—of the longer term. Public discussion of the problems involved is very important, particularly among scientists and technologists whose contribution to their solution can be very great.

The broad context of these problems is familiar. Consumption of energy on a large scale is part of the fabric of our society. Last year Britain consumed the equivalent of 342 million tons of coal, with oil (159 million tons of coal equivalent) and coal (131 million tons) the largest components: and in the past every extra 1% of GDP has been accompanied by increased energy consumption equivalent to about two million tons of coal. We have to plan far ahead to continue to meet these needs as securely, flexibly and cheaply as possible in a way which allows continuing economic growth. But whereas our consumption patterns reflect past access to cheap and plentiful supplies of energy, we must now accept that energy is an expensive commodity; that more active policies are needed nationally and internationally to achieve security and availability; and that much more attention needs to be paid to the way we use our energy, cutting out waste and improving efficiency.

Coal and oil

Development of our indigenous resources must be a key factor in our strategy. It is already apparent that these resources are very substantial and that, though we still do not know their full extent, they can make a very major contribution to our energy supplies and, more broadly, to the strength of our economy. But there is a wide range of important and testing problems to be solved in bringing them forward and in making the best possible use of them.

We have much the biggest coal industry in western Europe, despite its decline during the past decade or so, and the new energy situation has completely altered the framework in which its future is planned. It is already clear that coal must remain one of the main foundations of our energy policy and that the industry can look forward to a sure future. But a lot of work has to be done to ensure that its contribution is made most effectively. Just holding annual production from deep mines at its present level of around 120 million tons—and I hope to go above that if possible—will be a major task. One of my first acts on coming into office was therefore to announce the setting up of a full scale examination—involving government, the unions concerned, and the National Coal Board—of the contribution which coal can best make to Britain's future energy requirements and of the steps needed to secure this contribution. Good progress has been made and we hope we will have made an interim report by the end of this month.

Then there are all the resources of the North Sea. North Sea gas already supplies 90% of our gas consumption; and North Sea oil offers the prospect of a completely new source of oil supply, with all the advantages that that implies in the present world energy situation. It also offers us challenges, both in its development and, having brought it ashore, in making the best use of it. Nowhere else in the world has oil been brought up from these depths in these conditions, and great technological and engineering skill has to be brought into play to make sure that development proceeds as fast as possible. I am concerned not only to see that this feat is carried through successfully but also to develop policies which will ensure that oil and gas from the Continental Shelf are exploited in such a way as to confer maximum benefit on the community, particularly in Scotland and the regions elsewhere in need of development.

Nuclear prospects

Another major and difficult area is nuclear policy. Studies in depth and extensive deliberations about nuclear reactor choice have been in hand literally for years, without any clear consensus emerging. It is certainly an area of great technological complexity where the issues are not at all easy to weigh up. At the time of writing I have not come to, or announced, any decision. But I would stress that it is clear that there is no ideal solution.

The implications of the new energy situation are of course far reaching and certainly not confined to the problems of developing indigenous resources and nuclear power, and planning to meet long term demand. Many other issues are involved. One which I believe will, and must, increasingly impinge on the lives of everyone is the conservation of energy in the sense of cutting out unneces-

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sary consumption and improving efficiency in use. Energy is now an expensive commodity and this fact alone will bring home to many people the need to use it thriftily. But the government has a role to play too, and this is another area where I want to do what I can to speed up and encourage adaptation to the new energy situation.

In every one of the areas which I have mentioned the role which science and technology must play is of great importance, in many instances fundamental. Indeed it is no exaggeration to say that science and technology must provide the key to the longer term future. And, because of the time scales involved, we have to be very farsighted in our approach. Well directed and effective decisions on research are therefore an essential part of energy policy.

There can be no doubt that the main thrust of research and development on energy in this country must continue to be nuclear, looking towards the fast breeder reactor and then, we hope, towards the very long term prospect of controlled nuclear fusion as an energy source. Nuclear technology is by far the most promising avenue to pursue, in terms of meeting the energy needs both of the United Kingdom and of the world as a whole. We are therefore investing substantial sums in the nuclear field and are actively seeking international collaboration to ensure rapid advance at minimum cost. I recognise the misgivings which some people have about the safety of nuclear power. It is a matter to which I attach the greatest importance and I recognise my responsibility to uphold the very high safety standards which have been established in Britain.

Other possibilities

Nuclear power is not of course the only possible option. Readers of the technical press will be familiar with a wide range of other possibilities being championed: for instance 'natural' sources, using energy from the Sun, tides, wind, waves and geothermal heat. But the development costs of these different technologies are likely to be high. We already spend more than £100 million each year in the United Kingdom on energy research and development, and we do not have unlimited resources. Moreover we should not feel that the United Kingdom must develop every technology for itself; in many cases international collaboration makes good sense and in some cases the best course may even be to import the developed technology.

Engineers and scientists in my department have examined the whole range of these other technologies in the energy sector which we might back, and, in collaboration with other departments, they have made detailed studies of the most promising options. They have paid attention to the possible scale of each technology in relation to our energy needs, the cost and time for development, and the possibility of successful exploitation, as well as to the likely impact on the environment. Their conclusion has been that none of the various possibilities shows sufficient promise to warrant large scale investment in research and development at this stage. It has to be remembered that many of these new sources would be in competition with nuclear power, as a source of electricity, and not with fossil fuels; so that their attractiveness in economic terms is not affected one way or the other by the rise in oil prices.

Nevertheless, this is an area where assessment is a continuing process. I have, for instance, felt that some further work on recurring energy is warranted as a form of insurance policy and I have accordingly commissioned a more detailed study of the prospects of extracting energy from sea waves: wave power is not expected to compete economically with nuclear power but it is an energy source particularly suited to the United Kingdom, with surprisingly large potential. I have also recently set up a team of scientists and engineers at Harwell to examine the many technological options and to ensure that our conclusions are soundly based. I recognise the importance to our future energy supply of getting these conclusions right.

We must adopt a quantitative approach to the big and far reaching decisions that have to be taken over the whole field of energy. Primary weight has to be given to the normal economic tests; energy is not the only scarce resource and an economic appraisal—provided we use realistic prices and, where necessary, take account of resource costs—enables resources of many different kinds to be weighed in the same scales. We know, of course, that these tests never tell the whole story. The calculations are only as good as the assumptions on which they are based. And we must have at the back of our minds other more nebulous considerations; with some, like security of supply, satisfactory quantification may be difficult, and with some, like the needs of future generations for energy supplies, it may be virtually impossible. But we must take the best account of them we can, while bearing in mind that, if we do decide to go down paths other than those indicated by the economic tests, we must be clear we are doing so for good and sufficient reasons.

Finally we must face the problem of taking decisions involving very long time scales. Over a long period the whole framework can change radically. On occasions it may change abruptly, as it did for oil last October. We are, therefore, very much in the business of risk taking. Not only can we not be sure that we have the answers, we cannot even be sure that we know the problems. It is for these reasons that our energy strategy must be flexible, balancing between different fuels and different sources, steering between overcaution and recklessness. However much any of us may think that we have the right answer, we cannot know for sure.

Towards rational energy policies

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"It is nonsensical to treat coal, gas, electricity, oil and nuclear energy as separate fuels without regard to the overall needs of Britain, and that is what has happened up to now. We should set up a National Fuel and Power Council to develop a coordinated and comprehensive policy for all the industries concerned".

It was in 1957 that I was involved with a small group of people, including some distinguished scientists, in having a look at, amongst other things, the future policy for energy for the United Kingdom. We had then, of course, no knowledge of what was going to come nearly twenty years later, but we did make that declaration and nothing that has happened since has shaken my belief in it. In fact the recent events in the Middle

East, which brought the western world almost to its knees and certainly quadrupled the price of oil, has strengthened my conviction of the importance of a permanent Energy Commission; its terms of reference today should be to make the United Kingdom completely independent of energy supplies from abroad at the earliest possible date.

North Sea gas and North Sea oil, which will begin to come into Britain very shortly, are additions to the other available energy sources that we have, the main one of course being coal, with nuclear power moving up. It is now generally accepted that the rundown of the coal industry during the 1960s was a grievous error. We made ourselves more and more dependent on outside sources for our energy requirements and not only handed over to the Middle East oil-producing countries control of our industrial production, but added considerably to our balance of payments problem. We now have the opportunity of complete independence and excellent energy export prospects. But this has to be carefully planned, because the relationship between coal, nuclear power, North Sea gas and North Sea oil should be so arranged as to maximise the life of oil and gas, while the opportunity is provided for nuclear power to make a gradual increase to the energy requirements of the nation.

First, I believe we must begin with the coal industry and ensure that its size is no longer dominated by the price factor, which determined its future from the latter part of the 1950s to date.

There should be established a target output for coal for the next 20 to 30 years. Coal production means long term planning. There is not only the question of planning the exploitation of the reserves and the constant replacement of the reserves to make good the annual extraction, but also the much more difficult and sensitive problem of planning for manpower requirements over several generations ahead. This involves immense human problems and social problems and cannot be adequately planned unless there is a guaranteed annual output.

There are two things that are absolutely essential if one is to maintain energy independence for all time—energy planning independent of Whitehall and international research on energy resources. The present method of planning by the Civil Service within a department is hardly likely to succeed. There must be serious doubts about the efficacy of the present machinery in Whitehall for resolving the kind of problems that emerge when one is considering four suppliers of energy, and the contribution that each have to make over the years ahead, and the life expectancy and economics of each of those sources of energy. If the government were wise, it would set up an Energy Commission right away to oversee the whole energy field and be responsible for advising ministers on strategy and tactics. If the Department of Energy decides to produce an energy policy from its own resources, it will assuredly fail as miserably as its predecessor failed with the Energy White Paper of 1967. The resources of the ministry are inadequate to do the enormous task of this long term planning.

The Civil Servants concerned with the writing of the 1967 policy are no longer there to face the consequences of the impotence of the 1967 White Paper. Some are retired, some are engaged elsewhere. The Civil Service approach to problems lacks continuity, and this is an inadequate approach. No *ad hoc* consideration can possibly perform the task of a continuing review. What is needed is an Energy Commission, whose task it would be to advise the government on energy policy and undertake comprehensive studies. The commission would be a continuing authority, staffed by experts in fuel economics and the specialist technologies. The commission, having no particular axe to grind, would be able to produce objectively an energy policy planned entirely in the national interest. It would do the total sum, taking into account all the knowledge now available as to the quantities of indigenous gas, oil and coal, and the expected contribution of nuclear power.

It would be able to ensure that capital investment was directed into those channels of energy development that would give the best return in national terms, rather than in a narrow advantage of one fuel over another. The infighting between the publicly owned energy industries would cease, as their place in the energy pattern would be properly established. Only an independent Energy Commission, in my view, would get the juxtaposition of coal, gas, electricity, oil and even nuclear energy about right. In an imperfect world, we cannot expect perfection although we can strive towards it; but like everything else, striving without the right tools would be a near hopeless task. An independent Energy Commission would be the right tool to do the job. There can be no doubt that such a commission would be able to create the plans and oversee the direction of those plans so as to provide the United Kingdom with independent sources of energy by the early 1980s.

The second very important approach must be to endeavour to secure an international research team into energy resources. Some time ago President Nixon set out on this trail, but I think failed because he invited too many countries to be involved. What I think is important is to set up an International Energy Council, whose purpose would be to mobilise the necessary teams for research. The object of the international research team would be to examine the possibilities of solar heat, oil and gas from coal, and oil from oil shale and tar sands. In these areas lie vast quantities of energy and although a good deal of the technology is already known, and makes a good starting point, a substantial amount of extra research work is required which is both costly in money and requires a very large group of fuel scientists and technicians. There is already a wide variety of work going on in Britain and in America to devise new routes for synthesising chemicals, oil and gas from coal. Research work in Britain has been concentrated on the development of the solvent extraction of coal and on digest extracts primarily as a route towards producing low volumes of high value and specialised products from coal. In America the basic theme of this work has been to apply solvent extraction and existing hydrogenation and other refining techniques to the synthesis of crude oil and natural gas as bulk sources of energy of raw materials.

These differing approaches have been dictated by the rather different relative economics of the coal and oil industries on either side of the Atlantic and by markedly different views about the security of petroleum supply.

Twenty-five years ago American energy planners had grasped the significance of the imbalance between gas and oil consumption and reserve availability. Historically the route towards chemicals and gas from coal has lain in coking. A typical feature, however, of traditional gas production from coke is the low calorific value—about 600 BTU per cubic foot—of the resultant product. Further notable features were the need for gas and coke markets to be in volume balance, and the highly fragmented localised nature of the coke-based gas-making industry enforced by maximum economic sizes of coke production units. In contrast, the discovery of natural gas has created a demand for gas of high calorific value, rich in methane, distributed from a relatively small number of sources through a trunk pipe line system.

As the years of North Sea gas begin to tail off, some quarter of a century from now, and where the demand for gas increases beyond economic production rates from North Sea sources, it will be necessary to synthesise what the Americans so aptly call pipeline gas, which can be blended in with North Sea supplies.

Already then we can see by an examination of the research work of the United States, and what is being done in Britain, that there is a great area of common knowledge and certainly a unified desire to retain energy independence. I believe, therefore, that it is highly important to press hard for international research work in these fields. There can be no doubt that the research scientists on both sides of the Atlantic will reach conclusions far quicker by working together than if they work independently.

The summary of my conclusions is, therefore:

- To reach complete independence for energy at the earliest possible date, and certainly not later than the early 1980s.
- To have an Energy Commission to advise the government on the way in which this objective can be achieved.
- To seek the greatest measure of international cooperation on research into the whole field of energy resources and energy uses in order to ensure that, despite the increase in energy demands year by year, world requirements of energy will be readily available.

North Sea oil in a world context

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Dr F. Howitt, of BP, assesses the oil and gas reserves which Britain can expect to exploit from the waters around its coastlines. Although supplies are limited, there seems to be some room for optimism: even if no more oil is discovered there may still be a small supply at the beginning of the next century.

DESPITE the fact that not a drop of indigenous regular production oil has reached UK shores from the North Sea, many people, myself included, are counting chickens. I want to put the results which have been obtained so far into the context of the present and potential production of the rest of the world. My statements and predictions are based on information collected by myself and my colleagues in the British Petroleum Company Limited.

Our conception of the general geological framework of the North Sea area is illustrated in Fig. 1. This shows the basins of sedimentation which developed after peneplanation following the Variscan orogeny (at the end of Carboniferous times) and which played the major role in providing the source and reservoir potential for most of the oil discovered so far. A conceptional cross-section illustrating stratigraphical succession, with major structural elements, relative thicknesses and current exploration 'plays' is shown in Fig. 2. Only very major faults and folds are illustrated, and Zechstein salt movement is idealised. The section is, however, drawn to scale particularly through chosen boreholes, although there is a vertical exaggeration of 60. The line of section is meant to go up the central median region, but in order to show major oilfield types it has been necessary to offset in places.

Occurrence of reservoirs

Despite the heavy exploration effort, during which 275 boreholes have been sunk since 1965 in the southern North Sea Basin, and the consequent discovery of some 42×10^{12} cubic feet of gas reserves, discoveries continue in the Permian Rotliegendes Sandstone. This is the same reservoir as that in the Leman Bank Field[1]. The gas is probably derived from the carbonisation of the coal in the underlying Coal Measures[2], and the structures are mostly sealed by overlying Zechstein salt[3]. One major gas discovery—the Hewett field—produces gas from two levels of Trias sands, in a structure that provided no seal at Zechstein level for the escaping Carboniferous gas. Naturally, the development of Rotliegendes sand facies to form the pre-Zechstein reservoir is a crucial general factor. Glennie[4] has argued plausibly that the reservoir sand formed essentially as a result of wind deflation of alluvial fans which spread northwards from the Variscan mountains.

Drilling in the northern North Sea Basin (about 200 exploration wells since 1969) has shown that three major 'plays' exist. The first is in the BP Forties Field, where there is oil in the Palaeocene sandstones[5]. This also seems to be the case in the nearby Montrose Field. Gas and condensates reportedly has been found also in the Tertiary sandstones of the Frigg, Heimdall and Cod Fields. The second 'play', which was the first to be discovered, is oil from the domed and fractured Upper Cretaceous and Danian chalks in the Ekofisk group of fields in Norwegian waters[6], and in the Dan Field in Danish waters. The third 'play' is oil from both the Jurassic sandstones in the far northern waters, where the Brent Field was the first discovery, and apparently

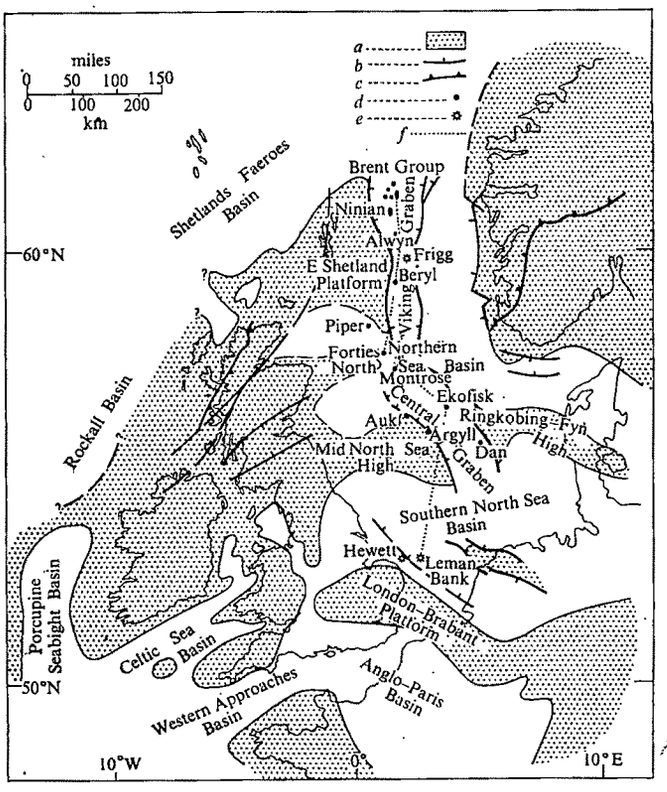


Fig. 1 Sedimentary basins around the British Isles, and the North Sea geological framework since the end of Carboniferous times. *a*, Stable pre-Permian areas; *b*, major faults; *c*, thrust faults; *d*, oil fields; *e*, gas fields; *f*, line of section of Fig. 2.

from the Moray Firth Basin, where Piper Field has been discovered. A further but smaller series of fields seem to be associated with older Palaeozoic rocks where they come into contact with unconformable Cretaceous rocks.

One striking fact revealed by the map of the location of all these oil discoveries, is that they lie within, or close to the edge of, the central, down-faulted area. The fault system is more complex than the simple graben representation (Fig. 1). Tensional and collapse forces developed throughout Middle and Upper Jurassic times after a regional central uplift. The tension was accompanied by volcanicity, mostly of Bathonian age, with at least one centre just to the north of the Forties oilfield. Individual fault blocks remained as positive areas until the end of the Cimmerian movements, after which most of them were covered by Cretaceous sediment. Relative upward movement continued in several instances, however, and some of these structures have uppermost Cretaceous resting on early Mesozoic or even older rocks. This major and complex unconformity is responsible for trapping the oil of the northern area in the underlying structures of Jurassic or earlier rocks. Fortunately, within the Viking graben, the Cretaceous overlying the unconformity is an almost continuous shale and mudstone facies, unlike the ubiquitous chalks of the south. Whether the hydrocarbon source is also in part the Cretaceous shales at the unconformity, or whether it is from indigenous Jurassic shale, is not certain. All of the oils, however, whether from Palaeocene, Cretaceous, Jurassic or earlier reservoirs, have similar properties. The sulphur content is of a low order—

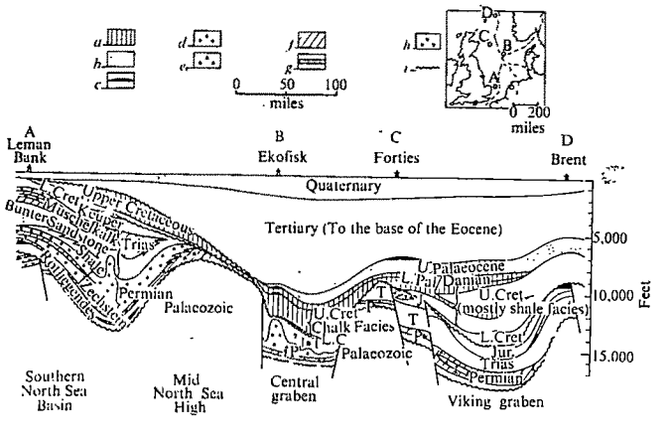


Fig. 2 Generalised and diagrammatic cross section (see Fig. 1) of the North Sea, from the Leman Bank gas field (south) to the Brent oilfield (north). *a*, chalk; *b*, sand or sandstone; *c*, oil or gas field; *d*, halite; *e*, anhydrite; *f*, dolomite; *g*, Coal Measures; *h*, volcanics; *i*, major unconformities. The horizontal scale is shown; vertical exaggeration ×60.

0.2–0.9% by weight—and asphaltenes are about 0.3–0.6% by weight. Gravities are in the high range (35–44°API, with most restricted to 35–37°API). Wax content is fairly high at 6.5–8.5% by weight, and the trace metals nickel and vanadium are lower than 2 parts per million (p.p.m.). The crudes give good yields of light and middle distillates but are unsuitable as a source of heating oils and bitumen. Gas contents are variable even between adjoining structures. Some are full of wet gas whereas others have oils that are undersaturated, and some reservoirs will therefore require supplementary energy so that the reserves can be recovered.

Estimating the reserves

Our estimates of proved reserves of oil are based largely on published information, including stated offtake plans and

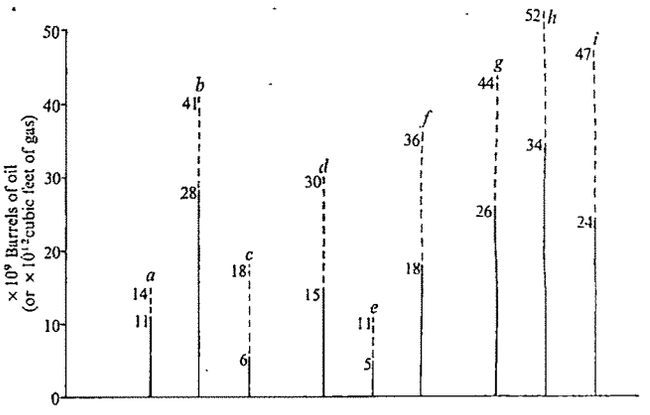


Fig. 3 Recoverable oil and gas reserves in the North Sea. *a–c*, Established reserves; *d–f*, additional reserves; *g–i*, potential ultimate reserves. *a*, Oil; *b* and *c*, non-associated gas, south and north respectively; *d*, oil and gas liquids, *e* and *f*, non-associated gas, south and north respectively; *g*, oil and gas liquids, *h* and *i*, non-associated gas, south and north respectively. Unbroken lines, reserves in UK waters; dashed lines, reserves in the remainder of the North Sea (in both cases: heavy lines, oil; fine lines, gas).

production potential, and they are supplemented from our own borehole and geophysical knowledge of the various structures. Table 1 lists the fields and discoveries, with estimates of recoverable reserves.

Of these discoveries only the main Ekofisk and Dan Fields are currently on production, but all of the named oilfields, with the possible exception of Josephine and Maureen, will probably be producing by some method by 1978. Supposing that the present commercial viability of discoveries depends upon sustained production capabilities of greater than 30,000 barrels of oil a day (or alternatively, 100 million cubic feet of gas a day), and considering that the discoveries of the northern North Sea have taken 100 exploration wells in the UK sector and 80 in the Norwegian sector, then the present success ratio is unusually high, at 1:6 (assuming that each of Ekofisk group of fields is a separate commercial discovery). It is probable that some of the wells were drilled only for stratigraphical information, and some may have been drilled with little

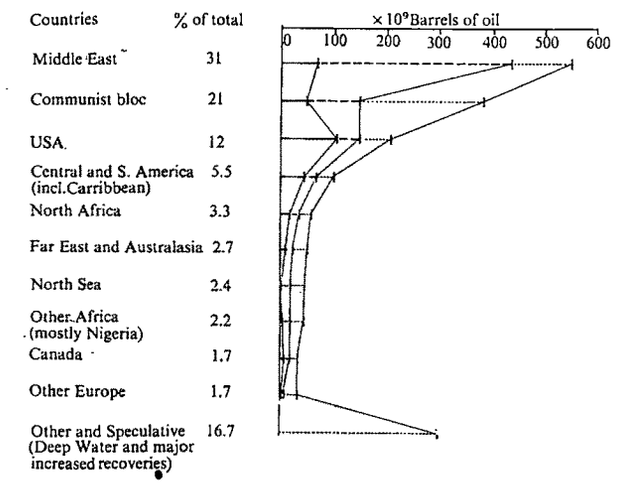


Fig. 4 World oil production, showing remaining reserves and possible future discoveries. Solid lines, produced reserves (300×10^9 barrels); dashed lines, remaining reserves (600×10^9 barrels); dotted lines, possible future discoveries (900×10^9 barrels). The potential ultimate reserves therefore total $1{,}500 \times 10^9$ barrels.

hope of success, only as a commitment to government for obtaining the licence. Some even, were probably drilled in the wrong place because of mistaken concepts of geology or geophysics. I assess that of some 220 prime prospects of present 'plays', about 85 have been explored. The proved reserves resulting from these tests in both Norwegian and UK sectors of the northern North Sea are >14 (for instance, 15)$\times 10^9$ barrels of oil, 18×10^{12} cubic feet of unassociated gas, and >1 (for instance, 2)$\times 10^9$ barrels of condensate (see Table 1 and Fig. 3). Using these statistics the ultimate reserves would seem to be 39×10^9 barrels of oil, 47×10^{12} cubic feet of unassociated gas, and 5×10^9 barrels of condensate (Fig. 3). Most of the major structures in the southern North Sea Basin have been examined, but remaining reserves there, although difficult to find, may amount to 11×10^{12} cubic feet of gas (Fig. 3).

Another, somewhat suspect method of reserves analysis, is based upon the volumes of basin sediment down to the economic basement. Weeks[7] categorised offshore sedimentary basins out to a water depth of 1,000 feet according to favourability for petroleum resources by area. His category A basins totalled 188,000 miles^2, and the average basin thickness was 2 miles, giving a total volume of sediment of 376,000 miles^3 in these basins. Weeks estimated that the A basins contained potential liquid petroleum reserves of 170×10^9 barrels recoverable by primary production methods, and, therefore, that average potential was 448,000 barrels a cubic mile. Similarly, his lower category B basins, of 1,657,000 miles^2, have an average potential of 160,000 barrels a cubic mile. The volume of sediments in the northern North Sea Basin is about 94,000 cubic miles down to economic basement. Assuming that this is a category A basin, there would be an ultimate recovery of 42×10^9 barrels of petroleum liquids. The close similarity of this result with my figure of 44×10^9 barrels of petroleum liquids is comforting, although perhaps fortuitous, as both methods rely on very doubtful criteria.

Our calculations of world oil production, reserves, and future possibilities split into well defined regions, are illustrated in Fig. 4, based on the work of Warman[8]. Since his calculations, more oil has been produced, of course, and a little more has been discovered, but our estimates of future possibilities are slightly less. Now, of the grand total of $1,800\times 10^9$ ultimate barrels, 300×10^9 barrels have already been produced. We believe, therefore, that some $1,500\times 10^9$ barrels could remain, of which 600×10^9 are proved reserves. Again, of the possible $1,500\times 10^9$ barrels, about 800×10^9 barrels are the remaining and possible future reserves of the Middle East and Communist bloc countries. A further 300×10^9 barrels could come from reserves in continental shelf areas in waters deeper than 1,000 feet, or from speculative and unforeseen breakthroughs in, for instance, primary recovery processes. The rest of the world therefore seems to have remaining and possible future reserves of 400×10^9 barrels, of which 140×10^9 have been proved and 260×10^9 are possible. The North Sea, with established reserves of more than 15×10^9 barrels of petroleum liquids and about 30×10^9 potential barrels would seem to rank well with the rest of the world apart from the Middle East and Communist countries (10–11%).

Table 1 Possible commercial discoveries in the North Sea

Country and block	Field	Estimate of recoverable reserves		
		Oil $\times 10^9$ barrels	Condensate $\times 10^9$ barrels	Gas $\times 10^{12}$ cu. ft.
Terhary 'play'				
Norway 7/11	Cod			
Norway 25/1 and UK10/1	Frigg			
UK 23/1	Lomond			
Norway 25/4	Heimdall		>1.0	>18.0
?UK 3/19	(Total *et al.*)			
Norway 25/2	(Petronord *et al.*)			
Norway 30/10-2	(Esso)			
UK 22/18	Montrose			
UK 21/10	Forties			
UK 16/28	Maureen	2.35		
Cretaceous 'play'				
Norway 2/4, 2/5 and 2/7*	Ekofisk Group	3.20		
Denmark	Dan			
Jurassic 'play'				
UK 211/29	Brent			
UK 9/13	Beryl			
UK 211/18	Thistle			
UK 211/26	Cormorant			
UK 15/17	Piper			
UK 211/23	Dunlin	>8.10		
UK 3/14a	Alwyn			
UK 211/28	Hutton			
UK 2/5	(Union *et al.*)			
UK 3/15	(Total *et al.*)			
UK 3/8 (and 3/3)	Ninian			
Norway 33/12	(Mobil *et al.*)			
Others				
UK 30/13	Josephine			
UK 30/16	Auk	0.35		
UK 30/24	Argyll			
	Totals	>14.0	>1.0	>18.0

* Includes Ekofisk, Eldfisk, Torfelt, W. Ekofisk, SE Tor, Edda, and Albuskjell.

Table 2 League table of oil producing countries in 1973

Country	Average production (millions of barrels a day)
USA	9.2
USSR	8.4
Saudi Arabia	7.6
Iran	5.9
Venezuela	3.4
Kuwait	3.0
Libya	2.2
Nigeria	2.1
Iraq	2.0
Canada	1.8
Abu Dhabi	1.3
Indonesia	1.3
Algeria	1.1
Others (less than 1 million barrels each a day)	6.2
Total	55.5

Future offtake patterns

Turning now to offtake potential to the end of the century, Fig. 5 shows what we believe could be the UK build-up pattern of yearly production, based on the proved reserves of 11×10^9 barrels of oil, and supplemented by the assessed additional reserves of 15×10^9 barrels still to be discovered. Superimposed is the Norwegian picture, built up from the 3×10^9 barrels of established reserves, supplemented by the assessed additional reserves. The patterns are built up from considerations of the time needed to prepare the facilities which are necessary for commercial production from particular fields after their discovery. Furthermore, in normal individual fields it is impractical and undesirable to produce at rates exceeding 10% of remaining reserves per annum. In most cases, if this rate is exceeded the ultimate recovery is decreased. It is, in fact, more realistic to anticipate a reserves production ratio not exceeding 15:1. The graphs of Fig. 5 take these factors into account in the total indicated for each year.

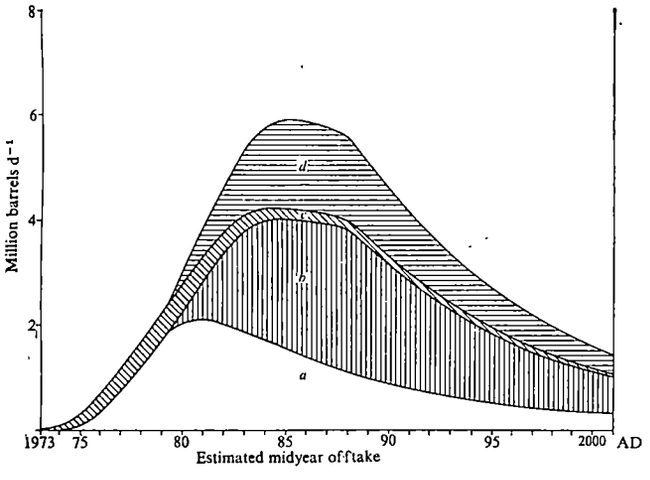


Fig. 5 Possible oil offtake pattern from the North Sea until 2,000 AD. *a*, UK offtake of proven reserves; *b*, UK offtake assuming a maximum discovery rate; *c*, Norwegian offtake of proven reserves; *d*, Norwegian offtake assuming a maximum discovery rate.

The present league table of individual producing countries, including the Middle East giants, is shown in Table 2, which has been derived from average figures for 1973 (ref. 9). Figure 5 shows that the UK will join this league before the end of 1978, and should remain in it until 1988, even if no more oil is discovered. If our estimates of finding the postulated undiscovered reserves are correct, then we shall still be producing about 1 million barrels of oil a day at the turn of the century.

In addition to the amount of oil present in the ground, the offtake pattern also depends upon the will of governments and peoples to extract it. The rate of extraction depends upon the availability of material, financial and labour resources—steel and concrete platforms, drilling units, well completion systems, separators, pipelines laid in deep waters, treatment and storage facilities, and so on—which are necessary to get the oil to the user. The unit cost of development is now running at £1,200 a barrel/day potential and, if inflation continues at the present rate, could escalate at 10% per annum. If the not inconsiderable exploration costs are included, then by 1982 a total expenditure of some £13,000 million will have been necessary to finance the oil development of the North Sea alone if all other resources, and the will, are available[10].

The possible developments on the western shores of the UK–Celtic Sea, Western Approaches, and west of the Shetlands, not to mention further out in the Atlantic, have not been considered here, so a little imagination is needed to increase the various parameters. Until a viable discovery is made I prefer to keep all options open. Reserves may be in proportion to those in the North Sea whereas, on the other hand, the various basins could be virtually empty of hydrocarbons, or contain only gas. Even using the suspect techniques of considering sedimentary volumes, however, the reserves do not seem very high. For instance, the W Shetlands basin out to a water depth of 1,000 feet contains about 13,000 miles^3 of sediment, and if this is a Weeks' category A basin it would contain 5.8×10^9 barrels of petroleum liquids. On the other hand, if it is a Weeks' category B basin it would contain only 2×10^9 barrels. Similarly, the Celtic Sea with about 9,800 miles^3 of sediment could contain 4.4×10^9 or 1.6×10^9 barrels, respectively. Even these theoretical and probably optimistic amounts do not significantly affect world statistics, and only increase my guess at the ultimate reserves of the UK sector by 14–40%. They are included in the category Other Europe in Fig. 4.

In painting this rosy picture of imminent oil availability to the UK, in these days of awareness of a possible world energy shortage, I do not wish to create a complacent and parochial attitude of "I'm all right Jack". I believe that the fortunate accident of nature that has provided these resources on the doorstep of the UK, also provides an opportunity to study and resolve the problems of longer term energy requirements. Nevertheless, even with good husbandry and no increase in requirements, the reserves I have postulated are finite and will have been substantially depleted by the turn of the century.

I should like to thank the Chairman and Board of Directors of British Petroleum for permission to publish this article.

1 Kent., P. E., and Walmsley, P. J., *Bull. Am. Ass. Petrol. Geol.*, **54**, 168–181 (1970).
2 Patijn, R. J. H., *Erdöl Kohle Erdgas Petrochem.*, **17**, 2–9 (1964).
3 Brunstrom, R. G. W., and Walmsley, P. J., *Bull. Am. Ass. Petrol. Geol.*, **53**, 870–883 (1969).
4 Glennie, K. W., *Bull. Am. Ass. Petrol. Geol.*, **56**, 1048–1071 (1972).
5 Thomas, A. N., Walmsley, P. J., and Jenkins, D. A. L., *Bull. Am. Ass. Petrol. Geol.*, **58**, 396–406 (1974).
6 Dunn, W. W., Eha, S., and Heikkila, H. H., *Oil Gas J.*, **71**, No. 3, 122–128; No. 4, 90–93 (1973).
7 Weeks, L. G., *Bull. Am. Ass. Petrol. Geol.*, **49**, 1680–1693 (1965).
8 Warman, H. R., *Geogrl J.*, **138**, 287–297 (1972); and in *World Energy Supplies* (Financial Times. London, 1973).
9 Anon., *Oil Gas J.*, **72**, No. 9, 138 (1974).
10 Birks, J., in *The North and Celtic Seas* (Financial Times, London, 1973).

Huge resources needed to exploit shale oil

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Although there is plenty of shale oil in the United States, treatment at the surface of the rock that contains the oil would require prohibitive amounts of water. But the alternative, production in situ, *is not yet a well developed technique.*

EARLY this year two oil companies together bid $210 million to lease from the federal government 5,000 acres of arid land in the remote north-west corner of Colorado. At that price, the land is almost as expensive as a prime piece of suburban real estate, and when the bid was announced it surprised not only much of the oil industry but also some of the government officials responsible for leasing it.

The reason why this rugged, largely unspoiled, part of the Rocky Mountains has suddenly become a boom area is simple: it contains more oil than the Middle East. The oil is locked into a band of rock, popularly known as oil shale, which varies from a few feet to about half-a-mile in thickness. According to the United States Department of the Interior, a region of 25,000 square miles which straddles the borders of Colorado, Wyoming and Utah contains about 1.8×10^{12} barrels of shale oil, about a third of which is in deposits rich enough to be exploited.

Already, the promise of this vast petroleum reserve has sparked off gold-rush fever in the small ranch towns in the area—motels are filled to bursting and the population is booming. The federal government, which owns more than 70% of the richest shale lands, is being pressed to speed up its commercial exploitation, and several newspapers—including the London *Times*—have suggested that Rocky Mountain oil shale could be the answer to the energy crisis, not only for the United States but for western Europe as well.

Unfortunately, however, such predictions are grossly over-optimistic, for there are several reasons why the Rocky Mountains will never upstage the Middle East in the international crude oil market, and in any case it will be a long time before shale oil starts flowing through pipelines and heating furnaces. Nevertheless, there is no doubt that shale oil could eventually supply a significant amount of the energy demand of the United States, and there is also no doubt that shale oil production will be accompanied by massive destruction of the environment.

The lease sale this January, which brought the staggering $210 million bid, was the first tentative step in a programme designed to test whether or not the shale fields should be thrown open for commercial development. A total of six such tracts of land have now been put up for lease to private industry—two each in Colorado, Wyoming and Utah—to test various methods of getting shale oil out of the rocks and to give the federal government an opportunity to see what environmental consequences are likely to result from complete commercial exploitation of the shale fields. If the tests prove successful, and if the environmental consequences are deemed 'acceptable', the Department of the Interior, which manages the lands, reckons that up to 1 million barrels of shale oil a day could be flowing out of the Rockies by 1985.

Although that amount is not to be sniffed at, it will represent less than 5% of anticipated demand in 1985 and according to several recent studies it could be close to the physical limit of production capability. The test operations being carried out both on the leased federal lands and on some private land will probably determine the likely potential for oil shale, but much will also depend on the extent to which the federal government becomes involved in the enterprise.

Deposits and extraction

It has long been known that massive deposits of oil are contained in the sedimentary rocks of an area known as the Green River Formation, and in fact some oil was produced from shale even before liquid petroleum was discovered in 1859. But in spite of about thirty years of research and development by the oil industry and the federal government, it has been uneconomic to produce shale oil on a commercial scale for the simple reason that the cost of extracting it from the rocks meant that shale oil could not compete economically with natural petroleum. But the economics were suddenly altered when the price of crude oil began to skyrocket and the Nixon Administration launched its pell-mell drive to make the United States self sufficient in energy.

Shale oil is locked into rock in the form of a solid hydrocarbon called kerogen. To get it out requires the rock to be heated to about 900° F (480° C) and the vapours that boil off to be collected. The result is a viscous, low gravity, moderate sulphur and high nitrogen crude oil which will probably have to be 'upgraded', partially refined, before it is piped to refineries.

Two different approaches to getting oil out of the sedimentary rocks of the Green River Formation have been tested so far, albeit on a modest scale. One approach involves mining the oil-bearing rocks, crushing them and heating them in a surface retort, and the other involves mining or blasting out an underground cavern and heating the shale in place by burning gases. The former method has been more extensively tested, and is reckoned to be closer to commercial application, but the second method, the *in situ* approach, could have significant advantages if it proves feasible on a large scale.

Three chief types of surface retort have been tested, all of which have been operated at the level of 1,000 tons of rock a day. Of these, it is reckoned that a design piloted by the Oil Shale Corporation (TOSCO) is closest to commercial application, but all of them could be scaled up to production levels over the next few years, according to a report prepared recently by the Atomic Energy Commission (AEC) for the Federal Energy Office.

The TOSCO process has as its central feature a rotary kiln into which is fed preheated shale crushed into pieces less than 0.5 inch in diameter. Retorting is carried out by means of externally heated ceramic balls, which are later separated from the spent shale and reheated. The products are drawn off and filtered. According to TOSCO, the process produces an excellent yield, a claim supported by the AEC study, and there should be little trouble in scaling it up to commercial production.

Another process being actively pursued by a number of oil companies is the so-called gas combustion method. This makes use of a vertical retort through which crushed shale moves downwards and gases, fed in at the bottom, move upwards. Air and some additional recycled gases are fed into the retort at a point about one-third of the way from the bottom, which causes the gases to burn. This retorts the shale, and oil vapours and gases, cooled by the incoming shale, emerge from the top. According to the Department of the Interior, "of the numerous retorts studied in the Bureau of Mines program, the gas combustion retort gave the most promising results". Seventeen major oil companies have recently begun to test a modification of the gas combustion process, and according to the AEC study, it will be about three years before the process reaches the stage that the TOSCO process has reached now.

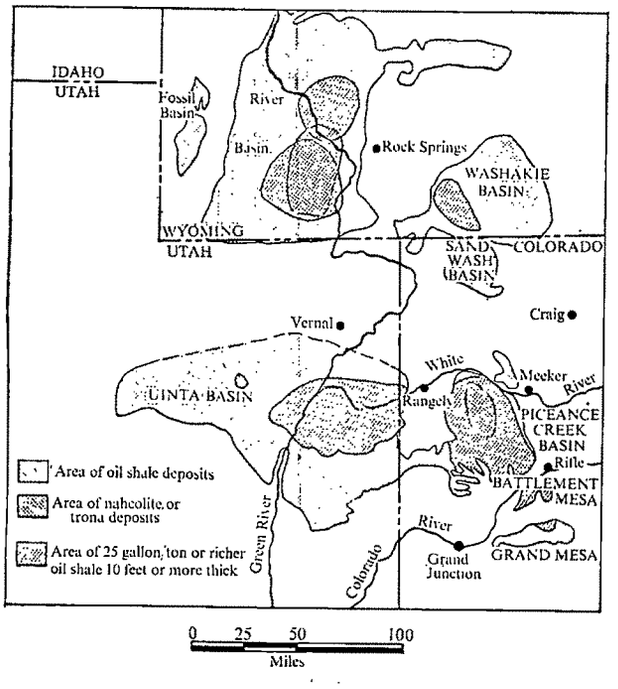


Fig. 1 Oil shale areas in Colorado, Utah and Wyoming.

The third method, the Union Oil Company process, also uses a vertical retort with a countercurrent flow of shale and gas, but in this case the shale is fed in through the bottom. Air is pumped in at the top of the retort and heat is supplied by the combustion of carbonaceous residue which remains on the spent shale. The process was tested in 1957 and 1958 on private land in Colorado, and Union Oil recently announced that it is planning to construct a 50,000-barrel-a-day plant which could go into operation by 1979.

Although the retorts are the only pieces of new technology used in the surface processing of oil shale, the scale of commercial operations will probably be limited by other factors, chief of which is the massive amount of mining that will be required to support even a 1-million-barrel-a-day enterprise.

The shale will be mined either by open pit methods (when it lies close to the surface) or by the room-and-pillar method, but whichever is employed a simple calculation indicates that it would have to be on a truly staggering scale. Assuming that the shale used in the surface retorts contains 30 gallons of oil per ton (one US barrel is 42 gallons), to extract 1 million barrels of shale oil a day with a recovery rate close to 100% would require the mining of about 1.6 million tons of rock. That is an enterprise similar in scale to the entire United States coal industry.

Environmental problems

A direct consequence of this massive mining operation is that there will be an equally massive amount of processed shale to dispose of once the oil has been removed. Since the crushing and retorting operation greatly expands the shale, the spent material cannot be simply fed back into the mines, and so it will be dumped into canyons (and revegetated?).

The spent shale will be bone dry and very dusty, and consequently it will not be easy to get rid of it. It is this aspect of the oil shale operations which most troubles environmental groups, and before the Department of the Interior decides to throw the entire region open to commercial development, it will have to be certain that the spent shale can be dumped and revegetated without undue environmental disruption, however that may be defined.

A second, and ultimately more intractable problem for a large oil shale industry, is the shortage of water in the Green River Formation. According to an environmental impact statement published by the Department of the Interior in August, 1973, a 1-million-barrel-a-day operation would require between 107,000 and 170,000 acre feet of water a year, and municipal development associated with industrialisation of the area would require another 14,000–19,000 acre feet of water. The impact statement suggests that about 340,000 acre feet of water is potentially available for oil shale operations.

This clearly does not give much room for manoeuvre and the AEC study flatly states that "the ultimate rate of production of an oil shale industry may be limited by the amount of water available, although the competition for water should not be severe for a 1-million-barrel-per-day industry". The study adds "a 3 to 5 million-barrel-per-day industry could possibly be established if all the water in the oil shale regions of Colorado, Utah and Wyoming were made available for oil shale development". That would mean, however, that agriculture, minerals processing and other energy industries such as the production of coal gas would have to be severely curtailed.

The chief consumers of water in the oil shale production process are the disposal of spent shale and the upgrading of shale oil so that it can be transported by pipeline. According to the Department of the Interior's impact satement, those two processes between them would use up about half of the water consumed in oil shale production.

Thus if water consumption and mining requirements can be reduced, the potential for oil shale production would be greatly expanded. And that is precisely why the *in situ* process is now being looked at very carefully.

Processing *in situ*

The first thing to be said is that *in situ* technology is not as advanced as surface processing technology, and in spite of some extraordinary claims made by companies involved in *in situ* research and development, there are many problems to be overcome before it can safely be assumed that the technology is ripe for commercialisation.

Nevertheless, one company in particular, Occidental Petroleum and its research arm, Garrett Research and Development Company Inc., has recently carried out a test of an *in situ* process which seems promising. According to Mr Richard D. Ridley, Garrett's manager of oil shale research, the process works like this. First, a room is mined out of the rock beneath and/or above the oil shale. The rock is brought to the surface, where it is either retorted in the conventional manner if it is rich enough in oil shale, or simply dumped and revegetated. The next step is to load explosive charges into deep holes drilled into the walls of the excavated rooms and fire them off so that as the oil shale breaks it will fill the room with tightly packed oil shale rubble.

The final stage is to retort the oil shale in place. This is accomplished by drilling a hole into the top and bottom of the rubble-filled cavern and circulating air through it. Initially, combustion is caused by pumping in natural gas, but eventually carbon left on the shale after it has been retorted provides the fuel. Shale oil runs into a sump at the bottom of the cavern, and it is eventually pumped to the surface.

Occidental has already tested its process on private land in Colorado and it will be conducting a larger test later this year. According to Ridley, if that test is successful, Occidental will be ready to move into commercial production. There are a few problems, however. For one thing, it has been suggested that the *in situ* process will cause subsidence, a criticism which Ridley flatly denies but which has been made by knowledgable oil industry experts in testimony before the House Science and Astronautics Committee. Another problem is that some of the oil shale is overlain by an aquifer which must be kept isolated from the underground retorting process. Again, Ridley claims that this will be no problem but other oil industry officials are not so optimistic.

Speed of exploitation

One indication of the fact that most of the oil industry is not ready to move into *in situ* processing is that of the six tracts of land put up for lease by the federal government, the two which are suitable for *in situ* methods failed even to attract a single bid. The Department of the Interior is now wondering whether to offer them for sale again, but in any case the lack of interest in the two tracts has already led to calls for increased government support for the development of *in situ* technology.

Strong arguments are in fact being raised for the federal government to speed up the exploitation of all the shale lands by immediately leasing more federal land than the six prototype tracts and by providing a variety of financial incentives to the oil industry, such as loans, price guarantees or tax breaks. Moreover, a bill has already been passed by the Senate which would, among other things, establish a joint government–industry corporation to build demonstration oil shale plants; a similar measure is now under discussion in the House of Representatives.

The AEC study recommends that since the potential for *in situ* plants is so high and since the technology is at present not very well advanced, the federal government should concentrate its efforts in this area by assisting industry to establish a plant capable of producing 30,000 to 50,000 barrels of oil a day, to demonstrate the technology on a commercial scale. "If construction began in mid-1975 such a plant could be in operation by mid-1977 and, if successful, could lead to an industry production capability of about 1.8 million barrels per day by 1985," the report says. And the AEC study also suggests that although there is every indication that industry will move by itself to establish commercial-scale surface processing plant, "there are no assurances that significant production by 1980 will result without a major role being played by the government".

Japan and the energy crisis

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The central problem facing Japan is how to reduce her growing reliance on imported oil.

FOR many years Japan had a plentiful supply of energy because of her extensive water resources and domestic coal. Towards the end of the 1950s, however, the import of petroleum (a relatively cheap commodity at that time, supplied mainly by countries in the Middle East) increased rapidly to meet the needs of industrial growth. The way in which the proportions of primary energy supplied by various means have changed over the years is shown in Table 1.

Petroleum replaced coal as the chief source of energy in the early 1960s and Japan entered the 'oil era.' Until the recent oil crisis, there was no difficulty in importing cheap oil from abroad and Japan's long coastline, with many good harbours, helped to keep the price relatively low.

The problem in Japan as regards energy supply thus has three major aspects. First, the dependence on oil imports has become so high that the supply is unstable and easily affected by the political and economic situations outside Japan. This is made even worse because more than 90% of the imports came from one region of the world.

Second, the absolute amount of petroleum consumed has become so high that Japan is consuming about 10% of that available to the whole world in spite of the fact that its population is less than 3% of the world total. There is no assurance that Japan will be able to maintain such a large share in the future.

Japan has an area of only about 400,000 km², about 4% that of the United States, and is consuming about one-third of the energy being consumed in the United States. That means that the density of energy (per unit area) is about nine times that of the United States; it also means that the degree of air pollution may be some ten times as great if the measures for preventing pollution are the same.

Japan was faced with a shortage of oil immediately after the declaration of an oil embargo by the Arab countries. Fortunately, the shortage was not too serious as regards quantities but it caused a sharp rise in the cost of oil, which is making itself felt in the cost of all energy sources. The electric power utilities, for example, are proposing to raise the price to the consumer by about 60% on average, which will have serious effects on the whole economy.

Thus Japan has been forced to reconsider the whole system of energy supply, taking the problems mentioned above into account. The first problem to be faced is the saving and the

Table 1 Supply of primary energy in Japan

	1955	1960	1965	1970	1975 (estimated)
Hydroelectric power (%)	21.5	15.3	11.3	8.3	6.6
Coal (%)	49.2	41.5	27.3	21.4	16.3
Petroleum (%)	20.2	37.7	58.4	67.2	72.8
Nuclear power (%)	0	0	0	0.6	2.4
Dependence on imports (%)	24.1	44.3	66.2	76.4	82.4
Total energy* (10^4 kl)	5,125	8,438	14,576	33,100	44,700
Ratio	1	1.65	2.84	6.46	8.72

* Petroleum equivalent, 10,000 kcalorie l^{-1}.

effective use of conventional energy. In considering the measures needed, a detailed analysis of the structure of the consumption of energy in the country has to be made. Tables 2 and 3 show the general structure estimated by the Ministry of International Trade and Industry (MITI) as of 1972.

Details of the measures for saving energy and using it effectively are being developed in industry and research institutions with the support of the government.

The second problem in overcoming the shortage and unstable supply of energy is the utilisation of domestic coal. The coal reserves in Japan are estimated to be about 20×10^9 tons. In the early 1950s, annual production reached 50 million tons a year but the increasing importation of cheap oil has meant that coal production has decreased gradually to the present rate of only 20 million tons a year.

Increasing the use of coal raises two difficulties. First, a huge investment will be necessary to increase the annual production again. The importation of coal is a possibility, but transportation and the requirement of a stable supply may cause problems. The second difficulty is air pollution. As a means of avoiding this, the gasification or liquefaction of coal are being studied. Both are still in the stage of basic research, and the government has started a development programme this year.

The third measure is the exploitation of new energy resources. Leaving aside nuclear energy, which is discussed later, development of various new resources is included in various government projects. There are many active volcanoes in Japan, for example, so there is plenty of potential geothermal energy.

Table 2 Structure of the demand for energy (%)

	Petroleum	Electricity	Others	Total
Manufacturing industries	32.11	15.33	17.41	64.85
Transportation	11.64	0.81	0.95	13.40
Home use	7.96	7.51	3.56	19.03
Others	—	—	—	2.72
Total	52.47	25.13	22.40	100.00

The development of this source of energy has a long history, but because of economic and technical difficulties, only two electricity generating stations are in operation: they have a total capacity of about 30 MW. In order to proceed any further, much developmental study is necessary, for example on deep well technology.

The government has also taken up the use of solar energy as its next energy project. Assuming that 1% of the solar energy falling on Japan is used at an efficiency of 10%, solar energy could provide the wherewithal to generate about 40 million kW of electricity. In Japan there are 2.5 million sets of solar water heating units installed in homes—equivalent to a few hundred thousand kW of electrical heaters. In combination with city gas or electricity, these units may be developed to more efficient devices for home heating and cooling. Other devices such as solar batteries and solar heaters for generating steam are also being examined.

One of the most important aspects of energy in the future is the protection of the environment. To this end, the use of hydrogen as a carrier of energy is under study as well as other means of obtaining clean energy, such as the removal of sulphur and nitrogen oxides from the exhaust gases arising from the combustion of petroleum and other fuels.

The work on the development of solar and geothermal energy, utilisation of coal and the use of hydrogen are all being carried out as part of a project called "Sunshine" which is a government-supported five-year programme. It has just been started and the budget for the fiscal year 1974 is about 2.3×10^9 yen (about $7 million).

Except for solar energy, however, there is a limit to the amount of energy available in each category and it is not high enough to meet the future needs of Japan. The snag with solar energy is that it will be a long time before it can be used in large quantities.

The deduction from all this is that nuclear energy is the only effective substitute for petroleum that is likely to be available this century. In Japan, the current installed capacity of nuclear power plant is some 1,750 MW, and some 15,000 MW more is under construction.

Recently, Mr S. Inaba, a Commissioner of the Japanese Atomic Energy Commission, estimated the structure of energy supply up to 1985 (see Table 4). The data given there do not reflect any official decision, but are estimated by Commissioner Inaba on various assumptions such as stable growth of the economy, the possible development of each category of energy and so on. The energy development programme is under discussion in various government departments and the long term plan for the programme is expected to be formulated this year. In any event, it is considered that nuclear energy will have a key part to play in the future Japanese energy scene.

Table 3 Demand for petroleum classified by end use as of 1971

	%		
Power	17.8		Mainly transportation
Heat	66.1	28.4	Industries
		22.3	Electric power generation
		15.4	Home use and so on
Material	12.8		
Others	3.3		
Total	100.0		

In developing nuclear power, however, there are many serious matters to be considered which are peculiar to Japan. Safety regulations for nuclear plants are very strict because of the high population density and the necessity for designing equipment that is resistant to earthquakes. Radioactivity pollution is a serious hazard especially because of the active off-shore fisheries. There are no parts of the coasts of Japan that are not concerned with fishing industries. It is also difficult to find suitable locations for fuel reprocessing and waste disposal.

Table 4 Estimated future structure of the energy supply in Japan

	1972 (Actual)	1975	1980	1985
Total supply (Petroleum equivalent, 10^6 kl)	366	447	603	800
Annual increase (%)	10.7	6.3	6.2	5.8
Petroleum (10^6 kl)	258	301	393	497
% of total supply	75	72	69	66
Coal (% of total supply)	17	17	15	13
Hydroelectric (% of total supply)	6	5	4	3
Nuclear power (10^6 kW)	1.8	7.4	28	60
Generated energy (kW h)	9.5	49	184	394
% of total supply	1	3	8	13
Others (% of supply)	1	3	4	5

As with other natural resources, Japan is short of indigenous uranium. Therefore, she has to develop nuclear power plants which use uranium as economically as possible. Accordingly, a demonstration power reactor of the heavy water type (165 MWe capacity) is under construction. There is also a fast breeder reactor programme. A liquid metal cooled experimental breeder reactor (50 MW thermal) is under construction and a demonstration fast breeder reactor (300 MWe) is being designed.

Japan has a large ship building industry which is interested in a future programme involving nuclear ships. A nuclear cargo ship of about 8,000 tonnes and with a nuclear reactor of 36 MW thermal has been completed and is awaiting sea trials.

Nuclear fusion is a promising field in the long term. In Japan, research has long been concerned with the basic side, that is, the

study of plasma. Recently, the research has become oriented towards the practical realisation of fusion and more resources are being allocated to it.

The present budget of the government for all the nuclear projects, including fusion, is about 67×10^9 yen (about $230 million) and it is estimated that industry is spending about the same amount of money. About 15% of the governmental budget is devoted to programmes of safety research and development.

Europe in turmoil finds time to discuss energy

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Compared with the United States, which imports only a few per cent of its total energy needs, and the Soviet Union, which is a net exporter of energy, Europe is heavily dependent on outside supplies.

IN Europe the nine countries of the economic community are in the process of piecing together an energy policy. Briefly, the plan calls for a reversal of Europe's energy supply position. Instead of 60% of Europe's needs coming from outside and 40% from indigenous sources as at present, the plan says that by 1985 only 40% should be imported and 60% originate within the community.

The reasoning is that the Nine will then have a greater measure of control over the energy received from outside and the price they have to pay, although clearly Europe will continue to be at the mercy of outside suppliers to a considerable extent.

In some ways it is back to the early 1950s when fears of a shortage of energy were instrumental in the establishment of the European Coal and Steel Community and of the ill-fated Euratom organisation. Now after two decades when energy has been plentiful and cheap, energy problems once again loom large in the European communities.

At present there is no ready-made framework for considering energy questions. There has been no common energy policy in the way that there is a common agricultural policy. A good thing some would say (although it is now being argued that the common agricultural policy has not been so inflationary for Britain as was at first feared) but the lack of an energy policy has meant that when a crisis strikes the European countries have no guidelines for action.

In fact each of the three communities in Europe has a finger in the pie. Euratom looks after nuclear energy, coal comes under the European Coal and Steel Community, and the European Economic Community itself (the EEC) deals with oil. The same goes for energy research. Euratom is looking into alternative forms of energy—the so-called hydrogen economy for example—as well as doing nuclear research, the Coal and Steel Community is supporting coal research and the EEC spends money on research into the development of oil.

Apart from the reduction in Europe's reliance on imported energy, the keystones of the proposed plan which was formulated by the European Commission are that oil should in future be regarded less as a fuel for burning and more as a raw material for the chemical industry, and that by 1985 the nine member countries' appetite for energy should be 10% below the present estimates for 1985 consumption, this saving to be brought about by using energy more sensibly than before.

Future mix

The planned distribution of sources of energy is shown in Table 1, with the present distribution for comparison. Taking the energy sources one by one down the table:

- **Coal.** Production within the EEC is to remain virtually steady—179 million tonnes (Mt) of oil equivalent in 1973 and 180 Mt in 1985. There will, however, be a reduction in output from the spent mines of north-east France, Belgium and Holland which will have to be matched by increased exploitation of the British and German coalfields. The rise in the consumption of solid fuel indicated in Table 1 will come from an increase in the utilisation of peat and peat coal mainly from Germany (25 Mt oil equivalent to 40 Mt) and a big increase in coal imports (21 Mt oil equivalent to 35 Mt).
- **Oil.** The main factor here is that the reserves in the North Sea should increase community oil production from a paltry 11 Mt in 1973 to 180 Mt in 1985, with a corresponding drop in imports if the demand for oil can indeed be kept down as shown in Table 1. The burning of oil in power stations and in central heating systems is to be discouraged, but it will have to continue to be burnt in cases where there is no foreseeable substitute—notably in road transport and aviation.
- **Hydraulic and geothermal energy.** The commission believes that there is not much scope for increasing the use of these sources in the community, so they will continue to play only a small part in the energy budget.
- **Natural gas.** An increase in exploitation of the gas beneath Holland and the North Sea will increase internal European production from 113 Mt oil equivalent to 225 Mt in 1985. Even so, imports will also jump from 4 to 150 Mt.
- **Nuclear energy.** A big leap forward in use is envisaged. This may not please the most extreme of the environmentalists.

Taken together these figures mean that energy imports to the Nine will slightly increase from 553 Mt oil equivalent in 1973 to 580 Mt in 1985, while the supply from indigenous sources will go from 369 Mt to 920 Mt, making Europe proportionally less dependent on external factors, but still requiring a considerable input of energy from outside.

At a New York conference on energy supplies, organised by the *Financial Times* and *Oil Daily*, the Director-General for Energy in the European Commission, M. Fernand Spaak, spelt out some implications of the plan: "This strategy requires the community to adopt and to implement a real supply policy for each source of energy within an overall energy supply strategy. Therefore the commission proposes a wide-ranging redefinition of the relationships between the public authorities and the energy industry, in particular those concerned with the transport, refining and distribution of oil and oil products. The objective is to

Table 1 European energy sources for 1985 compared with 1973 (figures in millions of tonnes of oil equivalent)

	1973		1985	
	Mt	%	Mt	%
Solid fuel	225	24.4	255	17
Petroleum	539	58.5	575	38.5
Hydraulic and geothermal	27	2.9	35	2
Natural gas	117	12.7	375	25
Nuclear	14	1.5	260	17.5
Totals	922	100	1,500	100

establish a code of conduct for the industry. The public authorities will monitor the industry's behaviour and will have the means of intervention when necessary".

Savings

The plan also envisages that high prices and a more rational use of energy will lead to a reduction in the annual growth rate of energy consumption by the Nine. Instead of an annual growth rate of 5.1%, predicted last year for the interval 1972–85, the aim is a growth rate of 4%. This makes the Nine's requirements for 1985 to be 1,500 Mt of oil equivalent, the figure used in Table 1, rather than the 1,660 Mt as estimated last year.

It is stressed in Brussels that this reduction does not mean that Europeans are going to go cold in the next ten years. Rather the commission says that by a more rational use of energy it means a reduction of energy input for the same level of output of useful energy. Table 2 shows the hoped-for pattern of consumption in 1985, leading to a total of 1,500 Mt of oil equivalent, compared with consumption if the higher growth rate continues. Figures for 1973 are given for comparison.

Table 2 shows that the European Commission has its eye on the improvement of the design of private dwellings. It



Fig. 1 Mr Fernand Spaak (left): a wide ranging redefinition of the relationships between the public authorities and the energy industry. Mr Henri Simonet (right), Europe's Commissioner for Energy.

has been fashionable for years to say that the thermal design of many houses leaves a lot to be desired, but next to nothing has been done about it.

In the transport sector the savings are to come from increasing the efficiency of existing means of transport and, more vaguely, by better town planning. In industry the commission is hoping for more efficient combustion of fuels and the utilisation of heat that would otherwise go to waste, coupled with a trend towards continuous processes and an end to the planned obsolescence of products.

Finally the plan calls for a European Energy Agency to watch over the security of supplies to Europe by developing existing sources and prospecting for new ones, as well as doing some stockpiling. But the history of the nearest thing Europe has so far had to an energy agency—Euratom—is not a good omen.

So far the plan has been well received. All the same the people in Brussels responsible for formulating it are not counting their chickens before they are hatched. Anything can happen, they say, while Europe is in its present state of shock. The most important precipitating factor has been the British wish to renegotiate its terms of entry into the EEC. With the minority Labour government which is calling for the renegotiation still looking surprisingly strong, this question seems likely to hang fire for many months. Then there is the nature of the joint European relationship with its protector, the United States, to be resolved. Furthermore, this spring there have been unstable political situations in more European countries than is usual at any one time.

Prospects

These factors are undermining confidence in Brussels, where there is apprehension that the energy policy will not be judged on its own merits or demerits and that it may become a pawn in some completely unrelated political squabble, as happened last year when Britain, then with a Conservative government, refused to talk about energy (the question then being the formulation of a joint, short term, way of dealing with the shortages brought about by

Table 2 Energy consumption in 1973 and 1985 (figures in million tonnes of oil equivalent)

	1973	1985 (assuming abundant energy and low prices)	1985 (assuming a more rational use of energy)	Difference
Industry	483	900	850	−5%
Transport	119	215	180	−15%
Private dwellings and so on	317	545	470	−15%
Totals	919	1,660	1,500	−10%

the Arab oil embargo) until Britain got more out of the EEC regional fund.

But so far the talks have gone well—perhaps unexpectedly well. During April the Commissioner for Energy, M. Henri Simonet, presented the plan to the Energy Committee, composed of the senior officials responsible for energy in each of the member states. The committee quickly gave its broad approval (which means that the Conservative line in refusing to talk about energy is not being followed by Labour), but there has been some quibbling about details.

The French and Germans are worried about the problem of marketing the coal once it has been mined if there is a deterioration in its price competitiveness, and the community may have to give aid to coal-mining in exchange for the security of supply endowed by coal.

The British, with their memories of the recent miners' strike and Mr Patrick Jenkins' exhortation that they should clean their teeth in the dark; pointed out that a lot of effort will have to be put into negotiations with the trades unions about rationalisation and wage policy. British

coal mining may not be able to fill the gap left by the closure of the continental mines. And doubts that the increase in production of European natural gas hoped for by the commission could really be achieved were voiced by the Dutch.

A more serious difference of opinion has blown up over the extent to which the activities of the oil companies should be monitored and even, on occasion, interfered with during the implementation of the strategy, as indicated in M. Spaak's speech. France, Italy and Belgium seem to be in favour of a greater degree of monitoring than at present and of the principle of more forceful governmental or community intervention, whereas Britain, Holland and Germany are supporting the oil companies' liberty,, in particular with regard to the companies' pricing policies.

The Energy Committee seems, however, to be unanimous in desiring that the plan to be put to a meeting of the Council of Ministers as soon as possible. With Europe still at loggerheads over other important issues what may happen then is anybody's guess.

Energy problems facing India

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If India is to avoid the worst effects of the increases in the price of oil she must make better use of her indigenous sources of energy.

INDIA was experiencing acute shortages of energy even before the first of the increases in oil prices had set the stage for a worldwide crisis. These shortages had to do mainly with gaps between demand and supply of coal and electricity, the former aggravated by neglect through increasing dependence on imported petroleum products and the latter by two successive seasons of deficient rains.

Higher oil prices added to India's economic woes (through adverse effects on the balance of payments position) at a time when she was trying to cope with inflation, runaway prices, and sizeable shortfalls in industrial and agricultural production arising from the failure of rains during 1971-72 and 1972–73, and consequent curtailments in power generation (hydroelectric) and imposed cuts in its supply.

A look into India's industrialisation programme (in regard to the technologies employed, the materials used as inputs, the pattern of production and so on) would reveal only a nominal or incidental relationship with the resources available in the country, or the overall needs and interests of the nation or her people as a whole. The tying of agricultural development programmes to the increasing use of oil-based inorganic fertilisers, instead of organic ones, is another case in point.

In general, one is struck by the poor correlation between Indian resources and actual sources in key areas. The area of energy is no exception.

Sources and resources of energy

India gets her 'go' from both commercial and noncommercial (traditional) sources of energy. In the former category are: coal, oil, gas and electricity; in the latter dung, firewood and vegetable wastes.

The present consumption pattern, as far as commercial sources of energy are concerned is: 50% oil (including natural gas), 29% coal and 21% electricity. Electric power generation at the end of 1972–73 was 61% thermal (coal, oil), 37% hydroelectric and 2% atomic (enriched uranium). Similar figures for traditional sources are difficult to arrive at and unavailable. But very rough estimates of 1962–63 figures were made by the first-ever Energy Survey Committee in 1965. They may still be indicative of the relative importance of various traditional sources (Table 1).

Of the noncommercial sources dung is still estimated to be providing nearly 50% of all cooking heat used in the country.

India's resource picture with respect to various sources of energy is as follows:

Coal. It is fairly abundant (except for metallurgical varieties). Known reserves (all types including lignite) are estimated at 83,000 to 100,000 Mt. Present annual demand and production (1973) are 95 and 79 Mt respectively.

Oil. Mineral oil is relatively scarce. The annual production of 7.5 Mt compared to the refinery consumption of 21.0 Mt in 1973 necessitated imports of 16 Mt (including petroleum products).

Electricity. (a) The potential for hydroelectric generation is abundant and has been estimated at 215×10^9 kW h energy a year. Of this, nearly 17% will have been harnessed when schemes underway are commissioned. Major problems are: uncertainties about rainfalls, high capital outlays, and long gestation periods required for completion. (b) The potential for thermal generation is great considering India's abundant coal reserves, provided she can increase its production. Gigantic thermal plants (near coal mines) of capacity up to 2,000 MW could feed a national grid to take power where needed. (c) At present usable nuclear fuel—natural uranium—deposits are very limited. Enrichment of uranium is not yet carried out in the country so fuel for India's only working nuclear power station at Tarapur in Maharashtra has to be imported. India's thorium deposits are among the largest in the world, but breeder reactor technology which is essential for turning available thorium into fissionable fuel, has not yet been developed.

Table 1 India's energy consumption (1962–63)

Commercial fuels			Noncommercial fuels		
Coal	Oil	Hydroelectric power	Dung	Firewood	Vegetable wastes
59.6 Mt	8.5 Mt	11.8 TW h	54.9 Mt	101.6 Mt	31.1 Mt
64.9 Mt c.e.	55.3 Mt c.e.	10.4 Mt c.e.	22.0 Mt c.e.	96.5 Mt c.e.	22.5 Mt c.e.

Mt, million tonnes; TWh, 10^9 kW h; lower figures are coal replacement equivalents for upper figures (Mt c.e., million tonnes coal equivalent).

Cattle dung. This is quite abundant. India has the largest cattle population in the world and milche cattle alone number 67 million.

Wood and vegetable wastes. It is difficult to estimate the extent of consumption of wood and its replacement in the country. But it is feared that poor sections of the rural population, searching for free fuel, may be indiscriminately denuding the landscape at a rate faster than it is being replaced. This needs urgent attention.

Alcohol. It can be obtained in fair amounts through the fermentation of molasses—a by-product of India's sugar industry (among the world's largest). By itself, or mixed with petrol, it could run internal combustion engines in cars and

other vehicles. Actually, that is exactly what was being done in the country in 1947–48 and 1961–62. Agricultural pumpsets can also be run on alcohol with only slight modification to their engines.

Genesis of problems

Most of India's present energy problems are of her own making. The oil crisis which is at the moment threatening to jeopardise the country's entire development effort through its economic consequences is no exception. The problems primarily relate to commercial sources of energy—oil, coal, and electricity.

In less than a decade, starting with the early 1960s, oil has been allowed to push coal into the background and become the energy base of India's economy. This is in spite of the fact that India is known to be richly endowed with coal but quite poor as regards oil.

The emergence over a decade of the present oil-heavy pattern of energy consumption can be traced to untenable assumptions, bad planning and erroneous policies. But it may have been egged on by a number of factors: relatively easy and plentiful availability of oil at favourable prices, until recently; growth of a pattern of production suited to elitist consumption based on imported technologies heavily weighted in favour of oil as a source of energy; characteristic Indian exercise of 'soft options' in importing and implanting as a whole (by not bothering even to effect modifications in imported technologies to the extent of making them suit Indian needs or conditions and reducing or eliminating their continued dependence on imported inputs), and so on.

All this happened despite warnings issued by the first Energy Survey Committee, as far back as 1965, against 'accepting or encouraging, the shift towards oil without adequate caution or consideration.

A 'gobar-gas' (gobar for dung) plant, was devised by the Indian Agricultural Research Institute (IARI) as far back as 1959. The plant can take in all forms of animal excreta and even human night soil. Septic tanks too could support such gas plants. Between 1961 and 1973 only 7,500 such plants were installed, but 20,000 more will be installed in 1974-75 according to a recent government decision.

India's entire rural and agricultural energy demand can be met through installation of such plants throughout the countryside. Along with the gas, organic manure obtained from residual slurry can be used in conjunction with our indigenous output of chemical fertilisers to increase agricultural production.

Questions of interest

A question of interest may be whether or not India will get any 'special deals' from oil-producing countries.

There are two aspects here: price and assured supply. As far as I am aware no oil-producing countries are going to give us special deals involving concessional oil prices anywhere near what they were before the latest Arab–Israeli war. Besides, it would be foolish to expect any of them to agree to do so.

The oil-exporting countries may, however, agree, in bilateral deals, to assure supplies of whatever oil we shall be needing and allow us to pay for them in ways that will not put unbearable pressure on our already difficult foreign exchange position. Some of the ways could be: barter deals; Indian technical assistance and training facilities in various areas for personnel from oil-supplying countries; soft loans on a long term repayment basis for financing our oil purchases and so on. (India has recently signed agreements with Iran and Iraq, but not all the details have been made public.)

Only time will tell how these arrangements, if indeed they are made on the lines indicated, work out in practice. Much will depend on the international situation in the Indian subcontinent. As long as the situation remains normal we shall have little difficulty with our oil arrangements.

Although one hopes for the best, the possible worst cannot be ruled out or ignored altogether. For example, if another conflict were to arise between India and Pakistan, most of our special deals with Arab nations (and Iran) are likely to be among the very first casualties, irrespective of the issues involved in the conflict or the merits of India's position. India must not overlook this aspect.

Fifth Plan and beyond

Unprecedented rises in prices in 1973 and early 1974 have dealt a shattering blow to most of the fundamental assumptions behind the Fifth Plan. While our planners are busy calculating and recalculating things over and over again—every time with higher prices—chances of prices stabilising at home or abroad, around levels assumed in the final Plan draft, would seem remote.

Energy-wise, things are going to be pretty tough during the period of the Fifth Plan. The problem of evolving a new pattern of consumption in which emphasis on oil is reduced to its proper size, as evidenced by its share in our import bill, is likely to keep us fairly occupied in the coming years. The consequent heavier demands on these two sources are bound to aggravate shortages further, unless some of the steps suggested in this article are taken up. Our performance during earlier Plans *vis-a-vis* targets, under far better conditions than at present prevail, gives one little cause for optimism about India's performance in coming years.

What lies beyond the Fifth Plan will, among other things, depend on how we tackle problems in the near future. If we panic over our oil problems and let our penchant for 'soft options' prevail over good sense, as we have often done in the past, the chances of our breaking out of the circle of energy shortages in future will immeasurably recede—notwithstanding some short term gains that may result from such an approach. Soft options here would mean maintaining our level of oil consumption and present oil-heavy energy policy intact by utilising various funds and lines of credit that are being established around the world, entering into unfavourable collaboration agreements with foreign firms for exploration of oil in India and so on.

If on the other hand, we decide to take up the oil challenge and meet it with firm steps and alternatives available to us from within the country, we could turn this crisis to eventual advantage even if no immediate gains result from this approach.

Petroleum products account for about a third of India's entire energy requirement. Last year we spent Rs500 crores (crore=10 million) to import 16 Mt of petroleum and products. For the same quantity we may have to pay Rs1,000 crores this year. This alone will wipe out half of our total export earnings during the year.

To cut consumption, which had become imperative, the Indian government decided to boost prices of all petroleum products. Two instalments of price increases sent petrol prices soaring from Rs1.74 to Rs3.15 a litre and kerosene from Rs0.75 to Rs1.15 a litre—making Indian petrol the most expensive in the world. (Actually the refinery cost per litre of petrol, even after all the increases in crude prices, is less than a rupee. The rest is all excise duty.)

Petrol and kerosene accounted for only about 11% of the total petroleum product consumption of 25 Mt; the rest (89%) was said to have been used essentially as economic inputs by various industries. Thus, industrialists have argued, any substantial cuts in imports of oil and related products would seriously hamper economic development of the country. The Fuel Policy Committee (FPC, set up in 1971 in the Planning Commission to project a long term energy picture for the country) has found, however, that fuel oil is one petroleum product whose consumption (16% of the 25 Mt used in the

country last year) can be drastically reduced without affecting the level of economic activity. Nearly 70% of the industries using fuel oil, according to the FPC, could switch over to coal without any technological difficulty whatsoever.

In the area of agriculture a 'new strategy', launched during the mid-1960s, sought an increase in output almost exclusively through costly inputs like oil-based chemical fertilisers, and diesel-run tractors and pumpsets. Another matchless piece of Indian planning.

Anyone who knows anything about Indian conditions would know that irrigation, organic manures and human manual labour are far more important and crucial inputs for our agriculture. Notwithstanding this, the consumption of chemical fertilisers, estimated at 19.7 lakh tonnes in 1972-73, is intended to be raised to 52 lakh t by the end of the Fifth Plan (lakh=100,000). This would involve imports valued at more than Rs500 crores in 1978-79 (excluding the crude oil costs). Compare this with the paltry Rs9.0 crores allocated for the development of local manurial sources in the centrally sponsored sectors of the Plan.

It cannot be disputed that chemical fertilisers have a useful role to play in Indian agriculture. But the importance and priority being accorded to them, and the total value of resources committed in this direction will be found to be totally unwarranted in any cost-benefit analysis.

Basically, as far as coal and electricity are concerned, it is a case of demand outpacing availability and supply (by 20% in the case of coal and 10% in the case of electric power). Factors like insufficient rains, maldistribution (of power) and problems involving movement of coal (from production sites to consumption sites far away) have aggravated shortages. There have been other reasons too.

Increasing interest in imported oil as a source of energy had serious and far reaching repercussions on coal use and production. From 33 Mt in 1950 coal production had increased to 67.7 Mt in 1965-66 when stagnation set in because of the increasing use of oil. The output of coal increased by only 8 Mt in the next four years and then dropped for two years in a row to 72.06 Mt in 1971-72. Contrast the 1972-73 production of 79 Mt with the Third Plan target of 98.5 Mt a year in 1965-66.

Power-wise, it has been claimed that the country could have had enough power to meet present demand (and even some to spare) had the Fourth Plan targets been achieved during the period of that Plan itself. We missed those targets, by an incredible 50%. Of the 9.3 million kW capacity targeted for commissioning during the Fourth Plan the actual achievement was only about 4.6 million kW.

Apart from a dismal failure to add new generating capacity, our performance with what we already have has been equally poor. For example, plant availability has been low and transmission losses, which one might think ought to have been decreasing, have actually been increasing. From an average of 17% in 1968-69, they were up to 18.8% in 1972-73, reaching 30% in some states.

Thermal stations have experienced difficulties in getting coal in a regular and guaranteed manner. Some older thermal plants are unable to get spares for some of their equipment since plants are outdated and such equipment is no longer manufactured in the countries from which they were imported.

Hydroelectric power generation has its own drawbacks, a major one being the utter uncertainty about rains. For example, for two years in a row now, abnormally deficient rains have necessitated sizeable curtailments in power generation.

Our nuclear power programme is still in its infancy. The only power plant in operation today is the 400 MW Tarapur Atomic Power Station in Maharashtra which was built by an American company on a turnkey basis. An unusually large number of problems encountered in its operation have rendered this first experience not an entirely happy one.

A second nuclear power station near Madras, when commissioned, will provide a far better opportunity for correctly assessing our potential and acquired capabilities in this new technology, since this station is to be a totally indigenous effort. But the true impact of nuclear power in India will be felt only when we have developed and mastered (fast breeder reactor) technology to convert our abundant thorium into fissionable fuel for use in power reactors.

Meeting the challenge

To meet effectively the energy challenge facing us at the moment we must do the following things right away: (a) Cut down oil consumption enough to bring import bill down to a reasonable level; (b) step up power generation and increase outputs of coal and mineral oil not only to meet normal growth in demand but also to neutralise the effects of cutbacks in oil consumption; (c) seriously consider making practical use of the 'total energy utilisation' concept in power stations and industries. (This concept visualises utilisation of all the heat produced by burning fuel. At present, power stations utilise only 30% of the heat to produce electricity and the rest is simply thrown away.) I believe that (a) and (b) could be achieved through three things: coal, alcohol and cattle dung. We can, in a number of cases (of industrial and other use) replace oil with coal without much difficulty. Alcohol, or a mixture of alcohol and petrol, can be used to run automobile and other engines with or without modification. (In the latter case the efficiency of the engine is slightly less.) Cattle dung—at present simply burnt after drying—can be fermented to yield combustible gas (methane) and residual slurry for conversion into organic manure, which is richer in nitrogen, phosphorus and potassium than fresh dung or so-called yard manure. The gas can be used for cooking, lighting and running engines to work pumpsets, tractors and grinders, and even for generating electricity.

Soviet thoughts on energy resources

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Nature's Soviet Correspondent shows that the Soviet Union is not being complacent about possible energy problems, large though her resources may be.

ENERGY supplies and resources have always held a prominent place in Soviet planning—Lenin, indeed, defined Communism as "Socialism+Electricity", and the first reaction to the 1973 energy crisis by the Soviet planners was the denial that this could in any way involve them. "The Soviet Union exports annually more than 100 million tonnes

of petroleum and petroleum products . . . The USSR invariably fulfils its contractual obligations and will fulfil them in the future", said the First Deputy Minister of Foreign Trade, Ivan F. Semichastnov, in an interview last January. "Our country is rich in fuel and energy resources . . . In the last 10–12 years the outputs of all forms of fuel have doubled," said the Minister of Power and Electrification Petr S. Neporozhnii in March[2].

Such comments, duly distributed abroad by the *Novosti* agency, would seem at first glance to indicate that a spirit of confidence prevails in the Soviet energy industry, and that the great industrial expansion envisaged under successive five-year plans is comfortably matched by routine and unhurried exploration and exploitation of the new deposits of fossil fuels.

A closer study of the reports, however, indicates a less propitious picture. The Soviet Union will fulfil its contractual obligations to deliver fuel but as regards her further expansion "this is not envisaged at the present moment"[1]. The vast resources of fossil fuel are "extremely nonuniformly distributed", being mainly in the eastern regions of the country with only 10% in the European part of the USSR and the Urals where 80% of Soviet industry is concentrated[2]. The almost daily accounts in *Pravda* of the completion of a section of pipeline or the commissioning of a power station suggest an underlying concern. So, too, do the *Novosti* reports, designed for foreign consumption, which from the beginning of this year have increasingly emphasised the healthy state of the Soviet energy situation. Coverage has varied from straight reports on new power stations, gas fields or pipelines to such details as the use of tea wastes to lubricate oil drills, air-cushion transport of heavy mining gear in the tundra, or the automatic deicing of high voltage power lines in the extreme conditions of Sakhalin Island. Indeed, many of the achievements commended are themselves an indication of the need to exploit all available power resources. Thus on March 5, 1974, *Novosti* reported the selection of a site for a 3,200 MW power station on the river Vakhsh in Tadjikstan (a site where seismic activity reaches magnitude 9, necessitating the placing of the station underground), followed by the description of a new town, Komsomolsk, to be built in the permafrost zone, near a rich deposit of coking coal—the town to house 70,000 inhabitants, the coalmine to produce an estimated 4.5 million tonnes (Mt) a year. In introducing a 'round table' discussion on the prospects of MHD generation of electricity, published in *Nauka i Zhizn'*[3], Academician Vladimir A. Kirillin, Chairman of the State Committee for Science and Technology, commented: "It is sufficient to say that the increase of the efficiency of thermal power stations by even 1% on the scale of our country at the present level of production of electric power would save approximately 3 million tonnes of fuel per annum . . . or . . . would produce an additional 10,000 million kilowatt hours of electrical power."

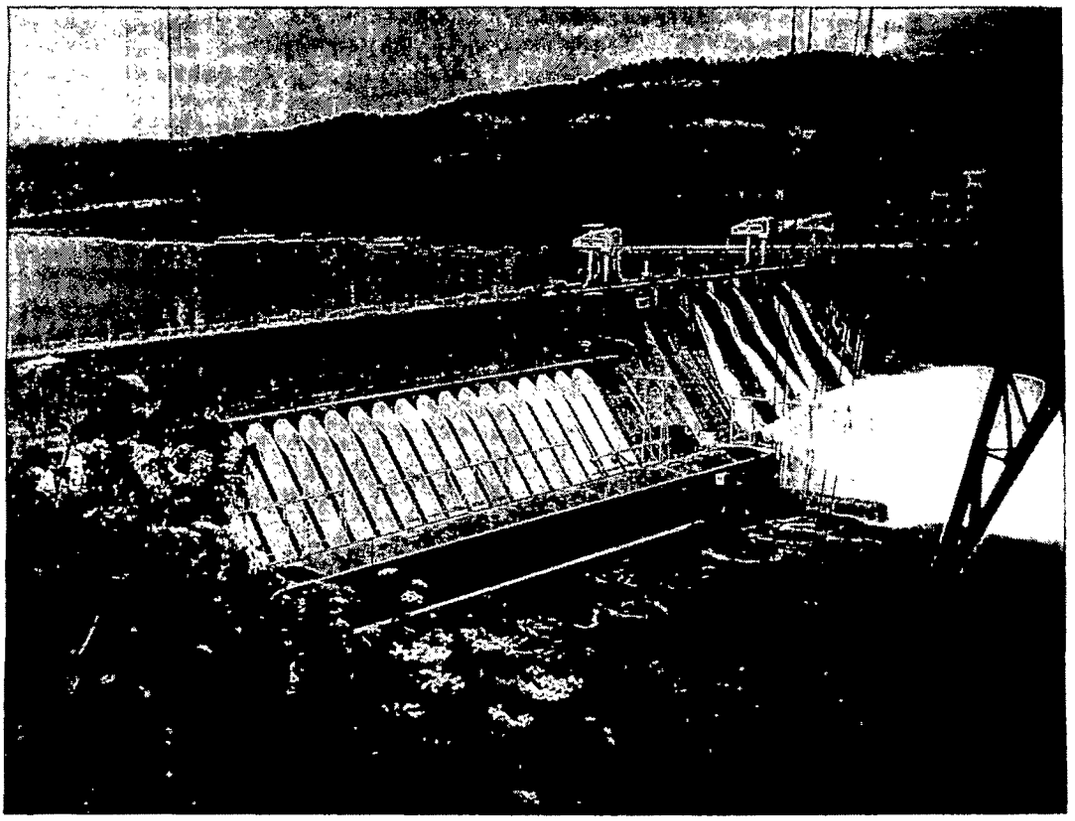


Fig. 1 The Krasnoyarsk hydroelectric power station on the River Yenisei in eastern Siberia.

Future supplies

It would seem, therefore, that in spite of the reassurances of the politicians, considerable thought is being devoted by the Soviet energy experts to the true state of their "inexhaustible' energy supplies. A lecture given by Academician Kirillin, during his recent visit to Britain[4] served only to emphasise this point of view.

At present, he said, more than 80% of the electric power in the USSR is generated by thermal power stations, using chemical fuels, mainly coal. Although the majority of the new power stations to be built will be based on nuclear power, a certain number of conventional power stations will be built in the future. These will almost all be coal fired; in spite of the increased proportion of oil and gas

in the overall fuel output of the Soviet Union (an increase from 51% to 65% in the period 1965–74), the natural resources of coal far exceed those of oil and gas. New gas-fired and oil-fired stations will therefore be built only where coal-fired stations would involve too great a pollution hazard, notably in cities. At present, indeed, there is an increasing emphasis on the importance of the coal industry in the Soviet Union, especially on open-cut mining, which makes the economic efficiency of coal production comparable with that of oil and gas.

(Some recent comparative statistics provided by the *Novosti* agency are of interest: the absolute output of coal in the Soviet Union is increasing by some 100 Mt every five years; the share of open-cut coal in the total coal output, it is estimated, will increase by some 30% in the current year. Considerable attention is being paid to lignite resources, as well as to those of 'black' coal; a recently discovered lignite seam in Byelorussia—conveniently near the industrialised areas of the USSR—is estimated to hold some 50 Mt of fuel, accessible by open-cut mining.)

The use of gas-fired and oil-fired power stations is also indicated, said Academician Kirillin, for peak-hour booster stations. This seems at first glance a somewhat strange admission; according to Minister Neporozhnii[2], the projected supergrid, which will unify the entire power production of the Soviet Union and the Comecon system into a single network, and which should be completed in the next 18 years, is intended, *inter alia*, to obviate the whole peak-hour problem. The considerable time differences between the various parts of the USSR should mean that with the unified supergrid, electricity could be transferred from power stations at local 'off-peak' to consumers in a 'peak' zone, thus saving some 35–40 GW of generating power (that is obviating the need to build power stations to that capacity). But Academician Kirillin seemed less confident. Questioned on the subject, he stated that only some 2 GW could be transmitted by the supergrid as envisaged, and that until superconducting transmission lines become a practical possibility, the need for booster stations will remain—an interesting example of the dichotomy between the claims of the planners and the tasks of the engineer.

Further elaborating on peak and semi-peak boosting, Academician Kirillin indicated that MHD (magnetohydrodynamics) stations are considered as a promising energy source in this field. Generation by MHD is, in fact, one of the specific fields of cooperation between Soviet and United States specialists, which has been developed under the open-ended agreement on technological cooperation of May 1972. A meeting held in Moscow in February and March 1974 resulted in the signing of a protocol on a programme of joint work to design, build and put into operation commercial MHD units. The Soviet team, headed by Aleksandr E. Sheindlin, Director of the High Temperature Institute of the Soviet Academy of Sciences, has already designed and constructed what they describe as an "experimental commercial" MHD generator, the U-25, and on the basis of this work, together with research data, they envisage the commercial operation of MHD stations by the mid 1980s with an efficiency of some 10–15% of that of conventional thermal power stations. Dr Bill Jackson, who signed the protocol on behalf of the United States delegation, observed that the problem of MHD generation is essentially now one of engineering rather than basic research and said that the U-25 generator marks a breakthrough on the engineering side. It should be noted, however, that the Soviet estimates on the efficiency of MHD generation are considered by many western experts to be unduly optimistic.

Nuclear power

Regarding nuclear power stations, the same optimism seems to prevail. In his lecture, Academician Kirillin stated that "most experts believe that extensive construction of nuclear power plants with fast-neutron reactors will not be a practical possibility before 1985"—a somewhat early date, even as a *terminus post quem*. The present generation of Soviet nuclear power stations are mostly of ^{235}U thermal neutron type, either water-moderated water-cooled vessel reactors, or channel graphite-moderated reactors. The latter are of special importance since they can be used as breeders of the plutonium required for the various fast breeder reactors which will form the next generation. One such fast breeder reactor, the BN-600 is already operating (at reduced capacity, because of teething troubles) at Shevchenko on the Caspian Sea, as a source of combined power generation and water desalination. A second BN-600 is under construction. In view of the difficulties which have arisen in the sodium cooling circuits of the Shevchenko reactor[5], it is noteworthy that Academician Kirillin observed that "probably it would be desirable, side by side with the creation of fast-neutron reactors operating with sodium coolant, to speed up the development of this type of reactor with a gas coolant."

Other sources

The other major source of electric power in the Soviet Union is, of course, water power. This has played a considerable part in the opening up and industrialisation of Siberia and the Soviet Far East. This programme is still continuing, with such major prestige projects as the 6 GW Krasnoyarsk power station (the largest hydroelectric plant in the world).

Hydroelectric power plants are now planned in conjunction with other projects—land reclamation, irrigation, and the planning and development of specific industrial centres (for example, the giant aluminium plant at Krasnoyarsk, which consumes one-third of the output of the hydroelectric plant). According to Academician Kirillin, projects are now under discussion for hydroelectric peak booster stations, in which the water is pumped up to a high level by a thermal power station at off-peak time, to be released again at periods of maximum demand. The high costs of such schemes, however, would limit their use only "to areas of favourable terrain".

Regarding such suggestions as the large scale use of solar energy or the Earth's heat (detailed projects for which appear from time to time in the semi-popular scientific press in the Soviet Union[6]) while admitting that their study constitutes an "urgent problem", Academician Kirillin considered that only with "principally new methods" could they yield adequate large-scale results, although on the small scale, for fruit drying or local heating, for example, some useful results have been obtained. Similarly, wind power, tidal power and the like lie, at present, outside the bounds of practical possibilities.

Although further research is needed in these fields, therefore, the main problem before the Soviet power generating industry at present is, in his opinion, two-fold: on the one hand, the more efficient utilisation of conventional thermal power stations—including the use of combined steam-gas turbines, and district heating schemes using the steam from power stations—and, of course, the improvement of anti-pollution measures and on the other, the greater development of nuclear power, including controlled thermonuclear fusion which, he considers, should be available as a source of industrial power generation by the end of the century.

[1] *Novoe Vremya*, No. 2, 7-8 (1974).
[2] *Novoe Vremya*, No. 9, 14-16 (1974).
[3] *Nauka i Zhizin'*, No. 4, 15-23, 40 (1974).
[4] *Power Industry of the USSR, Its Present and Future*, a lecture delivered at the Culham Laboratory of the UKAEA, May 23, 1973.
[5] *Nature*, **248**, 95. 468 (1974).
[6] *Priroda*, No. 5, 95-103 (1969).

Making energy value for money

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The National Industrial Fuel Efficiency Service Ltd, once a British Government body but now a successful private company, pays close attention to an energy problem that is often ignored.

EVER since the Industrial Revolution, people have tried to improve the use of energy, and countless patents have been issued for devices to economise on the amount of fuel needed to carry out particular work loads. Some of these, such as mechanical stokers and economisers, were invaluable; others were frankly useless, but by high pressure sales methods were sold in large numbers. Even today fuel users must be puzzled at the claims made for some articles which are alleged to save fuel.

Traditionally the industrialist has regarded his energy requirements as a factory service or, at best, as one of the tools of production. His main concern has been the final cost of purchased fuels or electricity per unit of production, insofar as this influenced his ability to compete. Since in most industries this cost has been a relatively small proportion of total production costs his concern has not been of very high priority.

Until recently the continuing availability of the necessary energy sources, in some suitable form, has been taken for granted. True, there have been some short term crises of supply of specific energy sources, but these have been of limited duration and the worst effects have often been mitigated by a temporary or permanent change to other available fuels.

The demand for energy all over the world has continued to increase without interruption since the end of the Second World War, and fortunately this increase was mainly met by the rapid expansion of oil production which, as far as Europe was concerned, meant the Middle East and African countries. The price of fuel oils dropped steadily in the postwar years and was stable for a surprising period in spite of steady rises in the cost of living and of industry's other raw materials. In turn this tended to stabilise other fuel prices, and indeed forced the contraction of the coal industry in the rest of Europe as well as in Britain.

Gas, regarded for all its life as a high price fuel which was only justified where cleanliness, convenience and improved production could justify its costs, dropped suddenly in price to industrial users after 1968 and became a serious competitor, in large scale use, to coal and oil. Unfortunately domestic consumers were not so lucky, as the very high cost of the conversion of their appliances to the use of natural gas together with other inflationary factors, prevented any real reduction in price to them.

Now, in less than a year, the price of oil fuels has rocketed, mainly because the Middle East countries have accelerated demands for participation and increased royalties but also because of the increased costs of such things as transport and refining. Oil fuels have increased roughly three-fold in price to industrial, commercial and domestic consumers alike, and the costs of other fuels have also risen rapidly partly under the influence of 'supply and demand' and also because of large wage increases and investment problems.

Almost at the last moment the industrialist is beginning to take a fresh look at his energy requirements and give this its true place in his priorities. After all, if these energy requirements are not met he will be out of business. In many cases almost for the first time, the industrialist is asking himself the questions:

- What is this industrial energy?
- What is the present situation?
- What should the World, the European Economic Community (EEC) and Britain do about it?
- What can individual companies do?

The answers to some of these questions involve a great deal of speculation. On his answer to the last question could depend the industrialist's competitive position or even his very existence.

We can hope that the recent discoveries in the North Sea will prevent any immediate shortage of supply, but the length of time granted to Britain will depend on the rate of exploitation. An accountant would argue that, if it were technically possible, the best financial recovery on the thousands of millions of pounds being invested would be to extract all the oil and gas in the minimum possible time. This would obviously more than satisfy British demand in these few years and the surplus could be sold to assist balance of payment problems. Such uncontrolled rapid exploitation would in passing be the final blow to the coal industry. There is a better, national argument to extend the period of exploitation, to keep the coal industry at a healthy and relatively steady level of production and so conserve resources to last over a longer period so that other sources of energy can take over as the North Sea's output declines. Nuclear power stations can, and will, be developed to reduce the demands on coal and oil for electricity generation but these need both capital and time to construct and we may well see other new forms of energy production developed such as solar, geothermal, tidal, nuclear fusion and wind power.

All these points so far have been concerned with the supply of fuels and energy, but disregard the effect of price. It is doubtful whether fuels will ever be as cheap again, relative to other costs, as coal and oil have been over the past twenty years. Although it has been argued that actual shortage is unlikely over the next twenty or more years, all fuels will be expensive. Of course it is easy for many companies to pass on the extra cost of fuels as just another of the rises in raw materials prices that cause the cost of their products to increase, and unfortunately many manufacturers seem to be doing just this. While demand exceeds supply, and for many companies this is the situation for a year or so ahead because of the reductions in output caused by the three-day week and cuts in electricity usage of recent memory, such a policy of passing on price increases works. This does not help the consumer, however, and it encourages wage claims.

Also, all our past history of surpluses and shortages indicates that fairly soon many manufacturers will have caught up with their order books and may be chasing customers. There are also services, such as hospitals, local authorities and government, and the domestic consumer, who manufacture no saleable product and so cannot pass on the large increases in fuel and energy costs. These must fall on the

whole community as increases in rates, taxes or personal spending.

Energy conservation can help by minimising the increases in costs of fuels and by reducing the demand so as to extend the period for which the finite resources of conventional fossil fuels will last. The National Industrial Fuel Efficiency Service, NIFES for short, as the most experienced organisation in the field has been able to demonstrate for more than twenty years that large savings can be made. Unfortunately during the era of cheap fuel and energy many companies and authorities have paid little attention to possible savings on a fuel bill which might have been less than 5% of their total running costs, preferring to spend their own staff time, use of outside advisory services and restricted capital resources on other facets of their costs.

A committee which considered fuel efficiency and conservation once said: "The committee reached the conclusion that the gap between fuel requirements and home production would continue to increase and there was the possibility that a critical shortage of fuel would arise. The committee recognised that the nation's economic progress and well-being would depend on fuel and power and that it was vitally necessary to make the most effective use of available supplies". How topical this sounds, and yet these opinions were those of the Ridley Committee of 1952. The wartime Ministry of Fuel and Power had operated a fuel efficiency service and this had continued after the war. Periodic bulletins on various aspects of fuel utilisation, issue of a regular newsletter and provision of a free first-aid advisory service by a staff of around 100 professional engineers and technicians was offered to industrial and commercial fuel users.

The results of these visits were so encouraging that the ministry decided to extend the service so that more detailed surveys of fuel usage could be carried out, both in the boilerhouses context and, more important as it turned out, in the realms of process and space heating. Fees were charged for these detailed surveys and a considerable demand developed from the inception of these 'mobile testing units' in 1948.

After the report of the Ridley Committee, the minister decided to set up a fuel advisory service which would operate outside the Civil Service structure, and so NIFES commenced operations in 1954. In the earlier years much of the running costs came from contributions from the National Coal Board, and the Gas and Electricity Councils, with small additional sums from certain oil companies and the Government of Northern Ireland. These contributions were progressively reduced as NIFES developed its own fee income and in 1969 the government took over responsibility, providing a much reduced and tapering three-year grant on the understanding that the company would become self-supporting.

In the early years, its activities were entirely concerned with fuel problems, using poor quality fuels in many boilers until better fuels became available, carrying out tests on existing boilers, furnaces and processes so that recommendations could be made to improve performance or reduce fuel costs, training boiler operators in correct firing and supervision and giving advice on numerous other fuel problems. Inevitably, as recommendations were made on existing plant and on its possible replacement, many clients expressed the view that the knowledge and experience of NIFES would be of great value to them when new factories or premises were to be constructed or when new plant was to be installed. The company was encouraged by such clients to move into the field of design engineering and advise on all aspects of such capital projects, and for many years now has been handling this field of consulting engineering as part of its activities.

Unfortunately this reduced grant meant that certain services in the education and training fields had to be curtailed, since they had been offered for many years at nominal fees to encourage interest in fuel efficiency and higher productivity. It was found that the demand dropped considerably when fees reflecting the true cost of providing such educational services were asked. One item of which the company is particularly proud is the Boiler Operators' Handbook, which was originally produced as the New Stokers' Manual in 1959 and has been revised and extended in several editions. Nearly 50,000 copies have been sold, and a steady demand still persists for this unique aid to the boiler operator. Nearly 17,000 men throughout industry and the public service have been trained in operation, maintenance and utilisation of plant, and more than 10,000 of these reached the standard of the City and Guild's Boiler Operator's Certificate.

At the end of 1971, the government had decided that NIFES should continue as a private company, but be organised so as to offer all the types of service then being carried out. It is pleasing to record that the staff were so confident of the viability of the company that they were able to agree amongst themselves to raise a share capital and give certain further financial guarantees so that the government agreed to sell the assets and goodwill. As a result the work of NIFES continued without interruption and with the same staff. Modest profits have been made in each of the first two years of private operation and the enginering staff has increased by around 25% since the government handed over ownership.

What is the present company offering? First, there is the enormous amount of experience. Many of the present staff have been successively with the Fuel Efficiency Branch and NIFES and others came from other industrial connections to NIFES many years ago, so that a good proportion of the staff have more than twenty years of experience in fuel, process, heating and allied problems.

The service has always offered to its thousands of clients an impartial advisory and testing service, at first concentrating on fuel and heat problems but very soon expanding into many allied fields. It pioneered the idea of energy measurement and costing and with a unique feedback system of its wealth of experience over the whole diverse range of industrial, government department, hospital and local authority work, it can claim to be able to present a client with the likely operating characteristics of a new plant before it is built. Unfortunately the myth of reliability of fuel suppliers has also been exploded in recent years by strikes and other industrial action and NIFES has helped many companies to install standby electrical supplies or dual-fuel systems, often showing that the cost of standby plant can be turned to profit by using it to avoid peak load charges.

In 1969 an independent survey of the results obtained by NIFES advisory and testing services was carried out and showed that in the first fifteen years of activity total fuel savings were at least 20 million tons of coal equivalent, and these are continuing savings because of alterations in process or production, or the running of existing plants at higher efficiency. The survey concluded that for each man-day spent on investigations and surveys by engineers, the resulting recommendations saved 20 tons of coal or its equivalent a year, much of which would continue to be saved each successive year. Since 1969 many further visits and surveys have been made, and a very conservative appraisal now suggests that some 30 million tons of coal equivalent have now been saved.

If the whole of industry raised its standards of energy utilisation, within practical limitations of scale, to that of the more efficient companies, an annual saving of more than £350 million could readily be made by known and proved techniques and practice which are financially viable even at today's energy costs. This is in fuel costs alone, and at 1974 fuel prices. There would also be inci-

dental savings in many cases because of reduced replacement, maintenance and labour costs, better working conditions and production improvements. Reduced pollution would also result from improved and more efficient fuel usage.

Heat and power surveys carried out over a reasonable sample of industrial groups indicate the potential savings shown in Table 1. The figures do not include the further savings possible by improving thermal insulation standards.

Table 1 Potential fuel savings

Industrial group	Mean saving (%)
Ceramics, brick, glass	15.0
Chemicals	18.0
Iron and steel	20.0
Engineering and metals	18.0
Textiles and leather	15.0
Food, drink and tobacco	15.0
Other manufacturing	21.0

The Thermal Insulation (Industrial Buildings) Act 1959 is one example of legislation which has made a positive contribution to energy conservation during the past 14 years. These standards are inadequate in the light of higher comfort standards, higher fuel costs and energy shortage. They are, in fact, among the lowest in Europe.

The regulations should be brought up to date by:

- Specifying lower standards of heat loss for roofs to reduce losses to half the present permissible figures.
- Extending these standards to cover walls also.
- Applying them to existing buildings, say over a period of seven years, as well as to new buildings.
- Extending the definition of buildings to include commercial and local authority buildings.
- Making some measure of control of space temperatures mandatory.
- Insisting on higher insulation standards for commercial and domestic buildings.

Such a policy would reduce the yearly usage of energy in Britain by at least 6 million tons coal equivalent and with rising fuel costs would be an economically viable proposition in the majority of installations. This takes no account of similar possible high savings on domestic premises.

There is an inbuilt financial incentive in energy savings. An exhaustive NIFES survey in 1965, covering industrial establishments, proved that a total capital investment of £2.5 million in new and modified plant yielded a saving of 300,000 tons coal equivalent a year. The payback period was two years for the sample taken. Although the capital expenditure nowadays would be substantially higher, fuel costs have risen much faster than the cost of energy-using equipment. (As the present high rates of interest have a deterring effect on investment in plant not directly associated with production, there would seem to be a case for reintroducing special investment grants for capital outlay on energy-saving equipment.)

Finally, a few examples where savings have been made. At a Midlands steelworks engaged in the production of stampings, tests were made on seven oil-fired bar heating furnaces. Defects were found on some burners, there was scope for further insulation and the design of the furnaces could be improved, even though the overall performance was as good as the average for this industry. With the cooperation of both operators and management, modifications were made. On a typical furnace, throughput was increased by 14% and oil consumption reduced by 15%, so that furnace performance measured in gallons of oil per ton of throughput improved by 25%.

At a gelatine factory a campaign was undertaken which covered reorganisation of the steam distribution, replacement of steam traps and improvements in recovery of hot water, tests on evaporators, boiling tanks and driers to improve their performance and reuse waste heat in other parts of the process, setting up a record system to examine daily steam usage in departments and reorganisation of the distribution of cooling water. This resulted in a reduction of 29.5% in the amount of steam used per ton of gelatine produced.

Whisky distilleries traditionally operated with stills heated from below by hand-fired furnaces using expensive large coal. For many years NIFES has cooperated with a number of distilleries in a quiet revolution in the whole whisky-making process. Several have converted their stills to steam heating, using highly efficient boilers, and to fullscale waste heat recovery systems to reduce dependence on rivers and streams for cooling water. At these converted plants, fuel savings of 50%, and in some cases slightly more, have been made without any change in the character of the whisky produced.

A company making glazed clay sanitary pipes were experiencing difficulties in drying larger sizes after the moulding process so that they could be fired and glazed in the kilns. After a series of tests modifications were suggested to drying procedures, using high humidity enclosed drying chambers for the first few hours. This reduced the proportion of cracked and unsaleable pipes from 45% to 5%, so that although there was little effect on fuel usage for drying, saleable production was almost doubled.

Nuclear fission as a general source of energy

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Heat from nuclear reactors can be used for several purposes other than the generation of electricity, for example as a source of heat for chemical processes or for producing 'synthetic' fuels.

THE total world consumption of energy is at present running at an annual rate of about 2×10^{17} kJ most of which is derived from fossil fuel sources. This is approximately 0.2 Q a year where the unit $Q = 10^{18}$ BTU $= 1.054 \times 10^{18}$ kJ is a convenient measure in talking of world energy consumption. The incident solar energy over the surface of the Earth is 5,200 Q a year. Estimated coal reserves would be sufficient to meet this rate of consumption for about 1,000 yr but the calculated

reserves of crude oil and natural gas would be barely enough to satisfy such a demand for 100 yr.

The pattern of utilisation of primary energy sources is, however, not compatible with their availability, oil and natural gas being preferred, to the extent that about two-thirds of our power is now derived from these. This has been a natural consequence of the fact that until recently they have been cheaper and more convenient to use than solid fuels and easier to transport over large distances.

Electricity plays an ever increasing role in the energy mix, but the present world consumption, about 6×10^{12} kW h a year, corresponds to only about 10% of the total primary energy supply. This ratio is certain to increase in the future but the extent to which it may do so is limited by a number of important factors which have to do with the problems of storage, portability and transmission of electricity. Economic considerations generally dictate that electricity should be generated in large centres from which it has to be distributed by overland transmission lines. The range of distribution is limited by the fact that such lines are expensive and losses are high. In this respect it is much more economic to transport energy over large distances in the form of liquid or gaseous fuel.

No large scale method of storing electrical energy at an acceptable cost has yet been developed and this, of course, results in poor utilisation of expensive electricity generating plant, arising from fluctuating load requirements. There is no corresponding problem in the storage of primary energy in the form of oil or gas, of course, and these may be converted locally into the energy form required as the demand arises, including the generation of electricity with relatively inexpensive small plant such as gas turbines.

Except in the case of railways, electricity is relatively useless in transportation because of the problems of devising reasonably light and compact forms of storage. These problems must be regarded as completely insoluble in the cases of aircraft and ships and practically so for fast long distance road transport. The reason is quite fundamental, namely, that only a liquid fuel can meet the requirement of energy carried per unit volume and mass in these cases.

The foregoing considerations suggest that however far we may go in utilising energy in the electrical form, there will always be a requirement for liquid or gaseous primary energy supplies in at least equivalent amounts. This is important in considering the possibility of utilising nuclear energy to replace the dwindling resources of fossil fuels. Up to the present, nuclear fission has been seriously considered only as a source of energy for electrical power generation, but as we have seen this will not meet the total demand for directly usable energy and almost certainly not even the major part of it. It is necessary in the circumstances to consider the ways in which the heat generated by nuclear fission may be more fully utilised to meet the overall needs and not merely to generate electricity to be supplied through the grid.

Scope for nuclear reactors

In looking beyond the use of nuclear reactors exclusively to generate electricity, it is necessary to examine possible applications in the following catagories:

(i) the direct use of waste heat in the thermodynamic cycles involved in generating electricity (low temperature processes);

(ii) the direct utilisation of the heat output of reactors in chemical industrial processes supplemented by electricity production (high temperature processes);

(iii) direct or indirect application of nuclear heat supply to produce synthetic liquid or gaseous fuels (high temperature processes or methods involving intermediate electrical power generation).

The average efficiency of energy conversion from heat into electricity at present is about one-third. In the application of nuclear fission this figure is barely matched by water-cooled systems, because of the low steam inlet temperature in the power cycle, not exceeding 300° C, compared with around 550° C in modern fossil-fuelled stations whose net efficiency may be about 40%. The residual heat, amounting to 60–70% of the total, is wasted and its dissipation often presents embarrassing problems. Considerable attention is now being paid to the utilisation of this reject heat, notably for district heating and possibly for desalination.

The widespread utilisation of the waste energy for district heating is inhibited by the problems of siting central electricity generating stations in appropriate population and industrial centres, where the demand is likely to match the availability of the heat. Social, technical and economic problems are certain to limit the scope for adopting this practice on a large scale, at least in the foreseeable future. Apart from this limitation, however, technical considerations have to be taken into account.

In normal steam cycles the peak temperature of heat rejection is low (~ 50° C) and effective use of the waste heat requires that this should be raised. Reduction in the efficiency of the cycle in producing electricity is acceptable if the reject heat is being utilised, so that it is permissible to raise the back pressure of the steam and the condenser temperature. This merely implies trading electrical energy for useful heat. It is, however, obvious that there is much less scope for doing this in the low temperature cycles of water-cooled reactors than in modern conventional steam cycles whose source temperatures exceed 500° C.

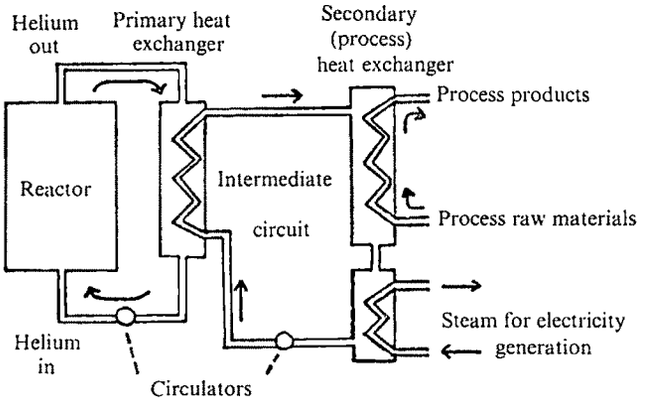


Fig. 1 Direct cycle with utilisation of waste heat. Helium temperatures: 1, ~950° C; 2, ~ 200° C.

The greatest prospects for utilising the reject heat of power conversion undoubtedly lies with the adoption of the high temperature reactor used in direct cycle with a gas turbine. In this system (Fig. 1) the helium acts as the working fluid in the thermodynamic cycle and expands through a turbine which drives the electricity generator. This power cycle, with a turbine inlet temperature of 950° C and an efficiency of conversion to electricity slightly in excess of 40%, would involve heat rejection at a peak temperature around 200° C. Such a high temperature would not only facilitate the utilisation of the waste energy of the cycle for district heating but would introduce possibilities for applying the heat, in a variety of industrial processes, Furthermore, in this system, the potential will exist for increasing eventually both the turbine inlet and the heat rejection temperatures while maintaining the efficiency of the cycle in terms of electrical power output. In this way it would be realistic to consider the application of waste heat in processes requiring heat (possibly as steam) at temperatures up to 250° C or perhaps higher.

Turning to the second category of possible applications of nuclear energy, we have a procedure in which the hot coolant emerging from a reactor is used to provide thermal energy directly in some high temperature industrial process. This is the so-called process heat application of nuclear reactors, which, clearly, is only meaningful if it is associated with the use of the high temperature gas-cooled reactor. So far the technical and

economic feasibility of using reactors to provide heat directly in this manner has only been considered for a limited number of processes, of which the steam reforming of methane and its application in iron ore reduction has received particular attention.

The application of nuclear heat in the steam–methane reforming process would involve using a nuclear reactor to heat helium to a sufficiently high temperature and transferring the heat from the helium to a mixture of methane and steam in an appropriately designed heat exchanger in which these latter gases would be brought as near as possible to the peak helium temperature, in the presence of a catalyst. A range of temperature from 750° to 950° C is estimated to be required for this purpose. The immediate chemical-reaction products in this process are hydrogen, carbon dioxide, carbon monoxide and residual steam and methane, which must be cooled to an intermediate level at which carbon monoxide can be reacted with additional steam to convert it to carbon dioxide. Further cooling to ambient temperature would be required in order to remove the carbon dioxide, residual water vapour and any higher hydrocarbons that might be present, leaving a mixture of hydrogen and surplus methane. Low temperature separation of methane from the hydrogen can leave the latter in a fairly pure state.

If the hydrogen were required to be used in ore reduction it would be reheated in the same plant, in counterflow regenerative heat transfer with the cooling products of the reforming process and further heat from the reactor helium added as necessary for the ore reduction.

The use of hydrogen and nuclear heat to reduce iron ore to sponge iron is an attractive alternative to the present process which uses coke both as a fuel and as a reducing agent in blast furnaces. This arises from the high cost and scarcity of coke in many parts of the world. But natural gas, which is likely to be a rapidly disappearing commodity early in the next century, can hardly be regarded as a satisfactory source of hydrogen in the longer term. Consideration of this brings one into the third and perhaps most important category of nuclear energy applications, that is its use to produce substitutes for the liquid and gaseous fossil fuels. It has been pointed out that these latter currently dominate the primary energy supply situation and that we must expect that the role of liquid and gaseous fuel will not significantly diminish in the future despite considerable increase in the utilisation of central station electricity. Because of this one should think in the future in terms of central energy stations which will export not merely electricity, but also liquid and gaseous fuels and that, in fact, the energy content of the latter may considerably exceed that carried by the former.

The ultimate key to the provision of substitutes for oil and natural gas lies in the intermediate production of hydrogen. In principle, this involves the absorption of energy in water to split it into its constituents. The established method of doing so, of course, is by electrolysis. In going from nuclear heat to hydrogen in this way, however, two efficiency factors are involved, namely, the efficiency of electricity generation and the efficiency of the electrolysis process. The former would be as low as 30% for water-cooled reactors, whereas the latter on the basis of existing technology is 70% at best, implying that the hydrogen product would carry only about 20% of the original thermal energy released. There is scope for eventually improving on this overall efficiency by a factor of two, partly by developments in the electrolysis process and partly by substantially increasing the efficiency of electricity production which should be possible as a result of longer-term developments of the high temperature reactor with direct gas turbine or dual cycles.

To achieve a hydrogen yield corresponding to more than 40% of the input thermal energy, however, would require the adoption of a pyrolysis process, directly utilising the nuclear heat to split the water. Direct thermal decomposition of water is ruled out in so far as it would require a temperature of about 4,000° C to give a high yield of hydrogen by this means. But it is possible to devise sets of chemical reactions involving various reagents whose net effect is to consume water and produce hydrogen while regenerating and recycling the reagents. Many such sets of reactions, involving three or four stages, are now known and can proceed at temperatures lying within the capabilities of the helium-cooled high temperature reactor; they offer the real possibility of producing hydrogen from water in a direct nuclear process heat application.

An alternative route to hydrogen production exists while solid fossil fuels remain in reasonable supply, which is likely to be the case over the next century, namely, the gasification of coal and lignite. The conventional steam–oxygen process of producing hydrogen from coal involves burning some of the coal with the oxygen to produce the heat required for the steam to react with the remaining carbon producing initially hydrogen and carbon monoxide, the latter being subsequently reacted with additional steam to produce carbon dioxide and further hydrogen. Methane is an alternative product obtained by reacting some of the hydrogen with the carbon dioxide, carbon monoxide or the coal itself. The heat used in the endothermic steam–carbon reaction, of course, is transferred internally from the coal burning in the oxygen in the same bed.

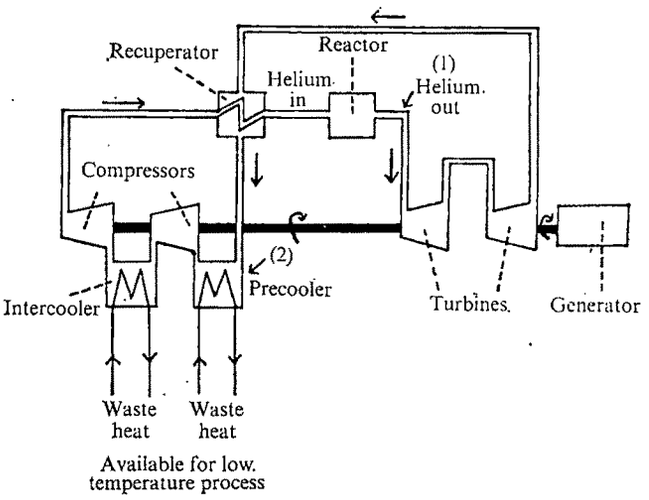


Fig. 2 High temperature process heat application.

The application of nuclear energy would involve externally heating the reaction vessel by means of hot helium instead of the internal combustion of some of the coal. By using external nuclear heating the yield of hydrogen from a given amount of coal could be about 45% higher than that obtained by the conventional combustion method. This important reduction in coal consumption would provide sufficient incentive to adopt nuclear heating which would otherwise have to be justified on questions of cost, with the much higher capital investment of the nuclear plant being weighed against the high cost of the additional coal required in the steam–oxygen process.

Considerable differences of opinion exist in estimating the temperatures required to gasify coal by reacting it with steam. Most experts consider that the peak temperature of the reacting materials would need to exceed 1,000° C for the process to proceed at an economic rate with hard coal and perhaps 900° C in the case of lignite. Lower temperature gasification is possible by reacting the coal with hydrogen to form methane (an exothermic process) and using nuclear heat in the steam–methane reforming process to produce hydrogen, part of

which is then recycled to react with the coal. There is a net production of hydrogen in this process which is the same for a given carbon consumption as in the steam gasification, with the alternative possibility of extracting this net yield in the form of methane. This process would be more attractive than steam gasification if it were not for the fact that a substantial fraction of the coal defies attack by the hydrogen and remains as a char. Success of the process depends consequently on the eventual possibility of being able to convert the char or otherwise use it as a source of carbon or energy.

Technical requirements

The discussion so far in this article has concentrated on the problem of using nuclear energy to produce substitutes for the liquid and gaseous fossil fuels which play such a vital role in supplying our energy requirements. There are many other possibilities for using high temperature heat from nuclear reactors to carry out industrial processes on site and many of these may prove to be commercially attractive. The important fact which emerges in a consideration of these is that they will call for substantially higher helium temperatures than are needed for steam cycle electric power generation. In the latter case a reactor outlet temperature of 750° C is ample to generate steam at say 550° C. If, however, one is to provide heat for chemical processes ranging upwards from about 750° C we have to contemplate the achievement of reactor outlet temperatures of at least 950° C. This would allow for the use of an intermediate heat transfer circuit, with a primary heat exchanger transferring energy from the reactor helium to helium in the intermediate circuit, and a secondary process heat exchanger transferring heat from the intermediate circuit to the reacting materials in the chemical plant. The purpose of the intermediate circuit is to provide a safe isolation between the slightly radioactive primary helium and the hot, chemically active materials in the process plant (Fig. 2).

The feasibility of achieving helium temperatures from the reactor core in the region of 950° C has already been firmly established. In fact, the AVR at Jülich in the German Federal Republic is now operating at that temperature and in the Dragon Reactor Experiment helium emerges from the hottest channels at temperatures in the range 1,000°–1,100° C. Developments in fuel and core materials technology lead to the expectation of mixed helium outlet temperatures perhaps as high as 1,200° C in a few years. The problems of handling gas at such high temperatures elsewhere in the reactor primary circuit, and to a lesser extent in the intermediate circuit, are, however, quite formidable. Thermal insulation will be an aspect presenting many difficulties but undoubtedly the major problems will be associated with the interface heat exchangers, particularly the primary ones. It may be necessary to depart from the more conventional use of metals and go to ceramics or graphite in heat exchangers where the surface temperatures exceed about 1,000° C.

There would also be temperature problems to some extent in the cooler arms of both primary and intermediate circuits of nuclear process heat plants. This is because it is not convenient to have helium above about 350° C in those regions which contain the circulators, control machinery, and so on. The helium temperature drop across the process heat exchanger may be only in the range 200°–400° C so that it will emerge too hot for convenient recirculation. For this reason it would be desirable to take the helium from the high temperature process through a further stage of useful heat removal, most probably in providing the steam to generate electricity so that the plant serves a dual purpose.

Availability of nuclear fuel

It has been assumed here that nuclear fission might be used as a more or less complete energy substitute for fossil fuels. Such an assumption, apart from technical and economic factors, is only valid if the exploitable resources of fissile material are many times greater than those of fossil fuels. The workable reserves of the latter are estimated to be about 200 Q which corresponds to about 2.5 million tonnes (Mt) of fission. In a situation in which sufficient fast breeders exist in sufficient proportion, this quantity of fission could be obtained from about 4 Mt of natural uranium which should be substantially less than that which should be ultimately available from high grade ores at around £10 per kilogram of uranium. Breeder reactors, in fact, could use uranium at a price two orders of magnitude higher, which would make available extensive low grade sources of uranium, notably the 4,000 Mt that exists in solution in the oceans. On this basis, the available fissile energy is incomparably greater than that contained in recoverable fossil fuels.

Unfortunately, the sodium-cooled fast breeder reactor on its own does not fit into the process heat strategy and would only contribute to it if the breeding gain of the fast reactors were sufficiently great to make up the breeding deficits of thermal-neutron high temperature reactors of at least equivalent energy output. (Exclusive use of sodium breeder of course would still permit the generation of hydrogen by the electrolysis route, but the installed capacity of the reactor plant would then be much higher than in the pyrolysis route.) Alternatively, a helium-cooled fast breeder reactor capable of attaining the same output temperatures would need to be developed. This is by no means out of the question.

In the absence of satisfactory fast breeders, however, a nuclear process heat strategy on a level of at least 1 Q per year could be supported with thorium cycle high temperature reactors operating in a thermal-neutral near breeding regime. These might require about 300,000 t of natural uranium and 15,000 t of thorium to yield 1 Q of heat and it would be economically feasible to use uranium from sea water in this application. To all intents and purposes this would constitute an indefinite supply of energy.

Wave power

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Solar energy is one form of income on which we can afford to live. Here is another proposal: the use of power from the waves at sea.

THE amount of power in a wave train can be estimated by calculating the change of potential energy as the water in a wave above sea level falls into the trough in front of the wave (Fig. 1). If the sea water has a density ρ, and the width of the wave front is W, then the mass of water in the half sinusoud above sea

level is

$$W\rho(\lambda/2)\,(H_{tc}/2\sqrt{2})$$

using the notation defined in Fig. 1. The height of the centre of gravity above sea level is

$$H_{tc}/2 \times 2\sqrt{2}$$

falling to an equal distance below. In such a situation the change of potential energy would be

$$gW\rho\,(\lambda/2)\,(H_{tc}/2\sqrt{2})^2 = W\rho g H_{tc}^2/16$$

It is known that the frequency of gravity waves in deep water is

$$(1/\lambda)\,(g\lambda/2\pi)^{1/2}$$

and, therefore, the rate of transfer of potential energy, or the power, is

$$W\rho g H_{tc}^2\,(g\lambda)^{1/2}/16\sqrt{2\pi} \qquad (1)$$

Progressive waves transport energy across the sea, and it is valid to say that the rate of transport of energy across some line is power. Power density can be specified conveniently in kW m^{-1} of frontage.

The Institute of Oceanographic Sciences publish valuable data on waves using instruments developed by Tucker and Pierce[1]. Draper and Squire[2] have analysed observations from Station India (59°N 19°W) which is characteristic of the western approaches to the Hebrides. The amount of power is prodigious. The relationships between various wave parameters have been explained[3]. For waves at sea it is more convenient to measure the period, T, than the wavelength, λ. Oceanographers like to use a height measurement, H_s, the significant wave height, defined as the average height of the highest third of the waves. In order to extend this discussion to include random waves at sea, H_{tc} of equation (1) can be converted to H_s using the root mean square displacement, D_{rms} : in a sinusoidal train

$$H_{tc} = 2\sqrt{(2)}D_{rms}$$
$$H_s = 4D_{rms} \text{ (ref. 3)}$$
$$\therefore H_{tc}^2 = H_s^2/2$$

Wavelength, λ, can be converted to period, T:

$$\lambda = T^2 g/2\pi$$
$$\therefore \text{Power} = W\rho g^2 T H_s^2/64\pi \qquad (2)$$

The power which could be extracted can be predicted (Fig. 2 and ref. 2). Computer programs can apply equation (2) to each cell of Fig. 2. The average power over a whole year is 77 kW m^{-1} of frontage. Model tests suggest that the fraction of energy which may be extracted depends on the depth of the installation.

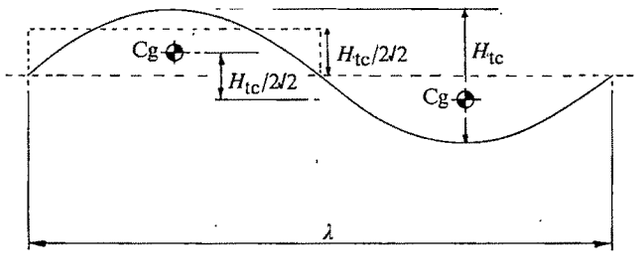


Fig. 1 Part of a progressive train of sinusoidal gravity waves in deep water. λ, wavelength; H_{tc}, trough to crest height; Cg, centre of gravity.

Above a depth d, this fraction is $1-\exp(-8\pi^2 d/T^2 g)$. New arrays can be drawn up for different depths, with each cell containing the contribution down to that depth. Unfortunately, some of the power comes in uncomfortably large amounts. A practical installation might need to be underdriven or submerged during the most severe weather. Further arrays for different power limits can be constructed with the contributions of high power cells reduced to some allowable maximum. Table 1 shows the results for combined depth and power limit reductions from three sea areas, and Fig. 3 shows the relationship between power density and wave period for the three observing stations.

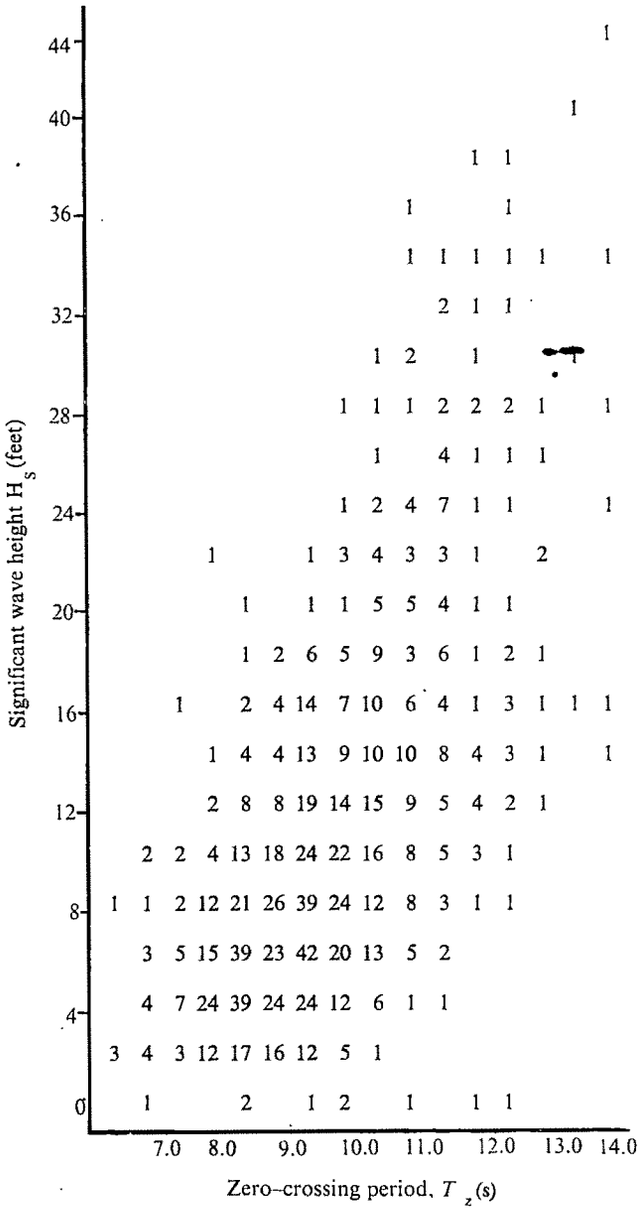


Fig. 2 Scatter diagram for whole year observations at Station India reproduced from ref. 2, showing the number of occurrences, in parts per thousand, of waves of a particular height and period.

Design problems

A search of British Patents shows about a hundred proposals of varying feasibility for extracting wave energy (see refs. 4 and 5). There was a spate of them after the First World War. Flaps, floats, ramps, converging channels and liquid pistons are all advocated, and much ingenuity is expended in accommodating tidal rise and fall. Model tests on some proposals, however, show poor efficiency. More recently, though, the successful operation of equipment at low power levels for buoy and lighthouse use, has been described. (Y. Masuda, and T. Yoshida, preprints.)

The essential problem is finding a method to convert dispersed, random, alternating forces into concentrated, direct force, using a mechanism which is efficient at low levels and yet robust enough to withstand the worst conditions. I believe that rigid connections to the sea bed are not possible on a large scale, and that the installation must be freely floating out at sea. As much of the equipment as possible should be below the surface. Concentrations of stress must be avoided, and power should be extracted smoothly.

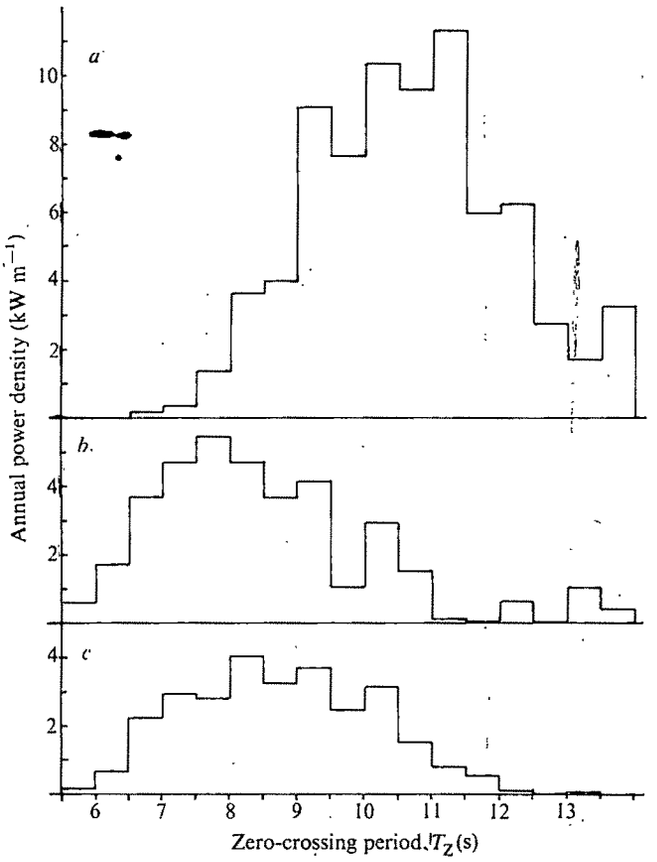


Fig. 3 Distribution of annual power density with period: *a*, Station India; *b*, MV Famita; *c*, Sevenstones.

Moving parts, are generally troublesome, but designs without them can only produce large volumes at low pressure differences, and need some kind of transformer for efficient conversion. I must, therefore, reluctantly accept the conclusion that the use of moving parts is unavoidable. I think that it is best to use rotating elements, and to protect mating surfaces from the sea. It will be difficult to gather in power from many small units moving independently, and so there should be a common framework for a number of them. As waves transmit energy to one another with very high efficiency, the water must be allowed to move in its usual circular pattern.

The first step is to get away from the idea of an object bobbing up and down, although, of course, it is this aspect of wave motion which is most apparent. Use of the to and fro movement would be much more rewarding. The energy passing through a vertical window is concentrated close to the surface and the water movements at all depths are of the same phase. I have tested a simple vertical vane pivoted about a horizontal axis along its bottom edge one eighth of a wavelength down. It works over a reasonable range of wavelengths and it shows an efficiency of about 40% when driving an electronic dynamometer. Roughly 25% of the energy is transmitted onwards, and about 20% is transmitted back to the source. It would, however, be much more efficient if, somehow, it could be designed so that it did not displace water astern and so that the amount of water displaced at any depth corresponded to the amplitudes of water movements at that depth. But is a vane of this kind possible?

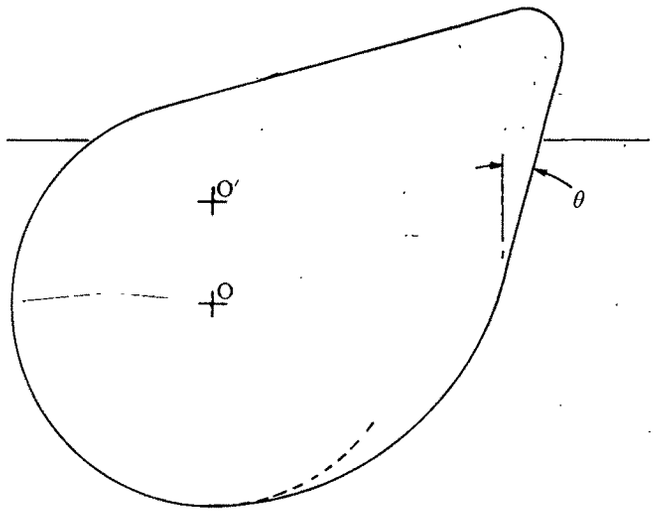


Fig. 4 An efficient vane shape designed to rotate about O. Waves come from the right.

The design

Consider the shape shown in section in Fig. 4. It rotates about its centre, O, and absorbs power from waves coming from the right. Its stern is a half cylinder centred at O, but from the bottom point it grows into a surface which is another cylinder centred about O′. This shape continues until it reaches an angle θ, to the vertical, at which point it develops into a tangent, which is continued above the surface. In my first model, O′ is one half radius above O, and θ = 15°. The efficiency for wavelengths of about eight times the diameter of the small cylinder

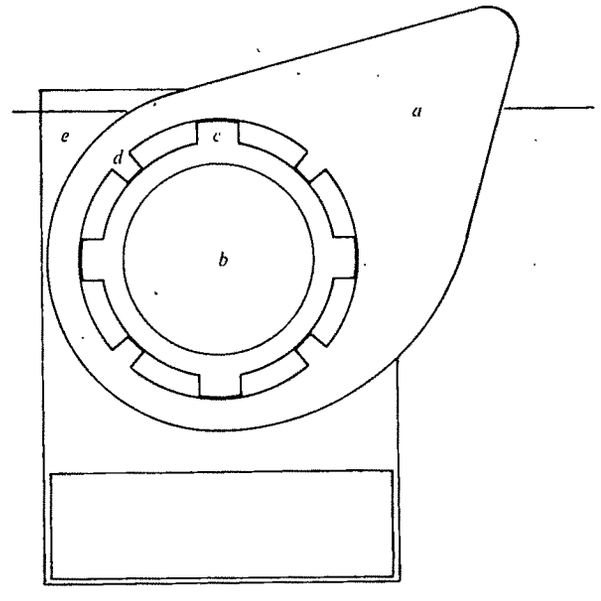


Fig. 5 A vertical section through vane and spline pump. *a*, vane; *b*, a hollow cylindrical member; *c*, paraxial ridges; *d*, inward facing ridges on the vane; *e*, vertical fin between this vane and the next. For the North Atlantic the diameter of the cylindrical portion will be between 10 and 20 m.

is over 80%. I intend to test the theory that a good vane should be able to extract nearly all of the power contained in the band of water above its depth of immersion.

When the vane moves there is no change in the displacement of the water behind it and the changing displacements in front of it rise from zero at the bottom of the cylinders to amounts close to those in the approaching wave. This means that the shape meets the necessary requirements. It is, so far, the most successful of the likely shapes that I have been testing in a wave tank. The models are coupled to a dynamometer consisting of two moving coils in a magnetic field. Velocity signals from one coil are amplified and sent to the other so as to oppose movement. Velocity and force signals are multiplied to indicate power absorbed by the vane and are compared with wave height measurements. This electronic simulation is very convenient for laboratory tests.

In full scale equipment at sea, the random rotations of a vane will produce unidirectional pulses of water flow through a special pump (Figs 5 and 6). The water is deoxygenated and decarbonated, and recirculates within the system. A vane is supported on, and rotates about a hollow cylindrical member with paraxial ridges on its surface. The outer faces of the ridges are supplied with high pressure water from an auxiliary pump, and they form hydrostatic bearings[6]. Between them there are inward facing ridges from the vane. The pair fit as a spline with close radial, but very loose circumferential clearance. The

Table 1 Annual power density for different combinations of depth and power limit.

Depth (m)	Power limit (kW m^{-1})		
a	100	200	300
5	22.7	24.0	24.1
7.5	29.0	32.3	33.0
10	33.5	38.6	40.0
15	39.1	47.0	49.8
20	42.4	51.9	56.0
25	44.1	55.0	59.8
b			
5	16.4	16.5	16.5
7.5	20.8	21.5	21.5
10	23.8	25.1	25.1
15	27.1	29.4	29.8
20	28.7	31.8	32.4
25	29.5	33.0	33.9
c			
5	10.9	11.0	11.0
7.5	14.2	14.5	14.5
10	16.6	17.1	17.2
15	19.3	20.7	20.8
20	20.9	22.7	22.9
25	21.8	23.9	24.2

a, Station India (59°N 19°W), total power = 77 kW m^{-1}; *b* MV Famita (57° 30′N 3°E), total power = 36.8 kW m^{-1}; *c*, Sevenstones of Lands End, total power = 25.8 kW m^{-1}.

spaces between the inward and outward facing ridges form four double acting pumps with nonreturn Macleod valves[7] in the walls of the ridges on the cylinder (Fig. 5). These valves were originally developed for use with blood pumps. They consist of an elliptical butterfly plate across a conical passage. The centre of rotation does not pass through the centre of pressure and so there is a strong closing action. In the open state they offer minimum occlusion and are the most efficient nonreturn valves that I know of. The working surfaces of the pumps are faced with ceramic, and are ground.

A common back bone for about 40 vanes is provided by the cylindrical member. An outer tube contains manifolds connecting the spline pumps to a common turbine at the centre of the structure. The high pressure manifolds (400 pound inch^{-2}) are made from 1 m diameter pipes (Fig. 6). Inside this ring of pipes is a large tube which forms the low pressure manifold and which returns water from the turbine to the pumps. The interstices between the pipes and the inner and outer tubes are filled with a mixture of lightweight concrete, glass fibre and resin. The ends of the pipes and tubes are swaged to allow a clamped connection of each length to the vertical fins fitted between vanes (Fig. 5). These fins house trim tanks, pressure smoothing accumulators, and the pipe work which couples each pump to the manifolds. At the bottom of the fins concrete ballast keeps the centre of gravity well below the metacentre.

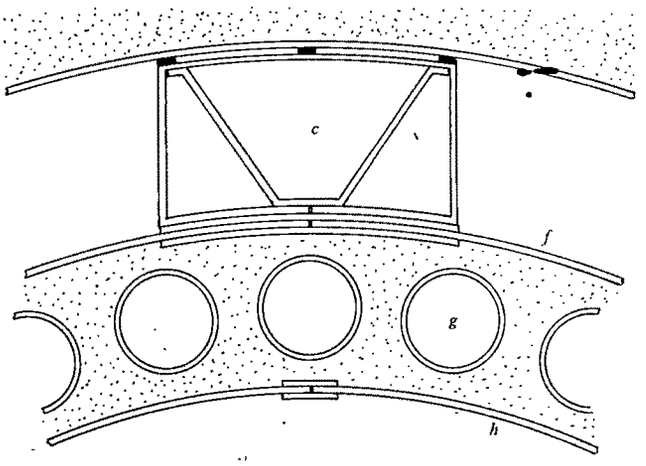


Fig. 6 Enlarged section of the spline pump, showing one ridge and the manifolds. *c*, Paraxial ridge shown in Fig. 5; *f*, outer tube containing manifolds; *g*, pipes of 1 m diameter; *h*, large tube, forming the low pressure manifold.

A crucial problem facing the designer of wave power machinery is the provision of a steady reference against which his waves can act. Many approaches use connection to the land but this design would operate out at sea. A stable reference can, however, be supplied by using a structure 0.5–1 km long. A short machine would pitch and heave and develop little force between vane and backbone. As the length is increased the installation samples a wider range of wave phases and becomes more and more steady. Stability is obtained from the inertia of the ballast weight, from the drag of the deeper parts in quieter water, and from the coupling of the machine to waves of opposing phase. The only drawback to using very great lengths is that the stress which can be developed by the worst conceivable wave rises with the square of the length. The largest predicted wave in 50 years in the North Atlantic would have a trough-to-crest height of 34 m and a period of 16.5 s. The surface particle velocity would be 6.5 m s^{-1} with a resulting stagnation pressure of 21,000 N m^{-2}. The shear forces and torsions which result from such monstrous waves would not be the most serious problem. It is the bending moments which are critical, and which set limits to the lengths which can be used.

Energy storage

In common with solar, nuclear, and wind power, wave power comes at times not necessarily convenient to the user. Although its seasonal availability roughly matches demand, the problems of storage and distribution require careful attention, and will have to be taken into account in the overall planning. Electrolytic production of hydrogen from sea water looks promising in this respect.

The installations could be self propelled. They could move in

line ahead, a low drag condition, out into the Atlantic, turn abreast to the waves, and be slowly driven back by wind and wave thrust, storing hydrogen on the way. Most of the hydrogen could be discharged at a shore terminal, leaving enough to get out to sea again. This mobility gives many advantages, and obviates the mooring problem.

Wave power is clean, safe, permanent and uses relatively simple and well known technology. It is likely to receive plenty of support after a bad nuclear accident but it would be prudent to have the basic research done now[8]. We are particularly fortunate in our resources of wave energy. The approaches to the Hebrides are probably the best site in the world. A few hundred kilometres of installation could meet the total present electrical energy requirements of the UK.

I would like to thank the University of Edinburgh and the Nuffield Foundation who supplied my workshop facilities, the National Institute of Oceanography, and my colleagues Drs O. P. Buneman, C. A. Greated, D. Mollison, and J. W. Ponton, for their help and criticism, and Sheikh Ahmed Zahi Yamani and OPEC for their continuing encouragement.

[1] Tucker, M. J., *Trans. Inst. nav. Archit.*, **98**, 236 (1956).
[2] Draper, L., and Squire, E. M., *Trans. Inst. nav. Archit.*, **109**, 85 (1967).
[3] Tucker, M. J., *Proc. Instn civ. Engrs*, **26**, 305 (1963).
[4] Dhaille, R., *La Houille Blanche* (4e *Journees de l'Hydraulique*), **II**, 421 (1956).
[5] Charlier, R. H., *J. marine tech. Soc.*, **3**, 59 (1969).
[6] Stansfield, F. M., *Hydrostatic Bearings* (Machinery Publishing Co., Brighton, 1970).
[7] Macleod, N., *One day discussion of Mechanisms*, 98–110 (Inst. mech. Engrs, London, 1971).
[8] Lovins, A., *World Energy Strategies* (London, Friends of the Earth Publications, 1973).

Prospects for hydrogen as an energy resource

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Storing energy in the form of hydrogen is an attractive possibility to provide fuel for transport and the reduction of iron ore. The main obstacle is the expense of the electricity needed to synthesise hydrogen.

THE recent restrictions placed by the producing nations on the global supply of oil, and the growing realisation that world demand for oil and natural gas is increasing at a rate faster than the discovery of new resources, have focused attention on the need for alternative energy resources. There is no immediate crisis in terms of proven oil and gas reserves, but development towards alternatives should start soon if the new technology is to be widely available when it is needed. As an indication of the time scale required for the introduction of a new energy resource it is worth pointing out that 18 years after the commissioning of the first Calder Hall reactor, nuclear power has still not achieved more than 10% penetration of the market for electricity production in the United Kingdom.

Forecast of the time when the rate of production of oil will begin to decline vary over a considerable period, and might be as early as the mid-1980s if historic trends continue: it is assumed here that the decline will certainly be evident by the end of the century. As we approach that time two primary energy sources are likely to assume over-riding importance: coal and nuclear fission. But synthetic secondary fuels are likely to be needed for special purposes and hydrogen could be one of these. Hydrogen is best regarded as a potentially attractive means of storing and transporting energy which can be provided from a variety of primary sources: it is therefore more analogous to electricity than to oil, although some of its most rewarding applications may be as a substitute for oil products. The hydrogen would be produced from water and in this process more energy is expended than is recovered in burning the hydrogen subsequently; this point will, however, assume decreasing importance as the production of hydrogen moves progressively towards dependence on renewable energy resources. Hydrogen attained industrial importance a few decades ago when coal provided the primary energy for water splitting; now it is produced in larger quantities from natural gas and oil: if it is to achieve the even more widespread use implied by the 'hydrogen economy' then it will have to be based on the energy input from nuclear fission reactors and subsequently there could be a gradual transition to nuclear fusion and solar energy resources.

Much has been written on the subject of hydrogen over the past few years, culminating in the nearly 100 papers at a recent conference devoted exclusively to the subject (see ref. 1). It is not possible to review all of this work here; I shall attempt rather to take a realistic view of the overall prospects and of some of the problems to be overcome.

Markets for hydrogen

As oil becomes a scarce resource and nuclear energy takes over, in principle all major energy users except air transport could be satisfied by the increased availability of relatively cheap electricity—much has been written on the advent of the all-electric economy. This would also require however, the development of major new technology, of which the long range electric road vehicle is perhaps an outstanding example. It is possible that when the costs of transmission and storage are added to the cost of production, under some circumstances hydrogen may become competitive with electricity for many uses. This possibility of interchange and substitution between several energy sources renders the whole subject very complex: the forecaster finds it fascinating and yet extremely hazardous.

One of the largest new uses for hydrogen could be as a fuel for road transport: in the United Kingdom this might ultimately amount to a requirement of several million tons per year. The competition will however be strong among several candidate fuel supplies:

- allocate oil to road transport as a priority item
- electricity, based on advanced batteries for energy storage on the vehicle
- liquified methane (natural or substitute)
- methanol
- hydrogen

Each of these possibilities has its advocates and, apart from the first item, each has a considerable range of development problems to be overcome. Perhaps a mixture of several solutions is the most likely outcome, with each fuel taking a particular share of the total market.

Practical experience has shown that the use of hydrogen requires little alteration to the internal combustion engine on

which so much development effort has already been lavished, and the environmental pollution from hydrogen would be much less than from petrol. It is feasible for the same vehicle to be engineered to be able to switch as required between a hydrogen and a hydrocarbon fuel supply. Increasing attention is now being given to devising the best means of storage of hydrogen on road vehicles. Storage as gas would be totally uneconomic but on present evidence there is no clear choice between cryogenic techniques and storage in metal hydrides. It is possible that hydrides will prove to have over-riding safety advantages. The most promising hydride system proposed so far is based on iron-titanium alloys, which are capable of absorbing and desorbing hydrogen at near ambient temperatures. There is much useful basic work to be done to arrive at the optimum hydride composition to meet criteria of high hydrogen density, low overall mass, avoidance of high operating temperatures, and the ability to withstand multiple recycles.

Economic forces will play a large part in determining whether hydrogen really can compete. There is a growing number of attempts in the literature to forecast the relative costs of the various fuels up to the end of the century, and as would be expected they cover a considerable range. For the United Kingdom in the late 1990s, the ranges in Table 1 summarise the production cost estimates. North American cost predictions tend to be a little lower, mainly in the range 100–140 (see ref. 2 for a useful comparative summary) pence per GJ for hydrogen by electrolysis. It is apparent from these figures that there would be a chance for hydrogen to become competitive with petrol towards the end of the century if the more optimistic rather than pessimistic assumptions prevail. Synthetic petrol from coal seems somewhat more promising but, as pointed out by G. V. Day at a conference on energy, Europe and the 1980s at the Institute of Electrical Engineers, London, May 1974, environmental restrictions and the availability of labour may restrict coal output to below the level required for the large-scale synthesis of petrol in addition to coal's more traditional role in electricity production.

There is potentially a much larger market for hydrogen as a substitute for natural gas for the majority of domestic and industrial purposes (possibly as high as 12×10^6 tons yr^{-1} in the United Kingdom) in which its energy content rather than chemical properties are important. As long as supplies remain available from the North Sea it will perhaps be impossible for hydrogen to compete on cost grounds (a factor of two to three lower than the cost quoted in Table 1 would be needed). But again towards the end of the century, these supplies may well become insufficient and hydrogen could present competition to replacement by substitute natural gas (SNG) from coal. Though at first sight it might seem possible to utilise the existing gas distribution system to distribute hydrogen, there may be very substantial problems in technology and in the logistics of a gradual changeover. Although this market is very large, therefore, it might not prove possible to penetrate it, except in the case of communities isolated from the national gas grid system. Detailed assessment studies of this aspect of the hydrogen economy are required.

There are specialised uses for hydrogen which may account for somewhat smaller demands, but which nevertheless would be large by current standards of production. One of these is the direct reduction of iron ore for steel making. This idea has been around for a long time and is currently attracting more attention because of problems created by the diminishing supply of metallurgical coke. In the United Kingdom the hydrogen demand could rise to the order of 10^6 tons yr^{-1} for this purpose. Second, imaginative designers of aircraft have also been proposing hydrogen as a substitute for aviation hydrocarbon fuel. Modern jet aircraft have voracious appetites for fuel: an average passenger in a plane with a load factor of 50% 'consumes' four times his own weight of fuel on a transatlantic crossing. Though it could be argued that this should be a priority outlet for oil products, some observers have claimed that there could be real technological advantages for hydrogen in aircraft use. Several preliminary design studies have been made of aircraft fuelled by hydrogen for both very high speed applications (Mach 5–6) and subsonic jumbo jets, but it is evident that much ingenuity will be required to avoid the very considerable advantage of low fuel weight per unit of stored energy being cancelled by problems arising from the high volumetric requirements for storage. If these design problems could be overcome, this could be an ideal use for hydrogen in transport, because fuel handling would be under strict control.

Finally, there is a hybrid fuel economy situation in which nuclear-based hydrogen could be used to adjust the C : H ratio of fossil fuels, as in the production of substitute oil or gas from coal.

Outlined above have been several contenders for the most likely new outlets for hydrogen if it can be produced more cheaply than at present and in much larger quantities. Assuming that the most feasible primary energy source for producing the hydrogen from water is the nuclear fission reactor and that the only feasible method on present evidence is by electrolysis using the nuclear-produced electricity (see below), it is of interest to consider the size of nuclear installation that might be required (Table 2). The number of reactors of the 1,000 MW class, currently a reasonably standard size for design and construction purposes, would be relatively small to meet United Kingdom needs compared with the likely installed capacity for electricity production by the end of the century.

Table 1 End-of-century fuel production costs in United Kingdom

	pence per GJ
Petrol (tax free) from crude oil*	140–220
Petrol from coal†‡	110–130
Hydrogen by electrolysis† (advanced technology)	135–175

* A range of double to treble the early 1973 costs is assumed.
† Extrapolated from a paper presented by G. V. Day at a Conference on Energy, Europe and the 1980s, at the Institute of Electrical Engineers, London, May 1974.
‡ Extrapolated from a paper by J. S. Harrison to the Management Group of the Society of Chemical Industry, November 1973. Coal assumed to cost double 1973 prices.

Table 2 Approximate number of 1,000 MW reactors to serve various potential UK markets for hydrogen produced by electrolysis

	No. of reactors
One million cars	3
Iron ore reduction (based on 1971 output level)	7
Fleet of 10 supersonic long-range aircraft	2

Methods of production

The outlets discussed above lie in the range 10^5–10^7 tons yr^{-1}: at the upper end these are large by present day standards for any single country. Two processes are regarded as competitors to make the best use of nuclear energy for the decomposition of water. The energy could either be used directly as high temperature heat to promote thermochemical decomposition, or the nuclear energy could be converted into electricity and used in an electrolysis plant.

Although the electrolytic route introduces the additional step of electricity production with an immediate loss of up to two thirds of the original energy, it is often regarded as the reference process. A large development programme would be needed, however, to translate current practice into a successful and economic plant of the size required.

Several authors have demonstrated that the major component in the cost of hydrogen from large plants would be the price of the electricity. In order to minimise the latter, the advantages

of scale would need to be realised and nuclear stations producing about 1,000 MW are envisaged directly coupled to the electrolysis plants. Each 1,000 MW complex should be capable of producing about 150,000 tons yr^{-1} of hydrogen. This is about 70 times the size of the largest unit yet built, and a very much larger extrapolation from the average electrolytic plant.

The problems of such very large plants have not yet been fully assessed. It is already clear, however, that conventional electrolysers in which virtually all the industrial know-how at present resides will not operate at a sufficiently high energy efficiency to render them economically viable. It would be necessary, therefore, to develop new types of cell probably incorporating porous electrodes and operating at high pressures. One of the most interesting new concepts in this field is the solid polymer electrolyte cell described by L. J. Nuttall, A. P. Fickett, and N. A. Titterington at the conference on hydrogen economy, University of Miami, March, 1974. This cell has been developed primarily as a means of producing oxygen for aerospace and submarine applications, but it is claimed that it can be adapted readily for large-scale hydrogen production. The electrolyte is a solid plastic sheet of perfluorinated sulphonic acid polymer which is a good conductor of hydrogen ions, and the cells can be constructed in such a manner as to produce hydrogen directly at high pressures, thereby avoiding extra compression costs which might otherwise be incurred in optimum transport and use.

An alternative to electrolysis which is stimulating rapidly growing interest is the decomposition of water by closed-cycle thermochemical processes. At least 10 research centres are now actively interested in this possibility and over thirty potential cycles have appeared in the published papers. The prize is a higher energy efficiency than is possible in the electrolytic route, perhaps approaching a factor of two higher under favourable circumstances. It is disappointing that none of the cycles described so far is an outright winner. The most substantial programme on thermochemical cycles is that at the Ispra laboratory of the Euratom Joint Research Centre. An illustrative example of the range of cycles which they have proposed is based on reactions of the iron chlorides:

(1) $6FeCl_2 + 8H_2O \longrightarrow 2Fe_3O_4 + 12HCl + 2H_2$ 650°C
(2) $2Fe_3O_4 + 3Cl_2 + 12HCl \longrightarrow 6FeCl_3 + 6H_2O + O_2$ 200°C
(3) $6FeCl_3 \longrightarrow 6FeCl_2 + 3Cl_2$ 420°C

Insufficient experimental information is yet available on these reactions for a conclusion to be reached concerning the cycle's chance of technical success. As with all other proposed cycles, certain difficulties can be foreseen already: reaction 1 needs close control of temperature to avoid a path to different products, reaction 3 presents chemical engineering problems arising from the volatility of $FeCl_3$. Important constraints which can rule out some otherwise promising cycles are imposed by the upper limit of temperature which can be provided by a nuclear reactor system (because of limitations on materials performance in heat exchangers), the economic need to avoid exotic materials of construction (difficult when handling metallic halides at high temperatures), the requirement for high rates of reaction to cut down the size of the plant items and the need to avoid the recycling movement of massive amounts of solid materials in the plant.

Electrolysis will allow the earlier introduction of large scale hydrogen production if other factors are favourable, but if a more promising thermochemical cycle can be devised this route could take over in the longer term. There is insufficient information available at present for the thermochemical route to be costed.

Conclusions

Despite growing current interest, the widespread introduction of hydrogen as an energy carrier is still well in the future. But such economic forecasts as can be made on rather scant evidence do not rule out the possibility that hydrogen could be competitive with alternatives such as petrol from oil or coal, or nuclear-produced electricity, by the end of the century. These estimates are based on the production of hydrogen by electrolysis, but if a thermochemical route emerges from the substantial research programmes now being carried out, it would have the potential of considerably improving the prospects.

The safety aspects of the widespread use of hydrogen require close examination, but in principle no major stumbling block is likely to emerge. The introduction of hydrogen could, however, best take place in the controlled environments represented by aircraft and commercial road transport fleets, and industrial plants for the hydrogenation of coal or the direct reduction of iron ore. Use in the private motor car and as a general substitute for natural gas seem further off.

Above all, the advent of the hydrogen economy must depend upon the successful further development of nuclear power to provide the cheap reliable primary energy source for water splitting. The electrolytic route can be linked to the need for successful development of the sodium cooled fast reactor to provide the cheapest electricity in the long-term, whereas the thermochemical route needs a fully developed high-temperature reactor system to provide the high temperatures required.

Both routes to hydrogen would require a large development programme, with the thermochemical route currently lagging because a universally agreed attractive cycle has not yet been devised. At the basic scientific level the thermochemical route presents an immensely stimulating challenge to chemists, chemical engineers and materials scientists.

1 Cahn, R. W., *Nature*, **248**, 628 (1974).
2 *Report of the NASA/ASEE Engineering Systems Design Institute, NASA Grant NGT*4 4.005.114 (1973).

Solar energy

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Studies have been made of a very wide range of possible ways in which solar energy might be used for domestic and industrial purposes. Some are already economically viable and the prospects for others are improving through intensive programmes of research and development.

MAN has been using solar energy purposefully for millennia and will continue to do so, since it is the driving force for agriculture. In the pre-industrial era, it was the predominant source of power also, for the windmills, watermills and sailing ships of the time derived their energy ultimately from the sun. These devices could not have driven the subsequent period of industrial expansion because, without growing to inordinate size, they could not have made available generally powers greater than a

few hundred kW. They were well suited to modest local needs, but could not serve the demands of industry, concentrated in the interests of high productivity. This became possible only with the use of the concentrated solar energy stored in the fossil fuels. Yet we may shortly have to return to an economy directly powered by solar energy as the brief era of cheap fuel comes to its inevitable end. In the long term we have no choice; solar energy is the only perpetual source capable of meeting the likely demands of the future. We have some options in the short term. The fission reactor could support us for a time and will certainly ease the problems of diminishing fossil fuels, though it is not universally welcomed and a shortage of fissionable fuel is already envisaged. A fusion reactor would presumably defer a general energy shortage for a very long period, but it is proving elusive. It might be sensible to enquire whether the resources allocated to fusion research could be more profitably applied to the exploitation of solar energy.

Available energy

The decline of the windmill and watermill is traceable to the low energy flows available to any given machine. Though the total energy arriving from the sun is immense, the flux is very small by present-day engineering standards. At the top of the atmosphere the maximum value is about 1.3 kW m^{-2}, but a variety of scattering and absorbing processes reduce this to about 0.9 kW m^{-2} at the surface. The difficulties of effective utilisation are increased by the seasonal and hourly variations, which are functions of latitude, and by the random fluctuations due to the weather. In summer,

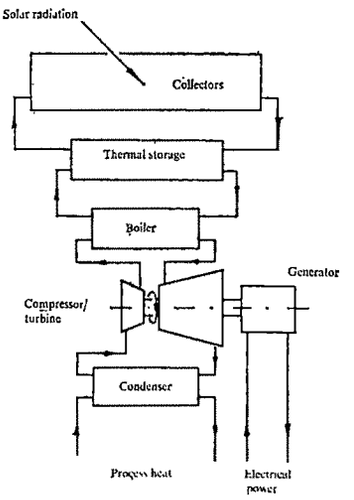


Fig. 1 Elements of thermal energy conversion system.

the increasing day length with increasing latitude helps to compensate for the longer air path so that in clear conditions the total energy received during one day varies only weakly with latitude. The winter isolation, on the other hand, is much affected by latitude; in Britain, for instance (latitude about 52°), a clear day in midwinter yields only about one-tenth of the energy of one in midsummer.

The total annual insolation (energy incident on a horizontal surface) is around 1.5 MW h m^{-2} over large areas of the earth and in places it exceeds 2 MW h m^{-2}.

The effects of climate, though substantial, are less than is usually supposed. The average annual insolation in Britain, with its notorious cloud cover, is nearly 1 MW h m^{-2}. Britons are usually surprised to learn that, on average, the United Kingdom receives more than half the energy available in Australia or two-thirds of that in the United States. The large droplet sizes of cloud in relation to the wavelength of the radiation causes predominantly forward-scattering, so that a sizeable fraction penetrates unless the cloud is optically very deep. At the same time, the radiation becomes diffused. Due to the high cloud cover in Britain an unusually high proportion—more than half—of the radiation reaching the surface there is diffused. This is important because this part of the radiation cannot be brought to a focus.

Large scale use

The entire world energy demand for human purposes other than agriculture is less than the solar energy falling on a region 100 km square in a favourable location. This is a trifling area compared with that of the world's land surface or even of the deserts, but it is very large on the scale of human constructions. Inefficiences in the energy conversion processes used would mean that even greater collecting areas would be needed. Nevertheless, serious study has been given to the design of 'solar farms' thousands of square kilometres in extent[1]. In these, the collectors concentrate the radiation so as to produce in a working fluid a temperature high enough to operate a

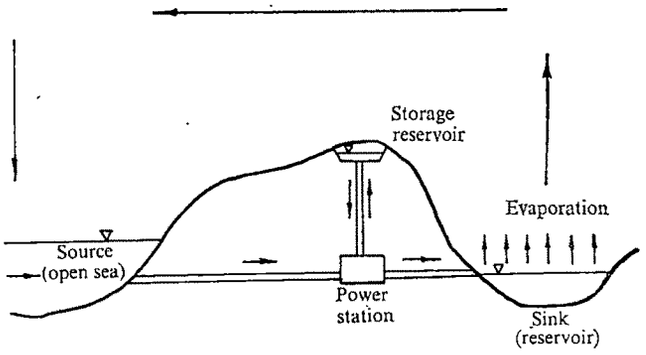


Fig. 2 Heliohydroelectric scheme, with pumped storage.

thermodynamic engine with respectable efficiency. An intermediate fluid might be used to link the collectors to a thermal storage element which permits a match to be obtained between the energy supply and the demand (Fig. 1). The cost of collectors is a significant factor in all uses of solar energy. When they are of the concentrating type, they need to be robust to maintain good fidelity of shape and resistance to wind forces and they must be steerable to follow the apparent motion of the sun. Useful temperatures can be obtained from two-dimensional parabolic troughs with the focal line orientated east–west, requiring only a slow seasonal adjustment to follow the sun's declination. It might be foreseen that these could be mass-produced in modules of moderate size which could in principle be indefinitely replicated to build up a large station.

The cheapest large scale collectors, in terms of cost per unit area, are reservoirs. A scheme recently advanced on this basis involves the Qattara Depression in the Libyan Desert[2]. It is proposed that this region should be connected to the Mediterranean, about 80 km away. The high evaporation rate, about 1.7 m a year, would maintain a free water surface 12,000 km^2 in area within the Depression at about 60 m below sea level. This would be sufficient to operate a hydroelectric plant of about 4,000 MW capacity, as shown in Fig. 2.

The ultimate in large collectors is the sea itself. Power plant exploiting the temperature gradient in ocean waters was first constructed in 1930[3] and the possibilities have been re-assessed more recently. Though the small temperature differences available inevitably mean low thermodynamic efficiency, the economics are by no means unfavourable[4].

Large scale systems such as these effectively combine the functions of collection and storage. A corresponding

arrangement on land might make use of the photosynthetic manufacture of combustible hydrocarbons. There is already a world shortage of timber and presumably of land suitable for traditional afforestation, but official studies in the United States[5] and Australia[6] both comment on the relatively small fraction of land area which would be needed for the intensive cultivation of fast-growing plants capable of furnishing a significant proportion of the energy demand. It is to be noted, however, that studies have yet to be made of the engineering problems of harvesting, transport and processing involved in such a scheme. These studies are urgently needed, since photosynthesis offers the most direct path to the manufacture of liquid and gaseous fuels which will probably continue to be needed indefinitely for transport.

A major objection to all schemes for the conversion of solar energy on a large scale at central stations is that there is very little freedom of choice on where they might be sited. Nearly always, they will have to be distant from the places in which the energy is to be used. This not only requires expensive distribution networks of power lines or gas pipelines, but raises the possibility of undesirable climatic effects arising from the transfer of energy on a large scale away from the places at which it has hitherto been absorbed. These and other factors have led many to the view that the proper scale of solar energy conversion is local rather than central. As this is a reversion to its pre-industrial use, it follows that some attention will be necessary to the methods of integrating such a source with the others that will be available, particularly in urban-industrial societies.

But, throughout the world, there is a huge potential requirement for devices with capacities ranging from a few watts to a few tens of kilowatts. About 80% of the Earth's population lives between the 40° parallels, including many of the poorest, but they can nearly all count on an energy flux exceeding 1 MW h m^{-2} yr^{-1}. It is more instructive to compare this, not with the million MW h per year required from a European power station, but with the level of local demand: the 1 MW h yr^{-1} of work performed by a pair of bullocks, or the 100 kW h needed to winnow the grain from a family's land. All the hot-water needs of a clinic and the refrigeration of its wards and theatres could be provided by the energy which falls on its roofs. The roof of a dwelling could furnish power for a loom or lathe; that of a barn enough for a pump, able to irrigate 10 hectares of land. A school's radio and television receivers could be powered by a device the size of a blackboard; a solar still the size of a duck pond could yield enough drinking water for a village.

Power for small local enterprises like these cannot be provided economically by large-scale generation at central sites, even by conventional means, largely because of the high cost of transmission where the population density is low. The cost of fuels for local power plant is also greatly inflated by distance, doubling in a few hundred km over land. It is being recognised that rural communities must be given more stability and, for this, work and amenity must be provided within the village. Solar-powered devices could substantially raise productivity in such local enterprises as weaving, timber sawing, paper milling, food processing and preserving, light manufacturing, plant maintenance, irrigation, fresh water supply, drainage and a host of other activities. By these devices, it may yet be possible to revitalise the village from within.

Local use

The conversion of solar energy for modest local demands has been the subject of experimental investigation for many years. It is known that solar stills, solar-powered engines and other devices were successfully operated a century ago. But they were unable to compete economically in an era of cheap fuel and their credibility suffered through the unguarded optimism of enthusiastic advocates whose claims were easily discredited. More recently, these devices have begun to receive sober engineering and economic analysis, exemplified by the contents of two specialist journals devoted to the subject: *Solar Energy* (Pergamon Press) and *Geliotekhnika* (available in translation as *Applied Solar Energy* (Allerton Press)).

Quite thorough studies have now been made of ways in which solar energy might be used on a local scale[7,8]. These include its direct use for heating and cooking; driving a thermodynamic system, as in distillation, desalination, air conditioning, crop drying and so on; providing a source for heat engines, refrigerators and heat pumps; direct conversion to electricity using thermionic, thermoelectric and photoelectric generators; and the promotion of photochemical and photobiological processes leading to the generation of electricity or the production of liquid and gaseous fuels and other chemical products. Some of these processes are already economically competitive and their position is bound to improve as fuels become scarcer and dearer.

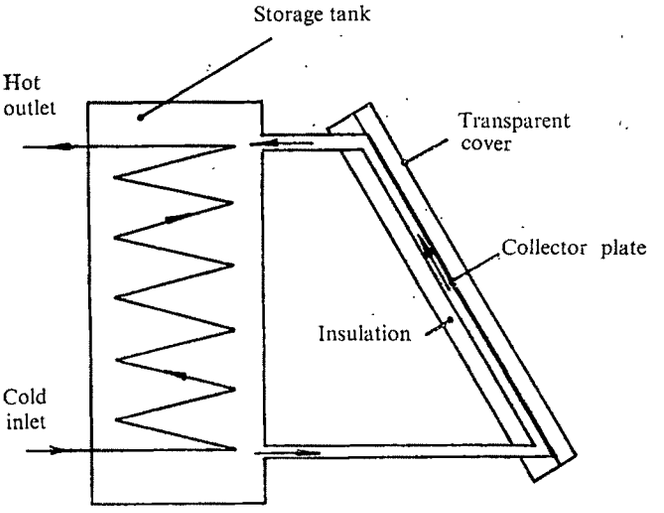


Fig. 3 Solar collector for domestic hot water system.

Flat-plate water heaters, of the kind illustrated in Fig. 3, are already widely used in many parts of the world and are now being marketed by several manufacturers in Britain. Up to about 60% of the incident energy can be transmitted to the water at useful temperatures (40-50° C) if the re-radiation is minimised by the use of glass cover plates, in the manner of a greenhouse, and of selective coatings on the absorbing surface which combine good absorptivity for solar radiation with low emissivity at working temperature. Heaters of this kind already make significant contributions to energy supplies for domestic, commercial and industrial purposes and their use is certain to grow[6]. Usually, they are linked with conventional heating systems and act as fuel savers rather than being designed to meet the entire heating load. It is estimated that in Britain they could yield about 10^6 MW h yr^{-1} for each 1% of dwellings equipped with them[9]. Much work is in hand worldwide to obtain a significant contribution to space heating and air conditioning from solar energy, and the design of buildings to exploit this is becoming a major preoccupation of architects. There is a history of building experimental 'solar houses' extending over some decades, in which Britain has had its share. In these, there has to be a means of energy storage to accommodate the seasonal and daily

variation in the energy available, and the fluctuations due to the weather. Thermal storage is obtained by changes in the sensible heat of a reservoir, which may be liquid or solid, or by latent heat exchange during a change of phase of the storage medium. Optimisation studies show that it is economical to obtain from the sun a significant fraction—typically ⅓ to ½—of the heating requirements of ordinary dwelling houses, even at quite high latitudes[10]. A typical domestic arrangement is shown in Fig. 4.

The economical provision of mechanical work and electricity on a small scale is proving elusive. There is little to choose in capital cost between low temperature heat engines driven by flat-plate collectors and more efficient ones with concentrating collectors. Both are still much more expensive than other sources such as the small diesel engine. But there are places where fuel costs are unusually high due to transport charges, where the net cost of work from solar engines would be competitive. These should be simple and robust and capable of being built using local resources. Their adoption will demand more from the entrepreneur than the engineer now.

On the other hand, much technical work remains to be done on direct conversion to electricity. Devices such as thermionic and thermoelectric generators require the maintenance of a temperature difference for their operation and hence have a restricted efficiency for thermodynamic reasons. Thermionic generators can operate effectively only with a high cathode temperature, perhaps of the order of 1,500 K, and this implies the use of concentrators of good optical quality, capable of tracking the sun accurately. This and other technical features have caused the thermionic generator to fall into disfavour for solar energy conversion, though it is capable in principle of quite good overall efficiency. Thermoelectric generators are subject to certain material limitations as well as thermodynamic ones, but continue to be of interest, even for use with flat-plate collectors, because of their simplicity. Photovoltaic cells, developed primarily for use in spacecraft, have reached useful conversion efficiencies—about 15% in full sunlight. Some, such as the GaAs/GaAlAs cells, function well in highly concentrated light. But these are single-crystal cells produced by very expensive processes. At a capital cost of some £10 to £100 per W of installed capacity, their use can so far be justified only in very specialised applications such as the powering of active buoys and other remote navigational aids and radio stations. Strenuous efforts are in hand to reduce the cost of photocells. This might be done by the development of large scale methods of production, perhaps of silicon ribbon which retains a single-crystal structure[11] or of thin-film polycrystalline cells. If capital costs are sufficiently low, quite small conversion efficiencies may be acceptable—the 8% reported for CdS/Cu_2S heterojunction cells[12], for example.

Outlook

It seems certain that non-agricultural uses of solar energy will continue to increase throughout the world, as the fossil fuels become scarcer and dearer. Already solar water heating is competitive in many countries and solar space heating seems about to become so. The outlook for conversion into work and electricity in the near future seems promising but is less certain. The principles are well established, and many devices have been built and tested in prototype form, but much more work remains to be done before they can have an assured place in the energy economy.

There is every sign that the necessary research and development work related to effective solar energy utilisation will be carried out promptly, at least as far as the needs of the developed countries are concerned. A US National Science Foundation report of 1972[5] recommended a commitment of $3,500 million in this area, justifying this with

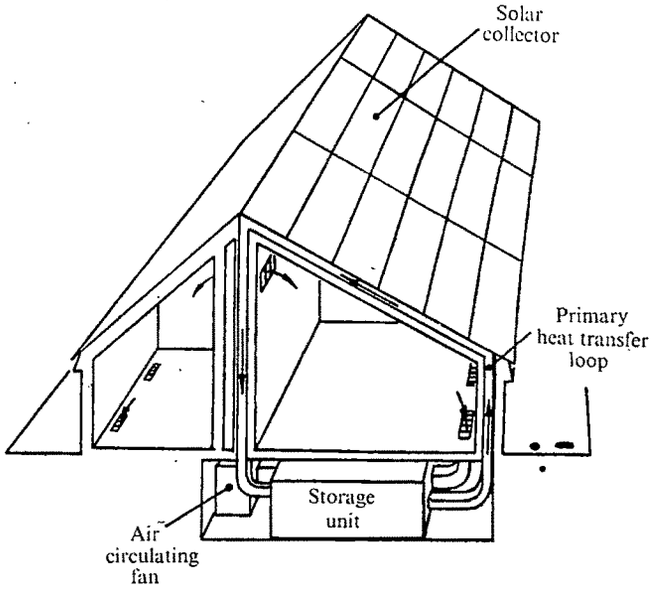


Fig. 4 Solar space heating system.

the contention that solar energy could provide 35% of the nation's heating and cooling requirements for buildings, 30% of its gaseous fuels, 10% of its liquid fuels and 20% of its electricity. These recommendations are being pressed ahead with extraordinary vigour; already the annual research budget for solar energy work has reached about $15 million. A somewhat similar programme, though not yet funded, was recommended by the Australian Academy of Sciences last year[6]. This suggests that solar energy could provide 40% of Australia's low grade heat and 50% of her liquid fuel within 25 years. The Soviet Union has maintained several centres for solar energy research for many years. Japan, long known for its widespread use of solar water heaters, is planning a major new solar energy programme said to be comparable with the American one.

There seems to have been little official support in western Europe though there has been some activity in the universities and the interest of industry is awakening. It remains to be seen whether this work will benefit the underdeveloped countries, which in many ways, are in most need of it. They usually have little indigenous fuel and are severely affected by rising fuel costs. On the other hand, they generally have ample solar energy, with little seasonal variation and a population which is still largely dispersed (but is drifting to the towns at an alarming rate). The encouragement of a greater use of solar energy in these countries could be the most sensible and effective way of giving financial and technical aid.

1. Meinel, A. B., and Meinel, M. P., *Physics Today*, **25**, 44–50 (February 1972).
2. Bassler, F., *Sol. Energy*, **14**, 21–28 (1972).
3. Claude, G., *Mech. Engng.*, **52**, 1039–1044 (1930).
4. Anderson, J. H., and Anderson, J. H., jun., *Mech. Engng*, **89**, 41–46 (1966).
5. *An assessment of solar energy as a national energy resource (Nat. Sci. Foundation NSF RA/N—73–001, University of Maryland*, 1972).
6. *Aust. Acad. Sci. Report* 17, (Griffin Press, Netley, South Australia, September 1973).
7. Daniels, F., *Direct Use of the Sun's Energy* (Yale University Press, New Haven, 1964).
8. Brinkworth, B. J., *Solar Energy for Man* (Compton Press, Salisbury, 1972).
9. Brinkworth, B. J., *Electronics and Power*, **20**, 356–359 (1974).
10. Lorsch, H. G., *Energy Conversion*, **13**, 1–5 (1973).
11. Currin, C. G., *Proc. 9th IEEE Photovoltaic Specialists Conf.* John Hopkins Univ., May 1972. (Institute of Electrical and Electronics Engineers).
12. Coste, G., in *Solar Cells* (edit. by Gordon and Breach, New York, 1971).

Tidal energy from the Severn Estuary

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Energy generation from the tides is widely regarded as economically less viable than that from conventional thermal alternatives. Although economic comparisons are unfavourable the potential overall improvements in network operation indicate the true value of new plant.

In operation, a tidal power scheme could possess much of the flexibility of highly versatile, conventional hydroelectric stations, and many types of project have been suggested. To assess in 1974 the preferred style of operation (not only for a tidal scheme but also for new thermal plant) necessitates anticipating consumer and generating demands beyond 1984, for it will not be until then, and for a long time afterwards, that any scheme planned now, whether tidal or otherwise, would be working. In particular, after 1984 a substantial nuclear component will emerge in the generating structure of all those industrial countries which opt for thermal energy schemes (few are even now able to rely on further conventional hydroelectric sources, and stations which require fossil fuels are unlikely to be favoured in large numbers). Coupled with the present crises in the provision of fossil fuels, and the complementary gross escalation of their costs, it is no surprise to find that the status of tidal power has, psychologically at least, improved markedly.

The last detailed, government-sponsored study of tidal energy in the UK was concluded in 1945; it was prompted by the additional power requirements of the war effort, and it leant heavily on the results of the only major UK study, of the Severn Estuary, which was completed in 1933. The schemes anticipated at that time were on a relatively small scale. A favoured site for the barrage was well up the estuary at English Stones, just below the present Severn Road Bridge (Fig. 1). The maximum power output would have been about 800 MW, over 10% of the maximum demand during the 1940s.

Canada, France and the USSR have since been studying more actively their own potential for tidal energy generation. The best site in the World, however, is probably the Severn Estuary. The Severn is not excessively deep or wide, the tides are almost the highest occurring anywhere, and it is near to centres which require a high energy load. But despite these advantages, which have been appreciated ever since the first studies, it is now more necessary to give close consideration to ecological factors than it was 30 years ago. Harnessing tidal energy must be seen to be not only economically and functionally competitive with other forms of generation but, in addition, must be environmentally acceptable in its own right. After all, the crisis in fuels is not so serious that all other considerations have to be overlooked, and in any case even the Severn Estuary–Bristol Channel could not provide more than about 25% of the present peak total demand for electricity, a ratio which will have declined considerably by 1984.

Energy potential of the tides

The rhythm of tidal variation from place to place, and from time to time at any place is well known. A few sites, like the Severn Estuary, the Bay of Fundy (Canada) and the Ile de Chausey (France) have exceptional spring tidal ranges of 14–15 m; and the range is over 16 m in Ungava Bay (Canada). These particularly high ranges only occur locally within each embayment, as a result of both focusing effects and a measure of resonance between the tidal and natural frequencies of oscillating water within the boundary of the estuary. Progressively lower maximum tides occur away from the optimum positions.

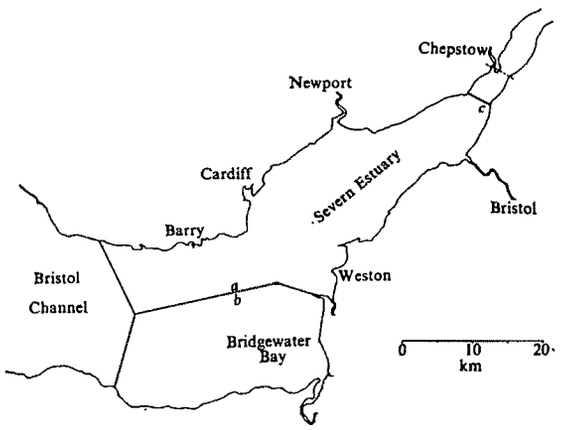


Fig. 1 Outline map of the Severn Estuary and Bristol Channel, showing the barrage scheme proposed by Martin in 1945. *a*, High level basin; *b*, low level basin; *c*, 1933/45 barrage proposals.

Much less favourable circumstances for energy generation apply during neap tides, when the range is typically about one half of that during spring tides. Inequality between the lunar and solar periods is responsible for this spring–neap–spring cycle, which takes nearly 15 days; it also gives rise to a tidal period of 12.4 h. Generation is therefore not possible over more than about 4 h during both the ebb and flood tide phases (Fig. 2), and the amount of energy, as well as the times at which it becomes available, vary from day to day. Nearly half of the output will coincide with off-peak times (peak demands for electricity now extend up to 14 h, from 0800–2200h).

Although, compared with generation from solar, wave and wind sources, the tides could be regarded as a relatively predictable form of natural energy, the form of output is still inadequate for electrical networks which depend heavily on thermal plant to meet a fairly predictable daily pattern of demand. To incorporate tidal energy into these networks an equivalent amount of thermal plant would have to stand by to carry the load when the state of the tide was not suitable for generation. This extra plant would have to be provided and run at low thermal efficiency (because duty periods are short), whereas continuous operation without the tidal scheme would produce the same output at a much improved efficiency.

Regularised output from a tidal scheme

The various limitations of the tides, quite apart from economical factors, have been a considerable barrier to

commercial development during this century. Earlier (tide mills can be traced back to 1180, at Dover), the consequences were accepted only in the absence of more reasonable alternatives. But methods of improving the regularity of output have been proposed, although they involve extra costs and energy losses:

- Power could be transmitted as and when available, either to the consumer by day or to a pump storage station. The latter alternative involves two reservoirs at differing elevations, interconnected through pump turbines. Many such installations now exist, storing energy from thermal power stations at night, for daytime release to meet short term peak load and emergency conditions. Over 75% storage efficiency can be achieved, so that six hours of pumping gives about four and a half hours generation at the same rate, when required. This technique was favoured in the 1933 and 1945 Reports. With tidal power, however, the additional costs fail to overcome the basic economic objections to tidal energy production.
- Two basins could be constructed in the estuary, each filled and drained when the tide is high and low respectively; energy would be generated by flow between the basins. This alternative was adapted by C. A. Martin (unpublished) for the Severn Estuary in 1945 (Fig. 1). Continuous output thus becomes possible; in Martin's scheme it varied between 3,350 and 850 MW. Unfortunately, however, publication of this imaginative scheme coincided with a slump in energy demand, and in any case it was ahead of its time in terms of the size of plant needed to provide for the normal annual growth rate in demand of that period.

Other schemes resembling that of Martin have followed as the scale of the second era of barrage planning has become more commensurate with requirements. For economical reasons, large thermal installations have also become fashionable: new stations, including nuclear plant, are, or soon will be, about 2,000 MW (in a 50,000 MW network). This trend is likely to persist as the nuclear proportion of the total installed capacity escalates towards 50% by the end of this century. After 1990 the nuclear energy component will therefore be greater in magnitude than the normal night time demand (equal to about 40% of the daytime demand). When this happens, all plant which use fossil fuels will have to be operated only during the daytime if nuclear stations are to be run continuously. Thereafter, the operation of nuclear stations would have to become flexible, involving not only further development and capital costs, but also a drop in the operating efficiency and extra fuel costs. This cannot be avoided unless excess energy is either discharged to the environment in the form of heat, or is supplied to pumped storage facilities.

I have already mentioned the fact that the use of pumped storage facilities allows energy to be regenerated as required, although bulk energy transfer from night to day should be distributed uniformly over the 12–14 h demand period if equivalent thermal plant (that is, the older, smaller and more uneconomic stations) are to be displaced entirely from the system. Given that pumped storage could be operated in this way, that is, in addition to the present duty of meeting relatively small variations in demand, there could well be merits in adopting it before a 'nuclear excess' situation arises. That would allow many of the more efficient fossil-fuelled stations which would otherwise have to be shut down at night, to be run continuously with an improved efficiency. In the limit, the ideal situation (which is not unobtainable in this case) would be to run all thermal plant, equivalent in capacity to about 75% of the maximum demand, on a continuous basis, and carry over from night to day accordingly.

The major objections to pumped storage installations are probably environmental. Creating reservoirs for any purpose receives little spontaneous sympathy from the community at large, especially when these cannot be made available for recreational purposes. Recently, the Central Electricity Generating Board (England and Wales) has experienced strong opposition to these projects; their new Dinorwic scheme in the Snowdon National Park in North Wales is regarded by amenity groups as aesthetically and environmentally unacceptable, because, they claim, it will spoil famous landscapes, and introduce extensive 'tide mark' scars as the water level changes more than 100 feet in four hours (three times the rate of rise and fall of the remarkable Severn Estuary tides).

Although, at 1,400 MW, the Dinorwic scheme will become the largest in Europe, its purpose will be more for short term supplementation of supply than for bulk energy transfer for consumption over 12–14 h. In fact, the latter style of operation would require 20 installations like Dinorwic to yield even 10% of a demand of 100,000 MW (the probable installed capacity soon after 1990). This is an unreasonable objective for pumped storage in its present form, even though 10% of installed capacity is still well short of the ideal referred to earlier. Understandably, alternative forms of pumped storage are being earnestly explored.

Martin's concept and estuarine pumped storage

The necessary difference between the water levels in the two basins of a pumped storage scheme may be achieved by using surface and underground reservoirs, which suggests that the sea and natural caverns should be exploited where available. An alternative would be to follow the principles adopted by Martin, and store energy by increasing the difference between the levels in two basins formed in an estuary. This variation has been evaluated by my colleagues and I, resulting in the type of scheme shown in Fig. 3 (the preferred energy capacity and geological conditions would dictate the actual location of barrages).

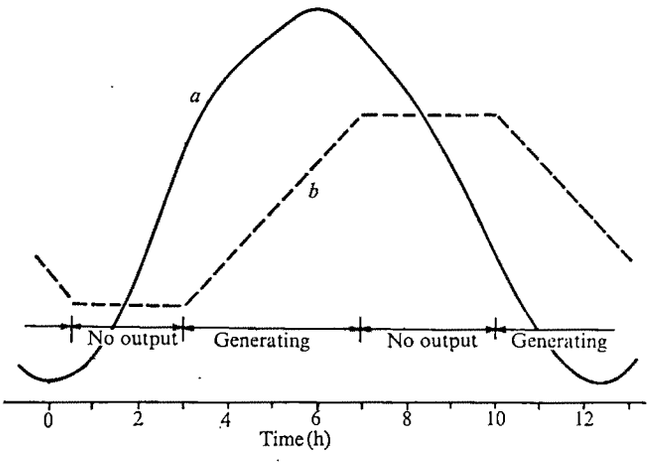


Fig. 2 Variations of tide level and the level in the basin, showing periods of generation.

The basic layout is somewhat different from that proposed by Martin (*et al.*), for here the second basin is small in area and sited entirely in deep water, so that the level can be reduced by pumping at night to well below low tide level, without the effects being felt on the coastline. The level in the larger basin is raised during the same night time period, but not to a level higher than that of the present high spring tide, thus avoiding problems of land drainage and removing the risk of floods which result from exceptional tidal surges.

From a scheme such as this it is possible to withdraw 4,000 MW at a constant rate (if that proves to be the preferred style of output) over the same 12-hour period every day, irrespective of the tidal range. The power from thermal

stations supplied to the scheme at night is also 4,000 MW, over a period of nearly 8 hours (the need for weekend variations in this pattern is recognised). This equality between the rates of pumping and generation makes the best use of available equipment. The scheme (Fig. 3) could be supplying 6% of daytime consumer demand by the mid 1980s, which, from one power station, is a reasonable increase in generating capacity. Similar but larger projects, operating in the Severn Estuary–Bristol Channel, could also be devised.

Economic and other factors

The economical value of a scheme of this type can be assessed on the following basis. For night time pumping using energy from thermal stations, the alternative to estuary storage on the scale of the scheme I have discussed (Fig. 3), would be three schemes each bigger than the Dinorwic project. In addition, harnessing tidal energy by the simplest of projects, akin to the 1933 scheme (Fig. 1) but involving the main enclosing dam shown in Fig. 3, would require a similar, additional amount of pumped storage potential. Making provision for this in an estuary, in such a way that firm daytime output is available, makes the scheme in Fig. 3 equivalent to thermal plant of the same capacity operated only by day. This equation should also take into account the fact that the tidal–pumped storage scheme permits other thermal plant to be run continuously rather than be cycled.

The combined estuary scheme would thus displace the equivalent generating capacity of nuclear plant having a cycling capability, that is an installation costing at least £1,400m—probably much more—at 1974 prices. This scheme would have a capacity of 4,000 MW and its operation could be achieved by the estuary project but without fuel costs other than for the purchase of energy at night from thermal stations. On this basis it is realistic to believe that the estuarine scheme is both economically preferable and operationally equivalent to the nuclear alternative.

Many other factors have to be considered in the moderately complex but technically straightforward operation of this type of estuary development. Basically, it is little more demanding on plant than are other tidal proposals, even though its performance is easily rendered compatible to energy requirements, through the pumped storage addition. Unlike the next round of nuclear stations the estuary scheme relies heavily upon proven technologies. Only two main components are involved, embankment construction, which has been explored in great detail, especially by the Dutch, and pump turbines. Several new types of hydraulic machine for extracting energy from small differential water levels have been devised recently, and these may be well suited to estuarine tidal schemes. Machines pioneered by the French in the 1950s, for the small tidal scheme at La Rance, Brittany, have now been well proven over many years of service, and would also meet this duty.

It is worth recording that the French project is similar in size and purpose to the Dounreay and Winfrith nuclear installations: all are about 250 MW and, although experimental, they are also useful contributors to the national network. To scale up their costs *pro-rata* would be as inappropriate as applying costs from La Rance to the proposed scheme (Fig. 3) in the Severn Estuary, or to the large Ile de Chausey project, for which La Rance was the pilot. In fact, Ile de Chausey was shelved as soon as La Rance was completed, because it was essentially a tidal energy project with the attendant disadvantages already outlined (it had been envisaged that there would be some pumping when the tides permitted).

Environmental considerations

The energy figures discussed here for the combined estuary installation, were evaluated within specified environmental restraints; more energy could be provided but this would create less acceptable conditions elsewhere. Land drainage and flooding have already been mentioned as factors limiting the height of the water level along the coastline. The high tidal range in this comparatively shallow estuary creates strong currents and exposes extensive mud flats at low water. Because of the currents, a large amount of sediment is held in suspension, and much of this settles out when the tide turns. Very little material seems to move seawards into the Bristol Channel, and therefore reservoirs constructed as shown in Fig. 3 should not suffer problems of sedimentation: the bulk of the material would be deposited over the area of the main reservoir in a layer consolidating to an average depth of only a few millimetres. Further inputs of sediment from the rivers could give rise to some local accumulations of deposits, but as there would be a 'tide' range of over 10 feet in this reservoir, currents of a strength comparable to those occurring in many estuaries and coastlines would still occur.

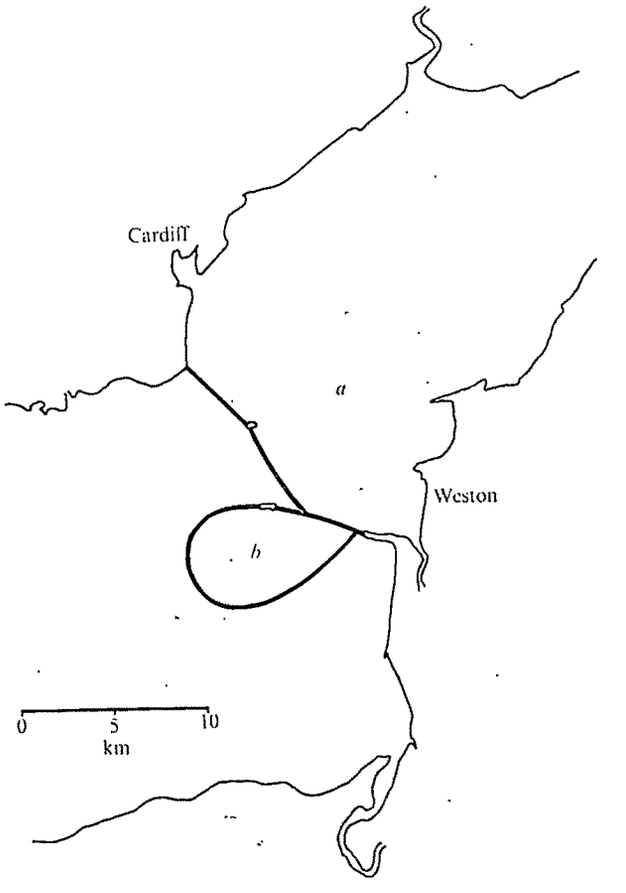


Fig. 3 Suggested barrage scheme: *a*, high level basin; *b*, low level basin.

The loss of the mud flats over much of the estuary will not be generally mourned, although there would be some ecological effects. The present combination of strong currents and high concentrations of suspended solids leaves little room for sustained marine life, and reduced currents and improved water clarity would, therefore, generally improve feeding conditions. This should promote greater fish populations, and these would pass through the large, slowly rotating pump/turbines.

Wildfowl must also be considered in any review of environmental factors. One of the most extensive areas of mud flat used for feeding is Bridgwater Bay, immediately seaward of the barrages outlined in Fig. 3. The tidal regime for this area would remain largely unaffected although there would be some reduction in the currents. Further up the estuary the substantial rise in mean water level caused by

the barrage, would become less significant than the higher levels created at present by the tidal surge. The low water levels, which are influenced more by the general bed profile of the river system than by tidal processes, would change relatively little. Thus, at the Slimbridge Wildfowl Trust, upstream of Sharpness, the pattern of flooding and exposure of feeding grounds might be influenced more by the 24-hour cycle of operations than by the modified range of water levels created by a barrage.

Overall effects

The future for tidal energy may be bleak, but an integrated estuary pumped storage project of the sort described offers worthwhile economic and operational advantages over a pure tidal development. In particular, because circumstances at present and in the foreseeable future favour such a project, it is timely to give the matter closer attention. Industrial, social and environmental matters must not be overlooked, not least because these could and should be permitted to benefit from such a project.

Some environmental aspects have been referred to above. On balance it seems that a net improvement over the present, somewhat suppressed ecological regime could be achieved, although more detailed studies of several aspects are required if there is to be an adequate picture of the probable changes resulting from a barrage. Many industrial and social factors also arise, such as benefits to shipping, inland road and rail communications, and the provision of a very large area for water recreation in a part of the UK that has managed to attract a population of three million despite the lack of social appeal of its estuarine focus.

It is to be hoped that energy planning in such circumstances would not be daunted unduly by the prolific interaction of seemingly complex factors, especially as even the potential power output need not be reduced significantly in order to provide other sectors of the community with wide benefits that are, in their own way, just as much in the national interest.

Nuclear options

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Dr G. R. Bainbridge is Manager of Technical Assessments in the Reactor Group of the United Kingdom Atomic Energy Authority. At the beginning of August he takes up his appointment to the new Chair of Energy Studies in the University of Newcastle upon Tyne. Here, he discusses the nuclear fission reactor options which Britain could take up during the next decade and gives an outline of the broad energy requirements for which nuclear reactors are needed.

BRITAIN took a first option which led towards the application of nuclear fuel to supplement traditional coal and oil supplies almost 30 years ago. It was to pursue techniques for controlling nuclear fission reactions on a large scale, thus making heat available, and manufacturing plutonium. Most of the nuclear research and development work in the world at that time was financed by military budgets. The construction of the Calder Hall plant, the first unit of which was completed in 1956, was no exception. But its most remarkable success was in the supply of by-product electricity, design data and operational experience for the later commercial nuclear power stations. It is easily forgotten that Britain achieved her first peacetime nuclear power objectives quickly and alone. It would be sad if transient commercial expediency led the country to take risks now by adopting nuclear reactor designs from abroad which are in some respects technically less sound than those produced at home.

Britain now gets 10% of her electricity from nuclear power stations (Fig. 1) which use natural uranium fuel and thus save 9 million tons of coal, or 5 million tons of oil each year. That is, however, only 3% of the total national fuel requirement (Fig. 2) and there is recognised scope for a more significant contribution as time goes on.

When a country is embarked, as Britain is, on a course of applying such a rapidly advancing technology as nuclear power, it is of benefit to review periodically and unhurriedly the available alternatives. The most important review of the 1970s is now in progress, and it could have very important consequences for Britain's nuclear power strategy. With a wealth of nuclear experience to her credit, the country is more favourably placed than ever before to survive any future shortages of fossil energy and rising fossil fuel prices, by arranging a strong nuclear power programme for electricity generation. Indeed, if a combined 'energy resources and use' strategy can be formulated to include a complementary plan for all of the available fuels, the 1980s should see Britain in a better position for energy than for many years past.

To reach that position, in which fossil fuels can be conserved or exported as well as being used rationally at home, I believe that action must be taken to provide most of our incremental electricity generating capacity, including replacement plants, in the form of nuclear power stations. This implies that 30,000–40,000 MW of nuclear capacity should be ordered in the next decade and built before 1990 (Fig. 3). A further 70,000 MW will have to be ordered

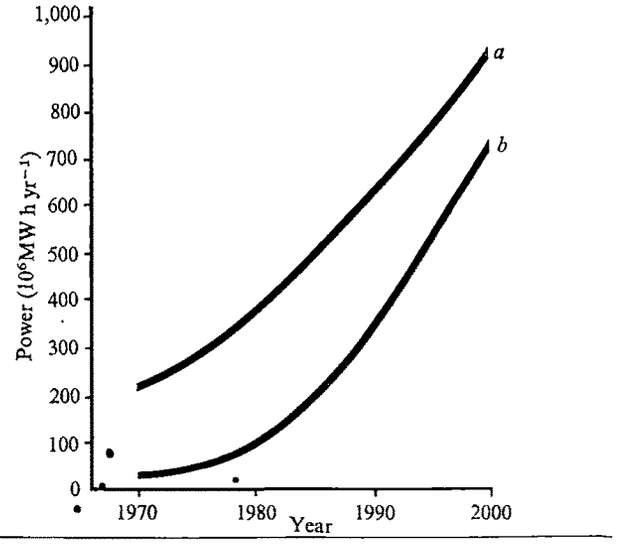


Fig. 1 The total electricity demand (*a*), and nuclear power supply (b), in the United Kingdom.

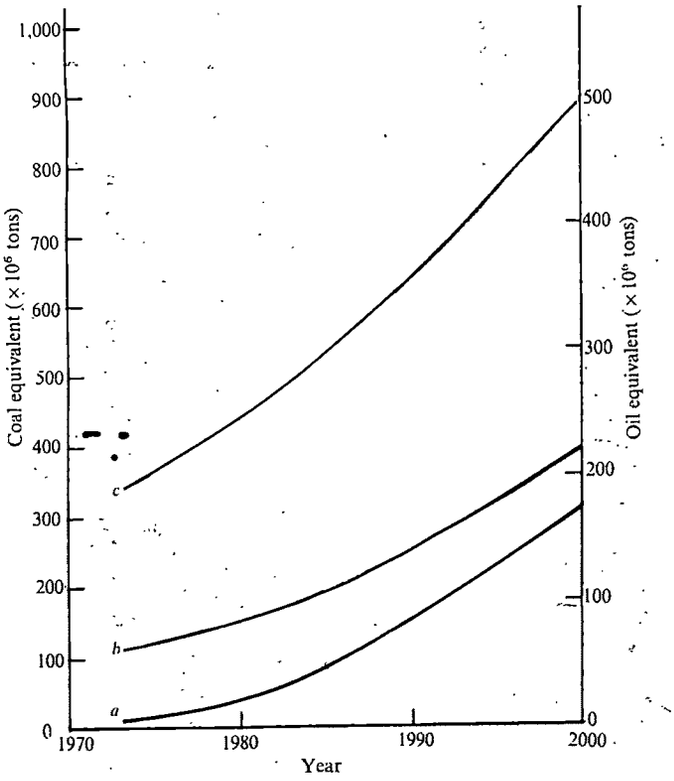


Fig. 2 Primary energy consumption in the United Kingdom. *a*, Nuclear electricity; *b*, total electricity; *c*, total.

between 1985 and 1995 for operation before the end of the century. That will enable nuclear power to supply in the region of 80% of the country's electrical energy usage (Fig. 2); around 30% of the country's total energy usage at the end of the century.

An expenditure of around £8,000 million will be required for the nuclear power station plant completed during the next 15 years alone, and £600 million will have to be spent on replacement fuel (Fig. 4). The task will not be easy to accomplish, even with a well established and financially strong National Nuclear Corporation (assuming that this is soon realised). The investment sum is over four times that of the first 20 years of commercial nuclear power. During that period there have always been two or more consortia, or design and construction companies, working with the Electricity Boards in order to get the initial 10,000 MW or so of nuclear plant into operation. But British industry as a whole has achieved similar objectives previously. The expenditures on an alternative oil station programme for the same electrical capacity would be £4,000 million capital outlay, and almost £7,000 million for fuel; 30% higher than the cost of nuclear plant in those initial fifteen years of construction and operation. At no time after about the third year of operation would the total expenditure on an oil programme be less than that of a nuclear programme. From then on nuclear fuel would cost only £1,500 million a decade, whereas the 550 million tons of oil, which would be used during the same period, would cost £16,000 million. Similar figures emerge if coal, and not oil, is considered as an alternative to nuclear power.

If this main nuclear programme objective is to be achieved, then the other nuclear commercial options will have to be allocated a much lower priority. It can be anticipated, however, that although the types of nuclear reactor selected as the best that Britain could build next may be suitable for unit designs with a nett output exceeding 600 MW, with minimal adaptations some of them might be provided in smaller unit sizes overseas. At present, power stations with nuclear units of 300±200 MW could be competitive with oil-fired designs, provided that the price of oil stays high in relation to that of nuclear fuel (at present about 6:1) and that the capital cost of oil plant relative to nuclear plant does not decrease (at present 0.7:1), (Fig. 5). For exports, which would relieve the pressures of commitment in a heavy home programme, a separate organisation of companies could be formed profitably, allied at home with the main National Nuclear Corporation, and overseas with interests already actively engaged in attracting nuclear power station business.

Other factors affecting the choice of reactor, such as the supply of industrial process and urban living 'comfort' heat, need to be considered separately from the main issues of this article. The provision of nuclear reactors for the propulsion of large, fast, container ships and oil tankers are also outside the main issues discussed here, as they would be additional and special in many respects.

If resources are to be used sensibly, and there is to be high morale and enthusiasm amongst those engaged in the

Table 1 Nuclear reactor options—parameters

	Gas-cooled reactors				Water-cooled reactors				Sodium-cooled
	Magnox	AGR	HTR	GBR	SGHWR	BWR	PWR	CANDU	reactor
Nominal reactor output available (MWe)	500–600	625	1,320	1,320	625	1,110	850	750	1250
Net efficiency (%)	32	41	38	35	32	31	32	32	42
Mean rating (MW h/t U)	3.1	12.6	112	250	19.8	25.9	38.2	22.1	202
Fuel	U metal	UO_2	UO_2	UO_2	UO_2	UO_2	UO_2	UO_2	U/Pu O_2
Mean fuel burn-up (MW d/t heavy atoms)	4,000	17,250	85,000	7% ha	22,050	28,000	32,000	9,600	6.9% ha
Fuel cladding	Magnesium alloy	SS	Pyrocarbon SiC	SiC	Zircaloy	Zircaloy	Zircaloy	Zircaloy	SS
Coolant and temperatures:	CO_2	CO_2	He	CO_2	H_2O	H_2O	H_2O	D_2O	Na
inlet (°C)	247	287	273	260	271	278	288	261	340
outlet (°C)	414	651	749	650	282	286	321	299	540
Coolant pressure (MPa)	2.9	4.1	4.2	6.6	6.7	7.4	15.2	11.0	—
(pound inch^{-2} gauge)	400	585	600	940	955	1,050	2,190	1,580	
Initial fuel weight (t heavy atoms/GWe)	1.011	195	23	9.5 24.7 70.0	159	126	83	142	Core 14.4 Ax B 14.7 Rad B 32.5
Replacement fuel weight (t heavy atoms/GWe y)	286	52	11	14.4 37.3 21.4	53	42	36	120	Core 11.4 Ax B 11.6 Rad B 8.6
Steam conditions at TSV:									
Temperature (°C)	400	538	538	538	277	286	266	254	490
Pressure (MPa)	5	16	16	16	6.2	6.6	5.5	4.2	16
(pound inch^{-2} gauge)	710	2,300	2,300	2,300	885	950	780	600	2,300

Table 2 Probable strategy

Year of order	1974	75	76	77	78	79	1980	81	82	83
Year on-line	1981	82	83	84	85	86	87	88	89	1990
Total nuclear capacity required ($\times 10^3$ MW)	2	3	3	2	4	5	5	4	6	7
AGR	1*	2*	1*	2†	3†	4†	2†	1†		
SGHWR	1		1							
HTR		1‡			1	1	2	2	6§	7§
Fast reactors			1‡				1	1		

* Likely to be delayed by late 1974 decisions, some giving way to water reactors or, even more likely, to coal and/or oil stations.
† Options based on further AGR/SGHWR experience.
‡ First commercial stations.
§ Options based on further HTR–FR experience and later, plutonium availability.

nuclear power station development and manufacturing industry, and if the paying public is to remain confident that nuclear power is in their own and the nation's best interests, then expeditious decisions must follow any review. This is particularly important when review procedures have been conducted at the highest political level, as has been the case in recent months. A Parliamentary Select Committee, an eminent Nuclear Power Advisory Board, four ministers and two governments have been involved.

Whether under the new policy, the established line of action is continued, or new, previously unforeseen conditions are met with a change of direction or pace, there must be adequate early publicity about the co-operation necessary for rapid progress. The present review must not end up as a little reported non-event, as did the thermal reactor and fast reactor reviews of a few years ago.

Nuclear reactor options

The objectives of low cost, plentiful, reliable, safely available nuclear energy must be kept in sight, for it is easy to be distracted and repeat old errors. Accepting these limited objectives, however, it is possible to argue that the logical progression in the use of gas-cooled reactor designs which Britain began to operate in the 1950s, could fulfil not only all her own needs but also those of some other countries. Table 1 gives typical parameters for gas-cooled reactors and for the main available alternatives.

Calder Hall was the first nuclear power station to feed substantial amounts of electricity into a national grid distribution system. The uprated output, now at 200 MW, regularly exceeds the original (perhaps conservative) intended output by 50%, and it also feeds process steam to the adjacent Windscale site. Moreover, its sustained performance over almost 20 years (an accountant's plant amortisation period) is, together with that of its twin station at Chapelcross, better than that of any other plant in the world exceeding 100 MW in output, whether nuclear or fossil fired. Year after year it exceeds 90% load factor even though it has to be shut down occasionally for refuelling. Such is the confidence that these plant can continue to operate at a low cost, that there is a current survey of the components which could be refurbished usefully in order to give the stations 20 to 30 further years of life.

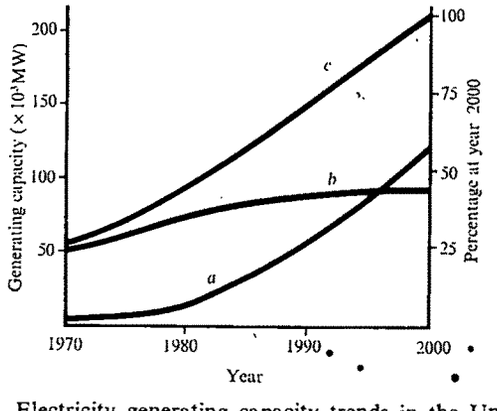


Fig. 3 Electricity generating capacity trends in the United Kingdom. *a*, Nuclear; *b*, fossil; *c*, total.

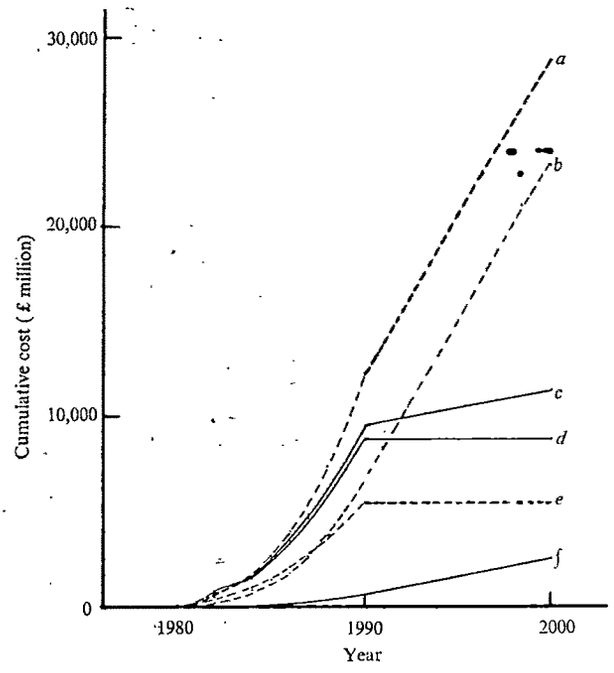


Fig. 4 Cumulative costs of alternative nuclear and fossil programmes completed between 1980 and 1990. *a*, Oil station plus oil; *b*, oil; *c*, nuclear station plus fuel; *d*, nuclear capital; *e*, oil capital; *f*, nuclear fuel replacement. This assumes that the cost of nuclear stations is £240 per kW (including the initial fuel charge); the cost of oil stations is £150 per kW; uranium costs about £6 per pound weight of U_3O_8; oil costs £30 per ton; there will be load factor of 70%; and a 10-yr programme supplying 37,000 MW of energy.

In 1955, when "A Programme of Nuclear Power" was announced in a White Paper, there was no doubt that the gas-cooled, graphite moderated, thermal neutron reactor, based on the Calder Hall, Magnox clad, natural uranium fuelled design, should be the first to be exploited commercially in Britain. During the 1960s, 18 reactors and associated plant in 9 stations were brought into operation (Fig. 6), providing an output of 5,000 MW. It was recognised then that other nuclear options would have to be taken before long, as it was expected that improved models would be built, and that a second type of reactor, liquid-cooled, might be used. So it turned out.

Following Hintonian guidelines to increase temperatures and efficiency and cut costs, the research and development design work was implemented during 1957–58 to enable the construction of an Advanced Gas-cooled Reactor at Windscale (WAGR). In order to limit expenditure its output was set at 35 MW of electricity, which it was believed would be sufficient to allow the core design to serve primarily as a test bed for higher rated, higher temperature uranium oxide fuel, slightly enriched in the more fissile ^{235}U isotope. It was also intended to provide data on the interaction between the graphite, the carbon dioxide coolant and the fuel, later determined as stainless steel clad, under onerous operating conditions. Since 1963, WAGR has been a prolific supplier of invaluable data for Britain's nuclear design teams. Yet it

has given a load factor averaging 84%, remarkable for a prototype plant.

The government, however, was prudently cautious in 1964 when considering the nuclear options for "The Second Nuclear Power Programme". As had been anticipated, other reactors had become available. These could use uranium more efficiently, and could reduce the capital cost of the power station and fuel to a lower level than had proved possible with the alternative Magnox (Mark 1) gas-cooled reactor designs. So tenders from British industry (in some cases including design features from foreign companies)

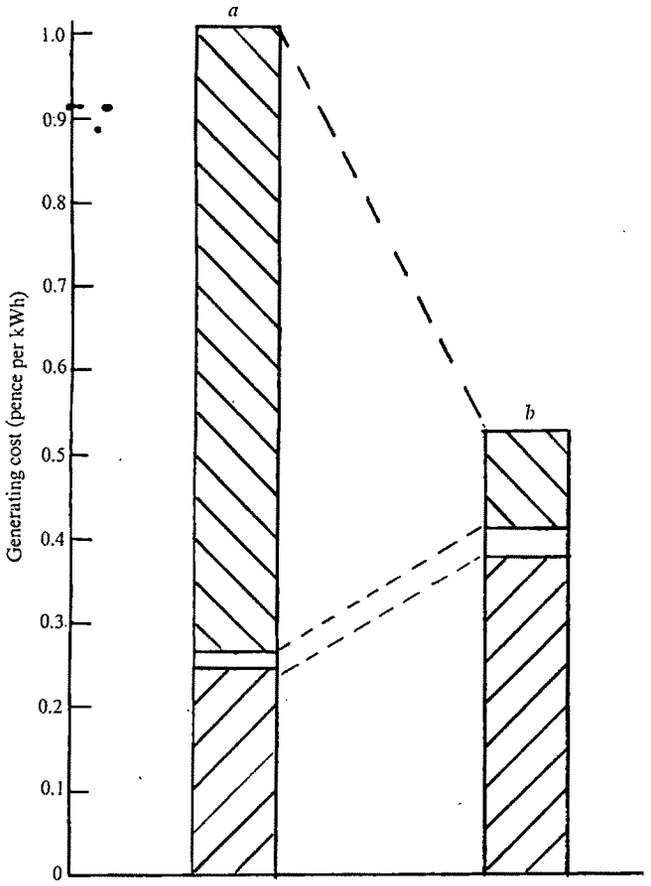


Fig. 5 Electricity generating costs in the United Kingdom. *a*, Oil; *b*, nuclear. In both columns the upper section represents the cost of fuel, and the lower section represents the capital outlay. The space separating the upper and lower sections represents the operational costs.

were considered, and an AGR (Mark 2) design was selected. The background of the assessment and the reasons for the choice were given in special Central Electricity Generating Board publications. A light water reactor design of boiling water type (BWR) was placed second, with pressurised water (PWR) and other AGR designs as runners up.

As a result of this, ten AGRs in five stations were ordered, to provide some 6,000 MW of output. The first two are now almost in operation. Though several PWR and BWR designs have been built more quickly overseas, many have suffered delays and extra costs. The AGRs use a steel cable prestressed concrete pressure vessel, an important safety development which was introduced into the last two Magnox stations. These have always been recognised as safe near urban locations. The LWRs of both kinds, however, have become the centre of a nuclear safety controversy in the United States.

The recent debate in the House of Commons, did not give a definite indication of the government's intentions in deciding between the wider range of nuclear options. Although the debate extended from mid-afternoon until late evening, nuclear power is a non-party issue, and the day was Thursday (with a quiet Parliamentary Friday to follow) and so only about 50 'specialist' MPs attended and there was no sensational result. There was, however, strong support for the British reactor designs and it was agreed that whatever decision was finally reached, any reactor built in the near future must be safe.

No one in the nuclear industry, Parliament, the Nuclear Power Advisory Board or the government seems to doubt that the development of a high temperature gas-cooled reactor (HTR) will eventually be a sensible course of action. The timing of the construction of the first commercial station, in relation to the progress of development work in the Euratom Dragon project and to advances overseas (not least in the USA) has yet to be settled. It seems unlikely, however, that the first, large, Mark 3 gas-cooled reactor unit could be ordered soon, which means that such designs will not contribute to commercial nuclear power until the late 1980s. Bearing in mind the continuing need for the improvement of designs using ceramic particle fuel and higher temperature coolant circuits (which have helium instead of carbon dioxide) this is a project which would suffer if rushed. On the other hand, the longer term development potential of the HTR could usefully receive more effort. Ideas for using HTRs to generate electricity with gas turbines, and the application of HTRs to provide process heat, now seem to be moving ahead more quickly as collaboration with other countries is arranged.

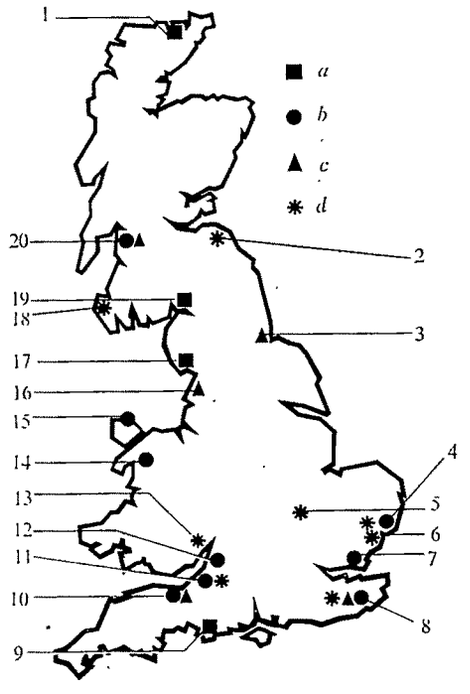


Fig. 6 Nuclear power in Britain. *a*, Experimental power stations; *b*, Magnox stations; *c*, AGR stations under construction; *d*, proposed stations. 1, Dounreay DFR and PFR; 2, Dunbar; 3, Hartlepool; 4, Sizewell 'A' and 'B'; 5, Molesworth; 6, Orfordness; 7, Bradwell; 8, Dungeness 'A', 'B' and 'C'; 9, Winfrith 'Dragon' and SGHWR; 10, Hinkley Point 'A' and 'B'; 11, Oldbury 'A' and 'B'; 12, Berkeley; 13, Portskewett; 14, Trawsfynydd; 15, Wylfa; 16, Heysham, 17, Calder Hall and Windscale Magnox and AGR; 18, Girvan; 19, Chapelcross Magnox; 20, Hunterston 'A' and 'B'.

The declared reluctance in some parts of the Central Electricity Generating Board to order more AGRs, can be expected to decrease as experience is gained with Hinkley Point B station and the South of Scotland Electricity Board's Hunterston B station. Both of these stations

are now almost in operation. Meanwhile, although forecasters have been perturbed by the low electricity demand of the past winter, it is felt that one or two stations of some other kind must be ordered soon to supplement firm power supplies in the early 1980s.

The case for building LWRs designed in the United States (particularly the PWR) in the meantime, has been strongly pressed by senior people in the CEGB and the National Nuclear Corporation (NNC)—a potential supplier under licence. Doubts that have arisen concern the safety of the thick steel pressure vessels and the pipework which comprise the coolant circuit, and the effectiveness of the emergency core cooling system (ECCS) in the event of a major fracture of the circuit. Much experimental work has yet to be done in the USA and other countries using LWRs, on both pressure vessel failure and ECCS during the next few years. There has been no publication of convincing evidence that, even if satisfactory safety solutions were known, any programme including the PWR would be better or cheaper than a programme using the British design. Factors bound to lengthen the programme and increase the costs are: import requirements, searching Electricity Board specifications, and the necessary quality assurance standards. It is important to avoid delays. For a large nuclear station, the interest charges on the committed capital, and the cost of alternative electricity supply into the grid system, can be of the order of 7% of the cost of the station for each month of delay. About 15 months of delay will double the cost. Project management and realistic construction programme planning are factors which are as important as plant design if improvement on past experience is to be obtained.

The case for building the British steam generating heavy water moderated reactor design (SGHWR) has been strongly pressed by the South of Scotland Electricity Generating Board and the Parliamentary Select Committee. The prototype 100 MW SGHWR power station which has been operating at Winfrith Heath in Britain since 1968, has resulted in an improved understanding of water reactors. The prototype incorporates many attractive features selected from the best of the earlier reactors. Not least of these is the modular pressure-tube construction of the core, avoiding the thick steel pressure vessel which characterises the LWRs. Experience in the operation of the Winfrith SGHWR has been good so that larger stations could now be built, with or without support from similar pressure tube reactor work in Canada, on the CANDU and boiling light water types. As in the case of the LWRs, however, many power station engineers consider that it is a retrograde step back to saturated steam conditions for the turbine-generators, particularly as the superheat steam machines now in use in all advanced British base-load power stations would be needed again later in the programme. It is possible, however, that because of the amount of nuclear power needed in Britain, there may be room for a few SGHWR designs. Interested countries abroad would then have some indication of the effectiveness of the design in larger scale operation than has been possible at Winfrith Heath.

In due course, fast neutron breeder reactors will be needed to ensure the better use of uranium resources. By converting ^{238}U (or ^{232}Th) into the more fissile ^{239}Pu (or ^{233}U) they can enable possibly a 50 fold reduction in uranium needs. They can also use waste ^{238}U from earlier isotope and uranium-plutonium separation plant operations, and ensure that natural uranium continues to be available at acceptable prices for the thermal reactors still operating.

Encouraging progress towards commercial sodium breeders is now becoming apparent, with the French Phenix and British PFR in operation. Only three factors could delay the establishment of commercial lead stations in continental Europe and Britain in the early 1980s: first, the inability to demonstrate that a programme of such reactors could be built with capital costs low enough to allow electricity to be generated as cheaply as is possible using contemporary thermal reactors: second, poor explanation to the public of the exceptional value of plutonium as a 'perpetual motion' fuel; and finally, poor explanation to the public of ways that plutonium can be managed in the fuel cycle external to the reactors.

Gas-cooled breeder designs would have been much further advanced if at the time of initial consideration prestressed concrete vessels had been available to ensure safe designs, which steel vessels could not. Gas-cooled reactors would have been a logical next step beyond the AGR and HTR. While a European Gas Breeder Reactor Association is now pursuing limited objectives in the fuel design and plant layout areas, large scale expenditures are unlikely, and progress is likely to be slow as long as success with sodium-cooled breeder reactors remains a possibility.

Probable strategy

The basis of probable future strategy (Table 2) is that gas-cooled reactors of the Magnox (Mark 1) type have worked well and given invaluable experience for later developments. It can be expected that gas-cooled reactors of the AGR (Mark 2) type will soon begin to work well and that the difficult problems of the earlier designs can be avoided through more careful specification of plant yet to be ordered, particularly concerning the boilers and the insulation of pressure vessel liner. It is agreed that development work towards gas-cooled reactors of the HTR (Mark 3) type should be continued because of its potential for electricity and process heat applications. Thus, gas breeder reactors (Mark 4) could be achieved, which in a large-scale, fusionless, national energy system of the distant future might have a place complementary to, or even supplanting, the most advanced sodium breeder reactors. They would not operate, however, before 1995.

A further basis of this strategy is that if water-cooled reactors are required, it will only be to a very limited extent, and there is not a justifiable case for importing their technology into the PWR or BWR designs at considerable cost. Proving their safety would give continuing difficulty in Britain. Sufficient progress has been made with the British SGHWR to exploit its capability now commercially in a few larger stations, while the AGR matures to full credence. An alliance of British SGHWR and Canadian knowledge with similar plant should mean that some overseas customers will become confident enough to order water-cooled power stations from the combined organisation. This might not occur if Britain and Canada continue to operate separately.

It does not ease the problems of a country to have too many parallel lines of development simultaneously. In particular, it is not beneficial to build contemporaneously too many prototype or lead power stations. With the choice of gas-cooling as the main line, and sodium-cooling as an option for the future, Britain would seem to have sufficient scope but not excessive problems. If there are benefits from water-cooled designs, as there seem to be in the small sized unit overseas market, these will come with slight extension of present commitments through SGHWRs. That obviates the greater effort required to understand and necessarily improve the LWR technology by importing PWR designs. Britain could and should buy technological expertise from abroad when it is lacking at home, but in the nuclear power field this is unnecessary at the present time. Indeed, she has available to other countries valuable gas-cooled, water-cooled and sodium-cooled reactor technology, which improved marketing could turn to benefit.

DNA fragments carrying genes for $tRNA^{Tyr}_{I}$

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The duplicated genes coding for $tRNA^{Tyr}_{I}$ have been isolated using site-specific nucleases and gel electrophoresis. An unduplicated sequence of 4–120 base pairs separates the two genes. Specific DNA fragments can be obtained in sizes appropriate for both functional and structural analysis of the $tRNA^{Tyr}_{I}$ region.

ONE approach to studying the detailed structure of a transfer RNA gene involves the chemical synthesis of the relevant deoxyribonucleotide sequences[1]. A major barrier to alternative approaches has been the problem of isolating genomic sequences of the appropriate size and purity. Several preparations of DNA enriched to various degrees for regions complementary to transfer RNA have been described[2–5]. In the experiments reported here, the genes for $tRNA^{Tyr}_{I}$ have been obtained as small DNA fragments using a combination of several site-specific endodeoxyribonucleases and polyacrylamide-agarose gel electrophoresis[6,7].

In *Escherichia coli*, three genes for $tRNA^{Tyr}$ have been identified. The gene coding for the major species, $tRNA^{Tyr}_{II}$, (70% of the total tyrosine accepting activity), maps at approximately 78 min (refs 8–10). The two genes which code for the minor species, $tRNA^{Tyr}_{I}$, occur as a duplication near the attachment site for bacteriophage Φ80 (refs 11–13). (The term gene is used here to indicate that sequence of bases coding for each tRNA. There is no evidence at present to indicate whether the two $tRNA^{Tyr}_{I}$ genes are transcribed as a single RNA molecule, as is found for tRNAs coded by bacteriophage T4 (ref. 14).) The $tRNA^{Tyr}_{I}$ genes are only distinguishable from one another in su $^{+}_{III}$ cells where one of them has a single base change in the anticodon region[15,16]. This suppressor phenotype has made possible the isolation of several different transducing derivatives of bacteriophage Φ80 as well as the isolation and biochemical characterisation of mutants deficient in various aspects of tRNA function[17–19].

Three phages have been used as sources of DNA. The phage Φ80h psu $^{+}_{III}$ (double copy phage or 'doublet') is a nondefective transducing phage carrying both genes for $tRNA^{Tyr}_{I}$. The phage Φ80h psu$^{+}_{III}$ (single copy phage or 'singlet') is a phage derived from the doublet phage by an unequal recombination event between homologous sequences, such that one of the two $tRNA^{Tyr}$ genes has been eliminated (see Fig. 6, inset). The phage from which the transducing phage were derived is Φ80h amb1 and will simply be referred to as Φ80h or parental[11].

DNA digestion profiles

The site-specific nuclease endo R·*Hin*d, (prepared from *Hemophilus influenzae* Rd according to the procedure of Smith and Wilcox[20]), was used to digest DNA isolated from Φ80h, the doublet transducing phage and the singlet transducing phage. When the limit digestion products are compared by gel electrophoresis, the three profiles are found to be congruent for a majority of the fragments (Fig. 1). Our conclusions here are based upon composite data obtained from the application of several different electrophoretic conditions, each chosen for its ability to resolve optimally a particular group of fragments. To facilitate comparison of the three DNA digests, a schematic diagram combining the data from several electrophoresis conditions is given in Fig. 2. Construction of this composite is accomplished by including an overlap of well resolved fragments in each experiment. The band differences between the three DNAs provide convenient landmarks for confirming the alignment of profiles.

There are three fragments which are unique to Φ80h DNA (A4, A6 and E4). There are five fragments which are absent from the Φ80h digest but which occur in both the singlet and doublet digests (A4a, A6a, D1a, F3a, F3b). These are called transducing-unique. In addition, there is one singlet-unique fragment (C1a) and one doublet-unique fragment (B10a).

Hybridisation

The ability to hybridise with ^{3}H-$tRNA^{Tyr}$ (ref. 21) has been tested for each of the seven fragments unique to the DNA of the transducing phages. These unique bands, and several others which are common to all three phages, were eluted separately from preparative-scale gels. The only fragments showing any significant hybridisation with ^{3}H-tRNA are the

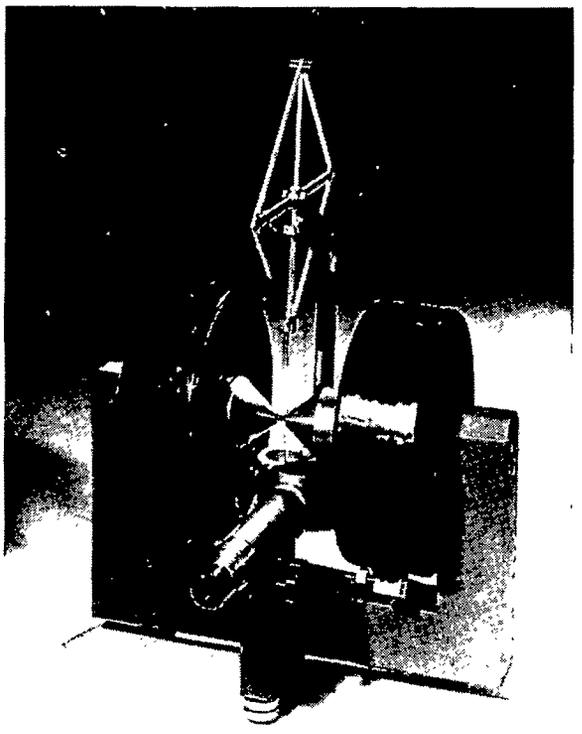
Table 1 Hybridisation of isolated fragments with ^{3}H-$tRNA^{Tyr}$

DNA type	DNA fragment	^{3}H (c.p.m. filter)	^{3}H (c.p.m./ pmole DNA fragment/filter)
Singlet	B 8,9	2.1	8.6
Singlet	B 11	1.9	3.6
Singlet	C 1a	28.4	56.6
Singlet	C 3	1.8	7.0
Singlet	C 4	0	0
Singlet	D 1a	0.8	3.1
Singlet	D 2,3,4	0.7	5.7
Singlet	E 1	0	0
Singlet	E 5	0	0
Singlet	F 3,3a,3b	0.1	0.4
Doublet	A 1,2,3	4.3	83.3
Doublet	A 4,5	4.0	10.0
Doublet	A 6,7	12.3	76.7
Doublet	A 8	3.0	16.7
Doublet	B 1	7.0	40.0
Doublet	B 2,3,4	9.7	56.7
Doublet	B 5	11.0	56.7
Doublet	B 6	9.3	50.0
Doublet	B 7	10.3	63.3
Doublet	B 8,9	58.0	395.0
Doublet	B 10a	194.7	1047.0
Doublet	B 10	60.0	330.0
Doublet	B 11	4.0	23.3
Doublet	C 1	1.3	10.0
Doublet	C 2	3.7	20.0
Doublet	C 3,4	4.0	23.3

DNA fragments were prepared from ^{32}P-labelled singlet and doublet DNAs (2×10^{3} c.p.m. μg^{-1} at the time of hybridisation) by preparative gel electrophoresis and electroelution from excised gel strips. After phenol extraction and dialysis, DNA was denatured in 0.2 N NaOH at 20° C for 15 min, neutralised with Tris-HCl, and brought to 6×SSC. Fifteen microlitres, containing 27 pmol of fragment were spotted immediately onto a dry 13 mm Millipore filter, which had been prewashed successively in 6×SSC and distilled water. DNA filters were exposed, while still wet, to ultraviolet light at 22 cm from a 15 W GE germicidal lamp (5 min per side), then dried for 3 h at 80° C under vacuum. To remove loosely bound DNA before hybridisation, filters were washed in 6×SSC, 0.1% Sarkosyl for 30 min at 20° C, then for 30 min at 67° C. Hybridisation was carried out in 6×SSC, 0.1% Sarkosyl, with ^{3}H-tRNA (2 μg ml^{-1}) at 67° C for 8 h. The ^{3}H-tRNA preparation was enriched for $tRNA^{Tyr}$ on B-D Cellulose[23]. Following hybridisation, filters were washed, treated with T_1 and pancreatic RNase as described[21], and counted in omnifluor.

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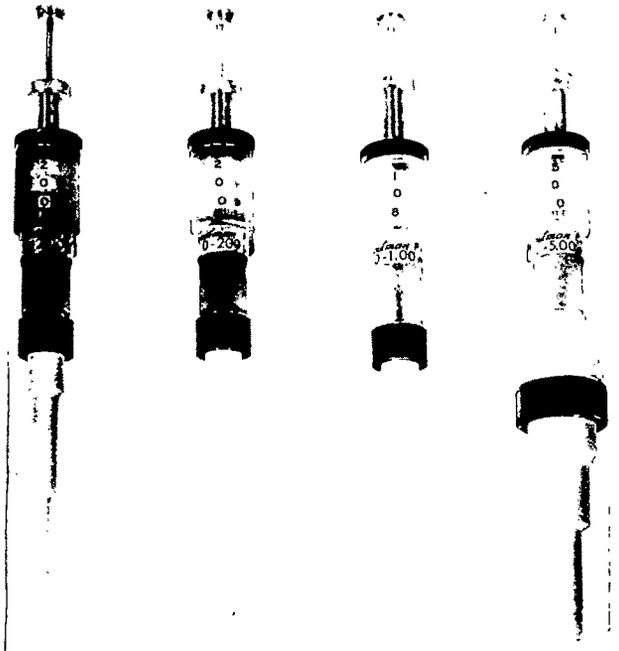


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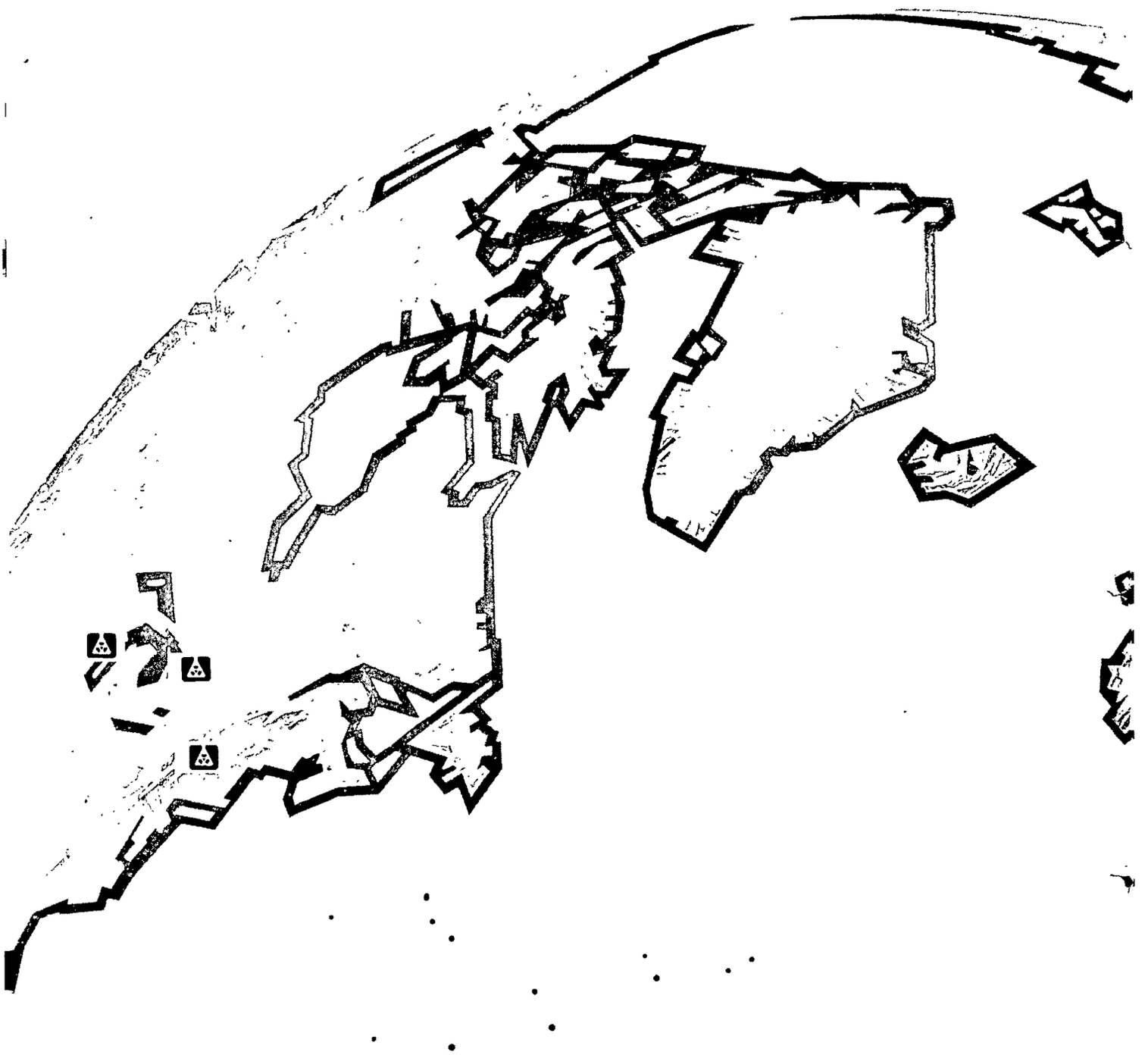
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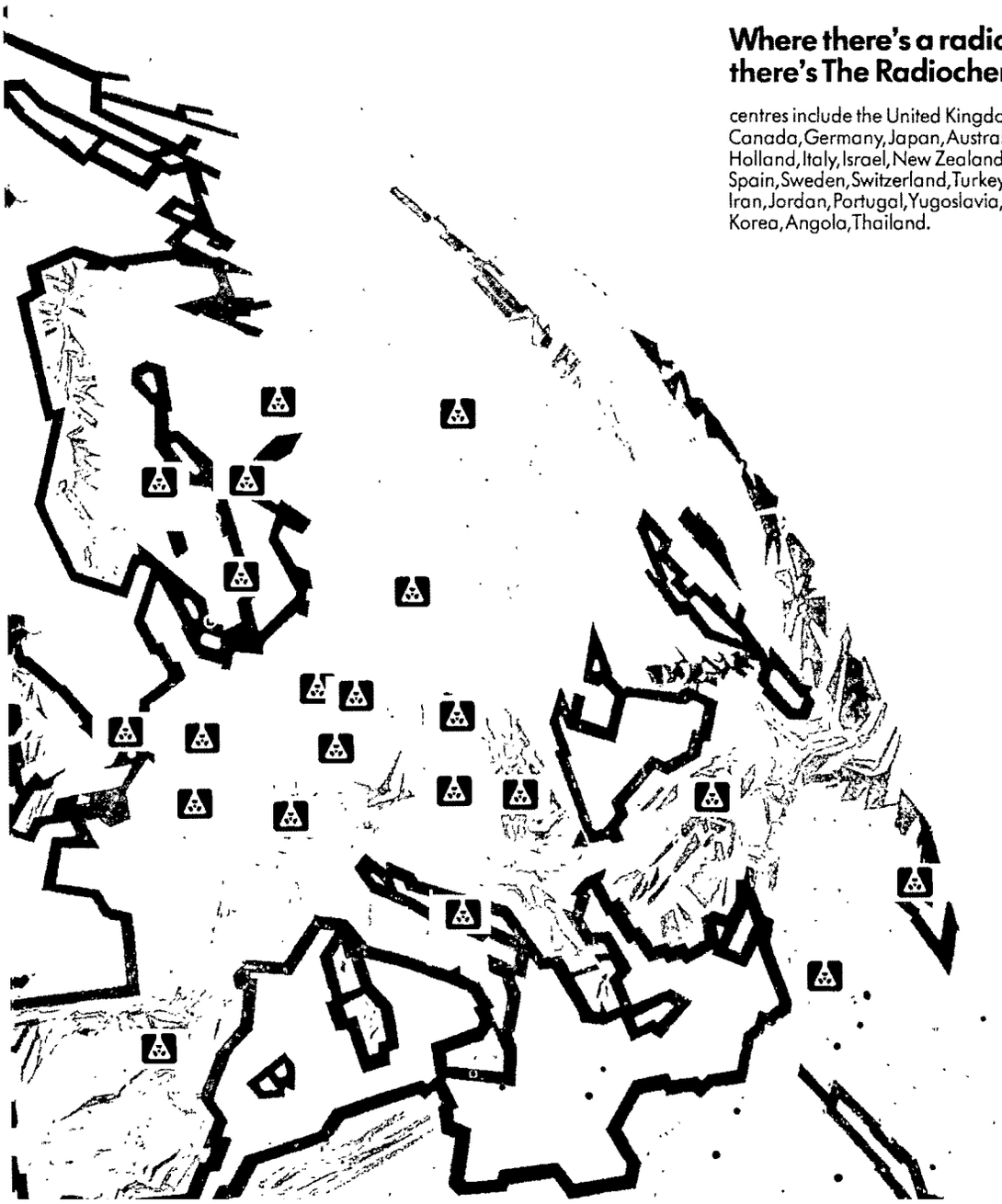
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Reactive Free Radicals

J. M. HAY

June 1974, viii+162pp., £4.40/$11.50 0.12.3335507

In this work the author has aimed to relate the reactivity of organic free radicals to their structure, and in particular to the type of orbitals occupied by their unpaired electrons. The treatment is more systematic than in previous publications.

Contents

Introduction. The structure of radicals. Bond dissociation energies. The formation and reactivity of radicals: non-polar effects. The formation and reactivity of radicals: polar effects. Autho index. Subject index.

Sub-Clinical Lead Poisoning

H. A. WALDRON and D. STOFEN

April 1974, xx+224pp., £5.50/$15.50
0.12.671650.1

Sub-clinical lead poisoning is a relatively new concept and to some extent a controversial one. This book aims to provide a broad and objective assessment of the information currently available, including work carried out in eastern Europe where the problem has been considered for longer than in the rest of the world. Accounts of the metabolism and pathology of lead in man and other animals, the signs and symptoms of classical clinical lead poisoning and the mobilisation of lead by man in historical and recent times, are given as background to the main study.

Parenteral Nutrition in Acute Metabolic Illness

edited by H. A. LEE

July 1974, xiv+454pp., £9.00/$23.25
0.12.441750.7

Here is an up-to-date account of the pharmacological, biochemical and clinical advances in the field of parenteral nutrition, which will be of prime importance to everyone involved in the management of "intensive care" patients, whether at clinical or laboratory level.

Contents

Introduction. Basic facts and requirements for intravenous nutrition. Physiopathology or injury. Clinical applications. Techniques and solutions. Studies of tolerance and toxicity. Author index. Subject index.

Microprobe Analysis as Applied to Cells and Tissues

edited by T. HALL, P. ECHLIN and R. KAUFMANN

June 1974, xx+435pp., £7.80/$20.25
0.12.319050.9

The present work consists mainly of papers presented at a conference on Microprobe Analysis as applied to Cells and Tissues held at the Battelle Seattle Research Center, Washington, USA during April and May, 1973. The papers explain and illustrate how microprobe techniques may be exploited to the limit of their present capability, and explore in detail the main technical problems, especially those of specimen preparation and of beam-induced damage in electronprobe microanalysis. Each presentation is concluded by a critical appraisal of the topic under discussion.

The Biochemical Mode of Action of Pesticides

J. R. CORBETT

June 1974, x+330pp., £7.20/$20.25
0.12.187850.3

This book provides a completely novel synthesis of current knowledge of how pesticides act at the molecular level. All fungicides, herbicides, insecticides and some veterinary products now in use, as well as some now superceded, but of special biochemical interest, are considered according to their mode of action. Information on uptake and breakdown is included where it is relevant to this underlying theme. Unlike most works in the field this text does not deal with fungicides, herbicides and insecticides separately. Rather the author considers it biochemically more logical to treat the compounds according to their molecular action, so highlighting relationships and common features and providing some explanation of selective toxicity.

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singlet-unique fragment, C1a, and the doublet-unique fragment, B10a (Table 1). There is occasionally a small amount of hybridisation observed in the bands immediately adjacent to C1a and B10a which is an artefact encountered in slicing the preparative gels and eluting bands. For example in Table 1 band B8,9 shows some evidence of hybridisation in doublet DNA where it is adjacent to B10a; however, in singlet DNA which contains C1a instead of B10a, the B8,9 band does not show any hybridisation.

The strongest statement permitted by the limitations inherent in the hybridisation experiment is that a particular fragment does carry a significant portion of the $tRNA^{Tyr}$ gene. It cannot be ruled out, on the basis of these experiments alone, that some other fragment(s) may also carry a portion of the $tRNA^{Tyr}$ gene which is either too small to be detected in the assay or which codes for those precursor sequences not found in the mature tRNA (ref. 22).

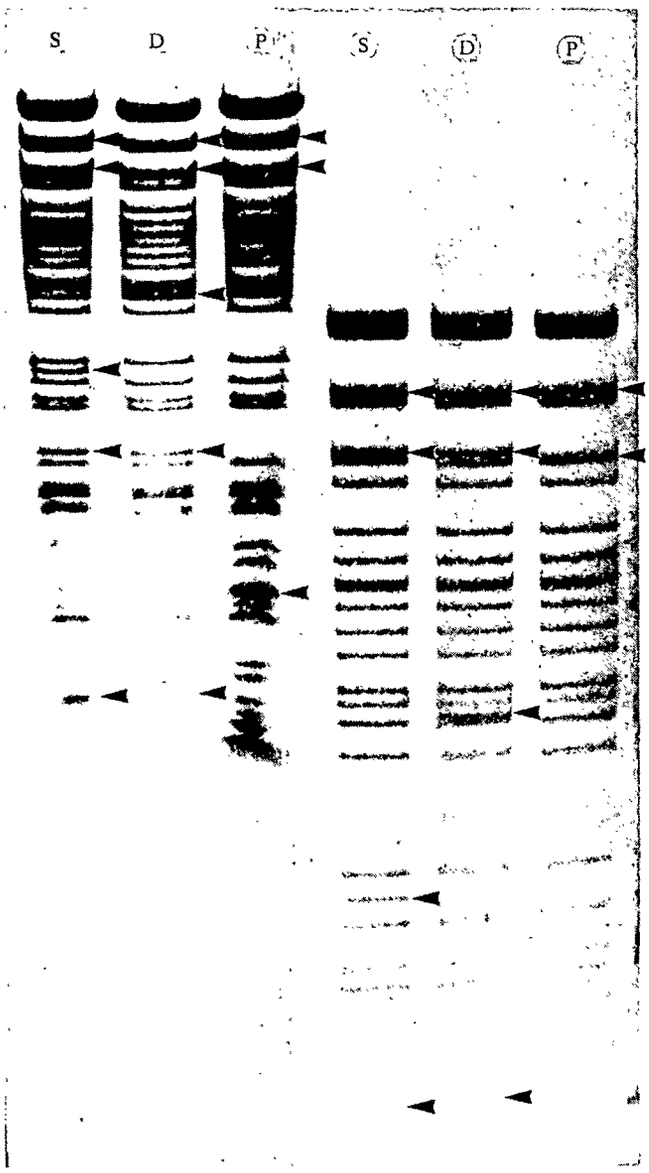


Fig. 1 Digestion profiles of Φ80h, doublet and singlet DNA produced by endo R·*Hin*d. Growth of phage and preparation of DNA were as described previously[23]. All endonuclease digestions were carried out for 4.5 h at 37° C in 10 mM Tris–Cl (*p*H 7.9), 6.6 mM $MgCl_2$, 6.6 mM mercaptoethanol, 60 mM NaCl with DNA at 200 μg ml^{-1} and using amounts of endonuclease sufficient to yield limit profiles. Unless specified otherwise all gels were made and run in Tris-Borate buffer (*p*H 8.3), have the composition 2% acrylamide (0.1% N, N′-methylene-bis-acrylamide), 0.5% agarose and were stained with Stains-all[7]. Electrophoresis was carried out at 200 V (11 V cm^{-1}) in EC apparatus flat plate cells cooled to 4° C. Digestions of Φ80h (P), doublet (D), and singlet (S) DNA were divided into two aliquots; lanes 1–3 each contain 40 μg of DNA and were electrophoresed for 3 h; lanes 4–6 each contain 10 μg of DNA and were electrophoresed for 6 h. Unique fragments (those not common to all three) are indicated by arrowheads (see text). For identification of the unique fragments see Fig. 2.

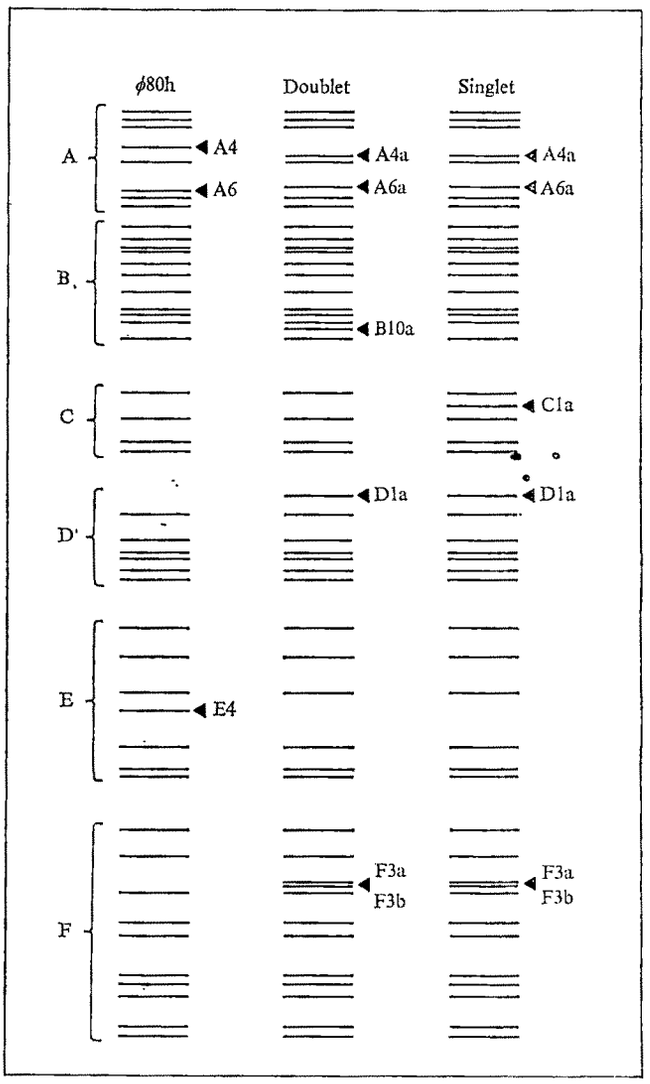


Fig. 2 Schematic diagram of *Hin*d digestion profiles of Φ80h, doublet and singlet DNAs. The relative migration distances are based on a 2% acrylamide–0.5% agarose 45 cm gel profile. Arrowheads indicate those bands unique to one or two DNAs (compare with Fig. 1). Band nomenclature is based upon the Φ80h profile. Bands falling into visually separated groups are assigned letters A–F, and fragments within a group are assigned consecutive numbers in order of migration distance. Labels for the fragments unique to singlet and/or doublet DNA are derived from the nearest non-unique fragment by the addition of a small letter (see ref. 23).

Integrity of $tRNA_1^{Tyr}$ genes

The question of whether all the sequences coding for the mature $tRNA_1^{Tyr}$ reside on the singlet-unique (C1a) and doublet-unique (B10a) fragments can be restated. Is there a nuclease recognition site within the tRNA gene(s) (including, in the case of the doublet DNA, any unduplicated intergenic region)? The following analysis is based upon two assumptions. First, the generation of the single copy transducing phage from the double copy transducing phage was by a mechanism not more complex than that deduced from the genetic data (ref. 11 and Fig. 6 inset). Second, if a nuclease-sensitive site exists in one region specifying $tRNA_1^{Tyr}$ it also exists in the other[16].

The distribution of fragments unique to each of the phage DNA digestions sheds some light on this question. The

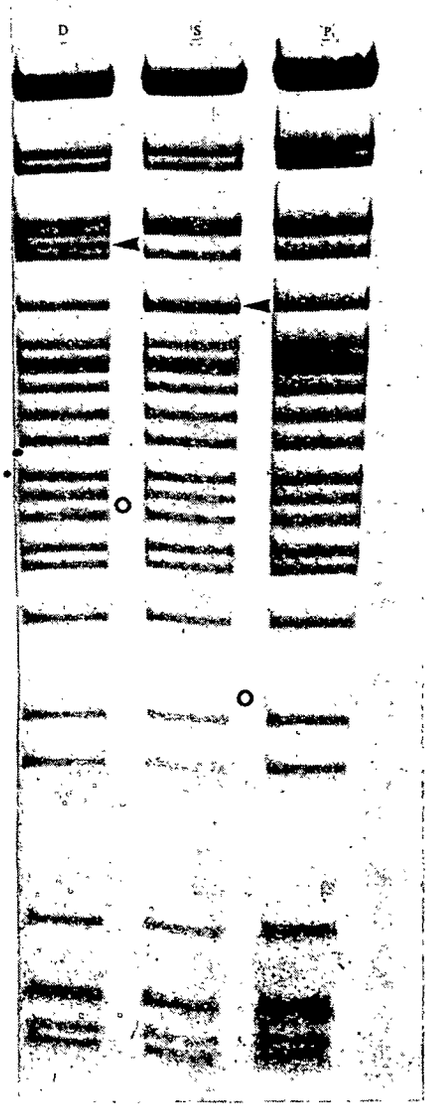


Fig. 3 Digestion profiles of Φ80h, doublet and singlet DNA produced by endo R·*Hin*c. Endo R·*Hin*c, purified from *Hemophilus influenzae* Rc (23) was used to obtain limit digestions of Φ80h (P), doublet (D) and singlet (S) DNA. Conditions of digestion and electrophoresis were as described in Fig. 1 except that electrophoresis was for 5 h. The *Hin*cII and *Hin*d (*Hin*dII and *Hin*dIII) digestion profiles of Φ80h DNA are identical except for four fragments unique to *Hin*cII and eight fragments unique to *Hin*d (ref. 23). Fragments unique to the *Hin*cII digestion are named for the nearest *Hin*d fragment followed by a small letter suffix. Arrowheads indicate the *Hin*cII doublet-unique (A7a) and singlet-unique (B1a) fragments which carry the $tRNA_1^{Tyr}$ gene(s). (In these gel electrophoresis conditions, B1a is not resolved from B1.) ○, Positions of the *Hin*d $tRNA_1^{Tyr}$ containing fragments, B10a and C1a—missing in a *Hin*cII digestion profile.

existence of three Φ80h unique fragments is a consequence of two nuclease recognition sites which exist in Φ80h DNA but which are missing from the DNA of both transducing phages. In the transducing phages the segment of *E. coli* DNA has brought into the phage genome five nuclease-sensitive sites generating the six unique fragments in each phage. The conclusion that none of these five nuclease-sensitive sites is within the $tRNA_1^{Tyr}$ gene(s) derives from the fact that the singlet and doublet DNA digestions have the same number of fragments. If there were a nuclease-sensitive site anywhere within or between the duplicated sequences, then the number of unique bands in the doublet DNA would be one greater than the number in the singlet DNA and the ability to hybridise ^3H-$tRNA_1^{Tyr}$ would have to reside in a band common to both phages.

Endo R·*Hin*d (the phosphocellulose fraction of *H. influenzae* Rd) contains two ATP-independent nuclease activities each specific for a different deoxyribonucleotide sequence (ref. 23 and H. Smith, personal communication). The nucleotide recognition sequence of the two enzymes, *Hin*dII and *Hin*dIII, have been determined using T7 DNA (ref. 24) and λ DNA (K. Murray, personal communication) respectively. The fact that neither of the nucleotide sequences recognised by these two enzymes is contained in the sequence of $tRNA_1^{Tyr}$ (ref. 16) or in the known tRNA precursor sequence[22] is consistent with the conclusion that no *Hin*d nuclease cuts are made within the duplicated $tRNA_1^{Tyr}$ gene sequences.

Hemophilus influenzae strain Rc contains a nuclease, *Hin*cII, with a specificity identical to that of *Hin*dII, but lacks any activity corresponding to the *Hin*dIII enzyme[23]. Therefore, those *Hin*d fragments produced by a *Hin*dIII cut at one or both termini are not present in a *Hin*cII digestion profile but occur as part of some larger, *Hin*cII-unique, fragment. The *Hin*d fragments B10a (doublet-unique) and C1a (singlet-unique) are in this category, and the fragments containing these sequences in a *Hin*cII digest are A7a and B1a respectively (Figs 1 and 3). These are the only doublet-unique and singlet-unique fragments in a *Hin*cII digest and have the same molecular weight difference as B10a and C1a (Table 2).

When the *Hin*cII fragment A7a is eluted and digested with Rd enzyme it gives rise to the *Hin*d fragments B10a, D1a, F3a, and F3b; whereas, the *Hin*cII fragment B1a gives rise to the *Hin*d fragments C1a, D1a, F3a, and F3b (A.L., C.F., and W.R., unpublished) (compare Figs 2, 3, and Table 2). These results further strengthen the conclusion that the fragments B10a and C1a originate from homologous regions in their respective chromosomes, and that they differ from each other only by that piece of DNA which was lost in the genetic derivation of the singlet from the doublet transducing phage (Fig. 6).

tRNA gene size

Although the full size of the $tRNA_1^{Tyr}$ precursor molecule has not yet been established, the presently known sequences require a minimum of 128 base pairs for the $tRNA_1^{Tyr}$ gene[22]. In further experiments a $tRNA_1^{Tyr}$ precursor 135–140 bases long has been observed (S. Altman, personal communication).

On the basis of the molecular weight difference between the doublet-unique fragment B10a and the singlet-unique fragment C1a (Fig. 4 and Table 2) it is estimated that the size of one $tRNA_1^{Tyr}$ gene plus any unduplicated intergenic sequence is approximately 200–260 base pairs. This number is somewhat larger than the size, 90–150 base pairs, estimated by electron microscopy of intact heteroduplex DNA molecules[25,26]. Estimates such as these represent an upper size limit, as the region of genetic duplication could be larger than the tRNA genes. If the crossover in the recombination event which

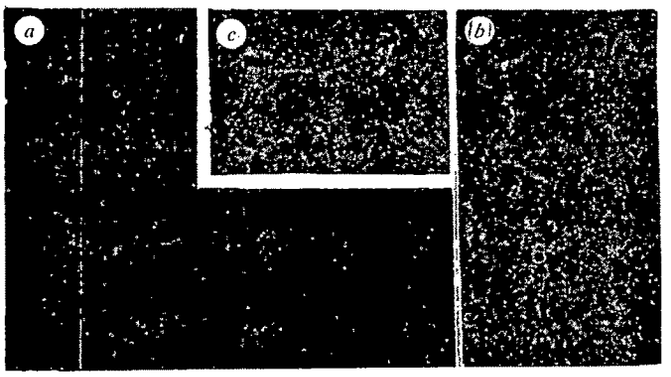


Fig. 4 Electron micrographs of (*a*) doublet-unique (B10a) and (*b*), singlet-unique (C1a) fragments. DNA fragments were prepared as described in Table 1. ΦX174 RFII circles, (*c*) (molecular weight 3.4×10^6 daltons) were included as internal standards for the contour length determination of fragment size. The electron micrographs were made by Nancy Brimlow in the laboratory of David Freifelder[28].

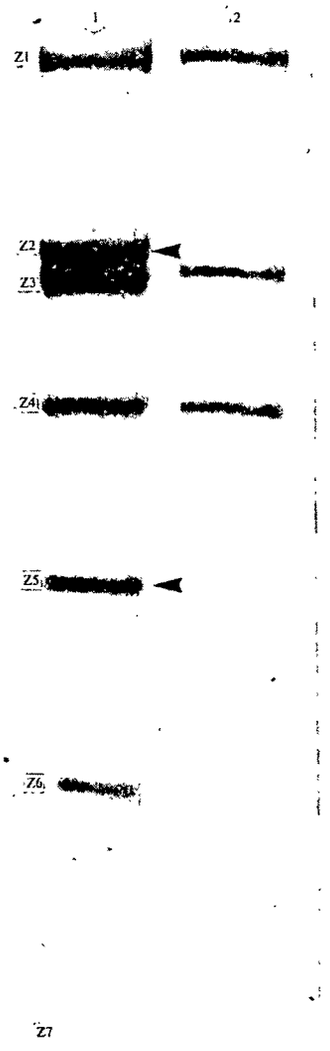


Fig. 5 Digestion of the doublet-unique (B10a) and singlet-unique (C1a) fragments with endo R·*Hae*. Endo R·*Hae* was prepared from *Hemophilus aegyptius* as described by Middleton *et al.*[27]. Pure singlet-unique fragment C1a was prepared as described in Table 1. B10a was obtained free from contamination by neighbouring fragments by isolating the *Hin*cII doublet-unique fragment A7a, digesting it with endo R·*Hin*d, and electrophoresing the digest to obtain the pure B10a product. Fragment B10a (lane 1) and fragment C1a (lane 2) were digested with endo R·*Hae* using the same digestion conditions as described in Fig. 1, and electrophoresed in a 5%–0.5% acrylamide-agarose gel for 3 h at 300 V. Fragments derived from B10a are numbered consecutively Z1–Z7. Fragments derived from C1a are assigned the number of the homologous fragment derived from B10a. Arrows indicate those fragments derived only from B10a: Z2 and Z5.

generated singlet from doublet took place outside the limits of the tRNA genes, then the doublet phage and singlet phage would differ by a correspondingly greater number of base pairs. This question will be answered in the course of the nucleotide sequence analysis of this region.

Taking the size of the tRNA gene plus spacer to be approximately 150×10^3 daltons (Table 2), the doublet-unique B10a and singlet-unique C1a fragments are two to three times the absolute minimum size necessary to contain the genomic regions coding for $tRNA_1^{Tyr}$.

To obtain a subset of smaller $tRNA_1^{Tyr}$ gene fragments, purified doublet-unique fragment (B10a) and singlet-unique fragment (C1a) were digested with endo R·*Hae* (from *H. aegyptius*) (ref. 27). The resulting fragments are well resolved on 5% gels (Fig. 5). Five of the seven fragments derived from the doublet genome have mobilities identical to those of the five fragments from the singlet genome. At the observed level of resolution this degree of coincidence can be taken as strong evidence (that is, as a fingerprint) that the two parental molecules, B10a and C1a, possess large regions of identical sequence.

Table 2 shows that, within experimental error, the molecular

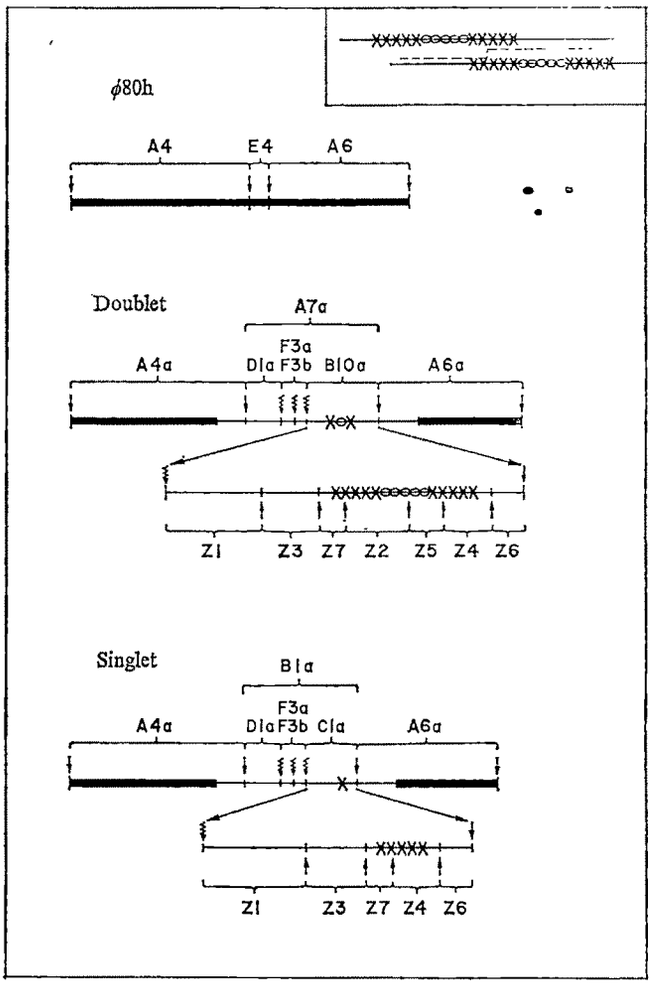


Fig. 6 A model, relating the fragments produced by *Hin*dIII, *Hin*cII and *Hae* nucleases. Homologous segments of the three phage genomes Φ80h, doublet and singlet are shown: phage DNA (▬), *E. coli* DNA (———), *E. coli* DNA duplicated in doublet phage and containing $tRNA_1^{Tyr}$ sequences (—X—), *E. coli* DNA found only in doublet phage, that is, nonduplicated intergenic sequences (—0—). The cleavage sites of the three different enzymes are marked: *Hin*cII or *Hin*dII (↓), *Hin*dIII (⇓), *Hae* (↑). The DNA fragments are labelled and drawn to the same relative scale, or expanded 5 times, as indicated (for assigned molecular weight values see Table 2). Of the four fragments derived from the *Hin*cII fragments A7a (doublet) or B1a (singlet), F3a and F3b are the only two which appear in a digest of intact singlet or doublet DNA with *Hin*dIII enzyme and must therefore be internal. As Z2 and Z5 are the only *Hae* fragments unique to doublet DNA, they must be adjacent and include all of the unduplicated intergenic sequence (—0—). (If there were no *Hae* sites in the unduplicated intergenic region only one doublet-unique fragment would be generated; two *Hae* sites in this region would give rise to three doublet-unique fragments, and so on.) The relative order of the other fragments is not determined nor is the location of the juncture between *E. coli* DNA and phage DNA. The size of each of the regions marked by (—X—) (the duplicated sequence) is between 135 and 230 base pairs and the size of the unduplicated sequence between them (—0—) is between 4 and 125 base pairs (see text). Inset: a representation of the out-of-register mispairing between identical doublet chromosomes, and an unequal recombination event (– – –) which gives rise to the singlet chromosome (ref. 11).

Table 2 Molecular weights of 'unique' fragments from $tRNA_I^{Tyr}$ region

*Hin*cII fragments	Phage derived from	Molecular weight (daltons × 10^{-5})	*Hin*d fragments	Phage derived from	Molecular weight (daltons × 10^{-6})	*Hae* Fragments derived from B10a† and C1a*	Molecular weight (daltons × 10^{-6})
			A4	Φ80h	14.0	Z1†*	1.75
			A6	Φ80h	11.0	Z2†	1.12
			E4	Φ80h	1.50	Z3†*	1.05
			B10a†	Doublet	5.6†	Z4†*	0.77
			D1a	Doublet	2.9	Z5†	0.54
A7a	doublet	10.5	F3a	Doublet	0.93	Z6†*	0.33
			F3b	Doublet	0.93	Z7†*	0.22
			A4a	Doublet	13.7		
			A6a	Doublet	11.3		
			C1a*	Singlet	4.1*	Sum B10a† fragments	5.78†
			D1a	Singlet	2.9		
B1a	singlet	8.3	F3a	Singlet	0.93		
			F3b	Singlet	0.93		
			A4a	Singlet	13.7		
			A6a	Singlet	11.3	Sum C1a* fragments	4.12*

Fragment molecular weights were determined by electron microscopy and/or comparison of relative electrophoretic mobility with SV40 *Hin*d DNA fragments of known molecular weight as described elsewhere[23]. The horizontal arrows indicate which *Hin*d fragments were derived from the *Hin*cII fragments A7a and B1a.

*, †, Derivation of individual *Hae* fragments from the parental *Hin*d fragments B10a and C1a.

weights of the *Hae* fragments add up to the molecular weights of the fragments from which they were derived. The most interesting feature of the *Hae* digestion profiles is the existence of two doublet-unique fragments, Z2 and Z5. Their presence is evidence that there is some number of base pairs—a minimum of four for the restriction site (K. Murray, personal communication), which occur as an unduplicated sequence between the two sequences containing the $tRNA_I^{Tyr}$ genes (Fig. 6).

$tRNA_I^{Tyr}$ gene fragments

These results indicate the potential usefulness and feasibility of successive digestion-fractionation cycles using different nucleases with appropriate site-specificities. The DNA fragments in Fig. 5 are the final product of three such cycles using the nucleases *Hin*cII, *Hin*dIII, and *Hae*.

A model showing how the relevant fragments produced in each of the three digestions might be related to one another in the intact genome is shown in Fig. 6. As shown above, the maximum size for one $tRNA_I^{Tyr}$ gene (including the intergenic sequence) is 200–260 base pairs. Taking the minimum size of four base pairs for the unduplicated intergenic sequence gives a maximum size of 256 base pairs for the $tRNA_I^{Tyr}$ gene. Taking the minimum size for the $tRNA_I^{Tyr}$ gene (135–140 base pairs) gives a maximum size of 125 base pairs for the intergenic sequence between the duplicated genes.

Each of the three endonucleases used in this work produces a different size $tRNA_I^{Tyr}$ gene fragment. The *Hin*d fragments B10a and C1a, which are two to three times the estimated size of the $tRNA_I^{Tyr}$ gene(s) (plus intergenic sequence), are the most favourable size for studying *in vitro* the regulation and maturation aspects of tRNA synthesis and also the overall structure of the $tRNA_I^{Tyr}$ genes. In both of these areas, the larger *Hin*cII fragments A7a and B1a may also prove useful. The seven *Hae* fragments, which are in the subgenic size range, are the most favourable for nucleotide sequence analysis.

The ability to isolate pure preparations of these fragments in chemical quantities should make a complete analysis of the $tRNA_I^{Tyr}$ gene(s) a feasible prospect. Similar experiments are being carried out with the gene coding for $tRNA_{II}^{Tyr}$, the major species, on the premise that this comparison may help to identify those features of gene structure which are important in the regulation of tRNA synthesis.

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[1] Khorana, H. G., *et al.*, *J. molec. Biol.*, **72**, 209–217 (1972).
[2] Brenner, D. J., Fournier, M. J., and Doctor, B. P., *Nature*, **227**, 448–451 (1970).
[3] Johnson, L., and Soll, D., *Proc. natn. Acad. Sci. U.S.A.*, **67**, 943–949 (1970).
[4] Marks, A., and Spencer, J. H., *J. molec. Biol.*, **51**, 115–130 (1970).
[5] Daniel, V., Beckmann, J. S., Sarid, S., Grimberg, J. I., Herzberg, M., and Littauer, U. Z., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2268–2272 (1971).
[6] Danna, K., and Nathans, D., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2913–2917 (1971).
[7] Peacock, A. C., and Dingman, C. W., *Biochemistry*, **7**, 668–674 (1968).
[8] Eggertson, G., and Adelberg, E. A., *Genetics*, **52**, 319–340 (1965).
[9] Orias, E., Gartner, T. K., Lannan, J. E., and Betlach, M., *J. Bact.*, **109**, 1125–1133 (1972).
[10] Squires, C., Konrad, B., Kirshchbaum, J., and Carbon, J., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 438–441 (1973).
[11] Russell, R. L., Abelson, J. N., Landy, A., Gefter, M. L., Brenner, S., and Smith, J. D., *J. molec. Biol.*, **47**, 1–13 (1970).
[12] Garen, A., Garen, S., and Wilhelm, R. C., *J. molec. Biol.*, **14**, 167–178 (1965).
[13] Signer, E. R., Beckwith, J. R., and Brenner, S., *J. molec. Biol.*, **14**, 153–156 (1965).
[14] Guthrie, C., Seidman, J. G., Altman, S., Barrell, B. G., Smith, J. D., and McClain, W. H., *Nature new Biol.*, **246**, 6–11 (1973).
[15] Goodman, H., Abelson, J., Landy, A., Brenner, S., and Smith, J. D., *Nature*, **217**, 1019–1024 (1968).
[16] Goodman, H. M., Abelson, J. N., Landy, A., Zadrazil, S., and Smith, J. D., *Eur. J. Biochem.*, **13**, 461–483 (1970).
[17] Abelson, J. N., Gefter, M. L., Barnett, L., Landy, A., Russell, R. L., and Smith, J. D., *J. molec. Biol.*, **47**, 15–28 (1970).
[18] Smith, J. D., Barnett, L., Brenner, S., and Russell, R. L., *J. molec. Biol.*, **54**, 1–14 (1970).
[19] Smith, J. D., Anderson, K., Cashmore, A., Hooper, M. L., and Russell, R. L., *Cold Spring Harb. Symp. quant. Biol.*, **35**, 21–27 (1970).
[20] Smith, H. O., Wilcox, K. W., *J. molec. Biol.*, **51**, 379–391 (1970).
[21] Landy, A., Abelson, J., Goodman, H. M., and Smith, J. D., *J. molec. Biol.*, **29**, 457–471 (1967).
[22] Altman, S., and Smith, J. D., *Nature new Biol.*, **233**, 35–39 (1971).
[23] Landy, A., Ruedisueli, E., Robinson, L., Foeller, C., and Ross, W., *Biochemistry* (in the press).
[24] Kelly, T. J., jun., and Smith, H. O., *J. molec. Biol.*, **51**, 393–409 (1970).
[25] Miller, R., Besmer, P., Khorana, H., Fiandt, M., and Szybalski, W., *J. molec. Biol.*, **56**, 363–368 (1971).
[26] Fiandt, M., Hradecna, Z., Lozeron, H. A., and Szybalski, W., *The Bacteriophage Lambda* (edit. by Hershey, A. D.), 329–354 (1971).
[27] Middleton, J. H., Edgell, M. H., and Hutchison III, C. A., *J. Virol.*, **10**, 42–50 (1972).
[28] Davis, R. W., Simon, M., and Davidson, N., *Methods in Enzymology*, **21**, Part D, (edit. by Grossman, L., and Moldave, K.), 413–428 (1971).
[29] Gefter, M. L., and Russell, R. L., *J. molec. Biol.*, **39**, 145–157 (1969).

LETTERS TO NATURE

PHYSICAL SCIENCES

Radio spectra of OH471 and OQ172

RADIO spectra for the highly redshifted $z \geq 3.40$ quasars OH471 (ref. 1) and OQ172 (ref. 2) are presented here for frequencies between 16.7 MHz and 85.3 GHz. The spectra are complex. Both have a peak at or near 1.4 GHz and a second peak near or above 85 GHz. Both have a low frequency cutoff, the one for OH471 being especially sharp. OH471 sometimes seems to be variable at 31 GHz on a time scale of days but the source appears to be stable at 10.7 and 22 GHz.

Although flux densities for these sources had already been published at a few frequencies, the unusual nature of the objects encouraged us to undertake further measurements at as many frequencies and epochs as possible in order to obtain much more complete data in both frequency and time domains.

All observations, new and previously published, are listed in Table 1. The spectra of the sources are shown in Fig. 1. Figure 2 is a plot of flux density against time for OH471, which has shown some evidence of variability. The observations involve more than 120 measurements at many epochs from 1963 to March 1974 at 25 different frequencies between 16.7 MHz and 85 GHz.

Flux densities have been adjusted to the Wills scale[3]. The flux density on this scale was obtained by multiplying values on the KPW scale[4] by 1.23 at 178 MHz, 1.10 at 750 MHz and 1.06 at 1.4 GHz. No adjustment was made at frequencies of 2.65 GHz and above since the difference between the Wills scale, the KPW and other commonly used scales is small (less than 5%).

Measurement details of the new observations are as follows:

Algonquin. Measurements with the 46-m Algonquin telescope were made on OH471 and OQ172 at 10.7 GHz between April and December 1973, and at 22.2 GHz during February 1974. The ARO 10.7-GHz receiver has a noise temperature of 120 K and a bandwidth of 100 MHz. The 22.2-GHz receiver has a noise temperature of 200 K and a bandwidth of 500 MHz. The observations were made by Andrew, Gearhart, Purton, Feldman and Marsh.

Bonn. Measurements were made with the 100-m telescope in July 1973 at 10.7 GHz and in August 1973 at 1.42 GHz with the results given in Table 1. Observers included Thiel, Fürst and Hirth.

Cambridge. The flux densities of both OH471 and OQ172 are below the 4C limit of 2.0 Jy at 178 MHz but an examination of the interferometer records yielded the values given in Table 1. In addition flux density measurements at 5.0 GHz of OH471 were made in June and July 1973 and of OQ172 in August 1973 and March 1974 with the 5-km telescope (Table 1) and accurate positions determined (Table 2).

Grakovo. Flux density measurements on OH471 and OQ172 were made during 1973 at decameteric wavelengths with the (Ukrainian) UTR-2 radio telescope. This telescope consists of two arrays of wideband horizontal dipoles arranged in the form of a 'T'. One array, oriented north-south, is 1,860 m long and has 1,440 dipoles. The other, or east-west, array is 900 m long and has 600 dipoles. The array operates over the frequency range 10 to 25 MHz in a five-beam mode.

Observations on OH471 and OQ172 with the UTR-2 telescope showed no detectable signal from which it may be concluded that the flux density of both sources is less than 10 Jy (f.u.) at 16.7 MHz and less than 5 Jy at 25 MHz. These observations support the conclusion that neither source has a low frequency component. The minimum detectable flux density is necessarily large because of confusion effects combined with the high sky background temperature ($\sim 10^5$ K).

Green Bank. Observations were made at 1.4 and 2.695 GHz with the 300-ft meridian transit telescope, using multiple-feed systems to determine accurate declination pointing corrections. The procedure has been described by Bridle *et al.*[5]. At 1.4 GHz, OQ172 was observed in February 1970 and OH471 in May 1973. At 2.695 GHz, OQ172 was observed in January 1973 and both QSOs were observed in April, May, and June 1973. Observations with the three-element interferometer were made in February 1974 at 2.695 and 8.085 GHz. The 300-ft telescope measurements were made by Bridle and the three-element interferometer measurements by Warner, Assousa, and Balick.

Kitt Peak. Observations at 31.4 and 85.3 GHz were made with the 11-m telescope at Kitt Peak during six days in March 1973 on OH471 and on four days in July 1973 on both OH471 and OQ172. Techniques were similar to those described by Conklin *et al.*[6].

Itapetinga. Observations were made during July and August 1973 and again in February 1974 with the Mackenzie University (Brazil) 13.7-m radome-enclosed telescope at 22.2 GHz. The receiver bandwidth is 100 MHz and the system temperature about 2,500 K. A double beam feed was used consisting of two horizontally polarised horns with beam separated in the sky by 9′. The data were corrected for atmospheric absorption and radome attenuation.

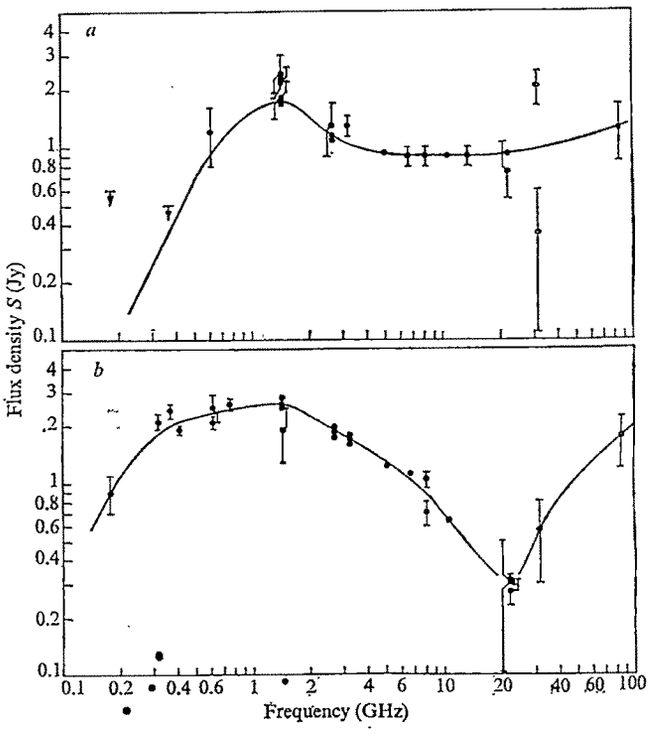


Fig. 1 Flux density (S) of OH471 (a) and OQ172 (b) against frequency. The points with no error bars have errors within the extent of the solid circle.

Measurements were made on seven days between July 25 and August 3, 1973, and on seven days in February 1974, for OH471 and on four days between July 25 and 29, 1973, for OQ172. Each measurement averaged more than 2 h of observing time. The measurements on OH471 were difficult because of the low elevation angle of the source (less than 21°).

Ohio. Records stored in the Ohio State University (OSU) Radio Observatory archives for epochs as early as 1963 were used to obtain flux densities for OH471; records back to 1966 were examined for data on OQ172. The observations were made with the OSU radio telescope with techniques as given by Fitch *et al.*[7]. Further observations of OH471 were made at 1.42 GHz with the OSU radio telescope in January 1974.

Stanford. The aperture synthesis interferometer was used during July 1973 for measurements at 10.7 GHz. Both sources were unresolved indicating that their angular diameters must be less than 10″.

Texas. The Broadband Synthesis Interferometer (BSI) was used to search for OH471 at 365 MHz on two occasions during May 1973 and on five occasions during January 1974. It was not found, but assuming that the source is constant at 365 MHz the upper limit is 0.5 Jy with 95% confidence. Techniques are similar to those described by Ghigo and Owen[8].

Table 1*a* Flux densities for OH471

Frequency	Flux density (Jy)*	Epoch	Observatory†
16.7 MHz	< 10.0	1973	Grakovo
25.0	< 5.0	1973	Grakovo
178	< 0.6	1962	Cambridge
365	< 0.5	1973-1974	Texas
612	1.2 (0.4)	1969.2-1970.2	Ohio[10]
1.40 GHz	1.70 (0.07)	1973.41	Green Bank
1.415	2.4 (0.5)	1963.9	Ohio
1.415	2.3 (0.4)	1966.8	Ohio
1.415	2.2 (0.4)	1969.6	Ohio
1.415	2.2 (0.4)	1970.2	Ohio[11]
1.415	2.3 (0.4)	1970.2	Ohio
1.42	1.73 (0.07)a	9 Aug. 73	Bonn
1.42	1.73 (0.07)a	12 Aug. 73	Bonn
1.42	1.8 (0.4)	1974.0	Ohio
2.65	1.3 (0.4)	1969.7	Ohio[10]
2.695	1.16 (0.03)	1973.28	Green Bank
2.695	1.10 (0.03)	1973.65	Green Bank
2.695	1.09 (0.05)	1974.1	Green Bank
3.2	1.30 (0.15)	1971.0	Algonquin[12]
5.0	0.945(0.025)	8 Jun. 73	Cambridge
5.0	0.945(0.025)	19 Jun. 73	Cambridge
5.0	0.951(0.025)	2 Jul. 73	Cambridge
5.0	0.927(0.025)	24 Jul. 73	Cambridge
6.6	0.90 (0.10)	1971.2	Algonquin[12]
8.09	0.90 (0.10)	1974.1	Green Bank
10.7	0.90 (0.10)	16 Apr. 73	Algonquin
10.7	0.95 (0.05)	22 May 73	Algonquin
10.7	0.81 (0.08)	25 Jun. 73	Algonquin
10.7	1.05 (0.11)	26 Jun. 73	Algonquin
10.7	0.87 (0.04)	27 Jun. 73	Algonquin
10.7	0.92 (0.04)	28 Jun. 73	Algonquin
10.7	0.88 (0.04)	29 Jun. 73	Algonquin
10.7	0.90 (0.04)	30 Jun. 73	Algonquin
10.7	0.88 (0.04)	1 Jul. 73	Algonquin
10.7	0.84 (0.04)	3 Jul. 73	Algonquin
10.7	0.89 (0.10)	24 Jul. 73	Algonquin
10.7	0.95 (0.14)	26 Jul. 73	Stanford
10.7	0.91 (0.04)b	26 Jul. 73	Bonn
10.7	0.87 (0.04)b	27 Jul. 73	Bonn
10.7	0.91 (0.04)b	28 Jul. 73	Bonn
10.7	1.01 (0.10)	24 Aug. 73	Algonquin
10.7	0.91 (0.10)	16 Sept. 73	Algonquin
10.7	0.92 (0.10)	12 Dec. 73	Algonquin
13.5	0.90 (0.10)	1971.2	Algonquin[12]
22.2	0.70 (0.25)	25 Jul.-3 Aug. 73	Itapetinga
22.2	0.80 (0.20)	19-27 Feb. 74	Itapetinga
22.2	0.87 (0.09)c	3 Feb. 74	Algonquin
22.2	0.91 (0.06)c	4 Feb. 74	Algonquin
22.2	0.90 (0.08)c	5 Feb. 74	Algonquin
22.2	0.89 (0.09)c	5 Feb. 74	Algonquin
22.2	1.01 (0.06)c	6 Feb. 74	Algonquin
22.2	0.89 (0.07)c	7 Feb. 74	Algonquin
22.2	1.02 (0.09)c	8 Feb. 74	Algonquin
22.2	0.89 (0.07)c	9 Feb. 74	Algonquin
22.2	0.83 (0.12)c	10 Feb. 74	Algonquin
22.2	0.95 (0.08)c	13 Feb. 74	Algonquin
22.2	1.10 (0.15)c	13 Feb. 74	Algonquin
22.2	0.87 (0.10)c	14 Feb. 74	Algonquin
22.2	1.00 (0.12)c	18 Feb. 74	Algonquin
22.2	0.86 (0.10)c	18 Feb. 74	Algonquin
31.4	2.07 (0.4)c	8 Mar. 73	Kitt Peak
31.4	0.36 (0.25)	11 Mar. 73	Kitt Peak
31.4	1.79 (0.5)	12 Mar. 73	Kitt Peak
31.4	0.77 (0.35)	13 Jul. 73	Kitt Peak
31.4	0.21 (0.7)	14 Jul. 73	Kitt Peak
85.3	0.5 (1.1)	3 Mar. 73	Kitt Peak
85.3	1.5 (0.6)	4 Mar. 73	Kitt Peak
85.3	0.65 (0.9)	5 Mar. 73	Kitt Peak
85.3	1.25 (0.4)	3, 4, 5 Mar. 73(av.)	Kitt Peak
85.3	0.85 (0.7)	17 Jul. 73	Kitt Peak

* 1 Jy = 10^{-26} W m^{-2} Hz^{-1}. Relative to Wills scale[3].
a, relative to 3C147=23.3 Jy; b, relative to 3C147=4.14 Jy; c, relative to DR21=17.8 Jy (peak with 1′.4 HPBW)
† Observations reported here, except where reference cited.

Table 1*b* Flux densities for OQ172

Frequency	Flux density	Epoch	Observatory
16.7 MHz	< 10.0	1973	Grakovo
25.00	< 5.0	1973	Grakovo
178	0.9 (0.2)	1966	Cambridge
318	2.1 (0.2)	1970	Arecibo[13]
365	2.4 (0.2)	1972	Texas[8]
408	1.9 (0.1)	1971	Molonglo[14]
606	2.1 (0.15)	1970	Arecibo[13]
612	2.5 (0.4)	1967.7	Ohio
750	2.6 (0.2)	1969.70	Green Bank[15]
1.40 GHz	2.78 (0.06)	1969.70	Green Bank[15]
1.40	2.58 (0.03)	1970.16	Green Bank[15]
1.415	1.90 (0.6)	1966.5	Ohio
1.415	2.2 (0.5)	1967.7	Ohio
1.415	2.6 (0.5)	1967.7	Ohio[7]
1.42	2.50 (0.08)d	12 Aug. 73	Bonn
2.65	1.70 (0.5)	1967.7	Ohio[16]
2.695	1.96 (0.1)	1970	Nancay[17]
2.695	1.85 (0.03)	1973.06	Green Bank
2.695	1.72 (0.06)	1973.28	Green Bank
2.695	1.81 (0.04)	1973.65	Green Bank
2.695	1.79 (0.05)	1974.1	Green Bank
3.2	1.60 (0.15)	1969	Algonquin [18]
3.2	1.78 (0.04)	1970.08	Algonquin[15]
3.2	1.74 (0.03)	1970.43	Algonquin[15]
5.0	1.22 (0.1)	1970	Green Bank[17]
5.0	1.22 (0.02)	Aug. 73-Mar. 74	Cambridge
6.6	1.10 (0.1)	1969	Algonquin[16]
6.6	1.12 (0.03)	1969.76	Algonquin[15]
6.6	1.12 (0.03)	1970.37	Algonquin[15]
8.0	1.05 (0.10)	1971	Michigan
8.085	0.70 (0.10)	1974.1	Green Bank
10.7	0.70 (0.10)	1969	Algonquin[16]
10.7	0.75 (0.05)	1969.19	Algonquin[15]
10.7	0.51 (0.03)	1970.50	Algonquin[15]
10.7	0.70 (0.07)	25 Jun. 73	Algonquin
10.7	0.54 (0.06)	26 Jun. 73	Algonquin
10.7	0.62 (0.04)	27 Jun. 73	Algonquin
10.7	0.66 (0.07)	2 Jul. 73	Algonquin
10.7	0.70 (0.08)	19 Jul. 73	Stanford
10.7	0.62 (0.02)e	25 Jul. 73	Bonn
10.7	0.62 (0.02)e	27 Jul. 73	Bonn
22.2	0.30 (0.20)	25-29 Jul. 73	Itapetinga
22.2	0.31 (0.09)c	4 Feb. 74	Algonquin
22.2	0.31 (0.03)c	5 Feb. 74	Algonquin
22.2	0.30 (0.03)c	6 Feb. 74	Algonquin
22.2	0.32 (0.04)c	7 Feb. 74	Algonquin
22.2	0.27 (0.04)c	16 Feb. 74	Algonquin
31.4	0.56 (0.25)	13-14 Jul. 73	Kitt Peak
85.3	1.78 (0.55)	16 Jul. 73	Kitt Peak

d, relative to 3C298=5.95 Jy; e, relative to 3C298=1.03 Jy. Otherwise as Table 1*a*.

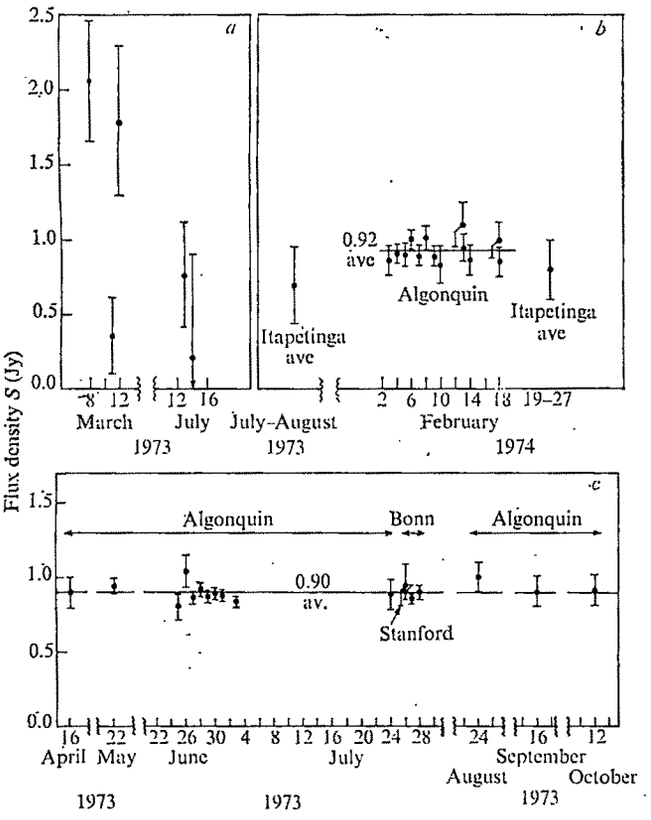


Fig. 2 Flux density of OH471 as a function of date for frequencies: 31.4 GHz(*a*), 22.2 GHz(*b*), and 10.7 GHz(*c*). *a*, All Kitt Peak data; *b* and *c* as labelled.

The spectra of OH471 and OQ172, shown in Fig. 1, are complex. Both have a low-frequency cutoff. The cutoff is particularly sharp for OH471. Both have a peak near 1.4 GHz and (if OH471 is not varying at 31.4 GHz) both have a second peak near or above 85 GHz suggesting at least two components.

The measurements on OH471 at 31.4 GHz made at Kitt Peak are suggestive of variability. But the observations at 22.2 GHz made at Algonquin show no evidence of variability and the observations at Itapetinga at the same frequency are inconclusive (Fig. 2). No observations were reported from other observatories for dates on which variability was observed at Kitt Peak so there is no direct disagreement. But the stability and greater precision of the Algonquin results are statistically inconsistent with the variability suggested by the Kitt Peak results if OH471 is a frequently fluctuating erratic variable as the later results imply. A frequency of 22.2 GHz is close enough to 31.4 GHz that a noticeable change might be expected to occur at 22.2 GHz accompanying a large variation at 31.4 GHz.

Measurements of OH471 between April and December 1973 made at Algonquin, Bonn and Stanford are all consistent with a constant flux density at 10.7 GHz.

Records from OSU of OH471 at 1.415 GHz since 1963 (Table 1) show no significant change in flux density until 1970. But between 1970 and 1974 there is a marginally significant decrease. So there may be slight variability at 1.4 GHz on a time scale of years.

Table 2 Radio positions of OH471 and OQ172

Source	α(1950.0)	δ(1950.0)	Observatory
OH471	$06^h42^m53.^s023(0.^s005)$	$+44°54'30''.89(0''.05)$	Cambridge (5-km teleslope)
OQ172	$14^h42^m50.^s479(0.010)$	$+10°11'12''.35(0''.15)$	Cambridge (5-km telescope)

According to Andrew *et al.*[9] there is a 90% or greater probability of sources with centimetre-excess spectra, like OH471 and OQ172, exhibiting radio variability. Variability is suggested in OH471 and it will be interesting to continue measurements of OQ172, particularly in the region 10 to 40 GHz, to find if any variability exists.

The spectrum of OQ172 (Fig. 1*b*) is complex. It would take four or five straight (power law) segments to give even a first order fit. The spectrum of OH471 is similarly complex and is further complicated by apparent variability at 31 GHz.

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[1] Carswell, R. F., and Strittmatter, P. A., *Nature*, **242**, 394 (1973).
[2] Wampler, E. J., Robinson, L. B., Baldwin, J. A., Burbidge, E. M., *Nature*, **242**, 137 (1973).
[3] Wills, B. J., *Astrophys. J.*, **180**, 335 (1973).
[4] Kellerman, K. I., Pauliny-Toth., I. I. K., and Williams, P. J. S., *Astrophys. J.*, **157**, 1 (1969).
[5] Bridle, A. H., Davis, M. M., Fomalont, F. B., and Lequeux, J., *Astr. J.*, **77**, 405 (1972).
[6] Conklin, E. K., Andrew, B. H., Wills, B. J., and Kraus, J. D., *Astrophys. J.*, **177**, 303 (1972).
[7] Fitch, L. T., Dixon, R. S., and Kraus, J. D., *Astr. J.*, **74**, 612 (1969).
[8] Ghigo, F. D., and Owen, F. N., *Astr. J.*, **78**, 848 (1973).
[9] Andrew, B. H., Medd, W. J., Harvey, G. A., and Locke, J. L., *Nature*, **236**, 445 (1972).
[10] Kraus, J. D., Gearhart, M. R., and Ehman, J. R., *Nature*, **243**, 94 (1973).
[11] Brundage, R. K., Dixon, R. S., Ehman, J. R., and Kraus, J. D., *Astr. J.*, **76**, 777 (1972).
[12] Wills, B. J., Kraus, J. D., and Andrew, B. H., *Astrophys. J. Lett.*, **169**, L87 (1971).

[13] Jauncey, D. L., Niell, A. E., and Condon, J. J., *Astrophys. J. Lett.*, **160**, L31 (1970).
[14] Hoskins, D. J., Murdoch, H. S., Hazard, C., and Jauncey, D. L., *Aust. J. Phys.*, **25**, 559 (1972).
[15] Ross, H. N., thesis, Univ. Toronto (1972).
[16] Kraus, J. D., and Andrew, B. H., *Astrophys. J. Lett.*, **159**, L41 (1970).
[17] Witzel, A., Véron, P., and Véron, M. P., *Astr. Astrophys.*, **11**, 171 (1971).

New position of Cen X-3 from Copernicus

THE X-ray source Cen X-3 (3U1118-60) is part of a pulsating, eclipsing, binary system[1,2]. The third Uhuru catalogue[3] gives a best position for the source of α (1950) = 11 h 18 min 55 s, δ (1950) = −60° 19′ 5″ with a 90% confidence error box of area 7 × 10^{-4} deg². At present this X-ray source has not been positively identified with an optical counterpart although several candidates have been suggested— (refs 4–6 and L. Webster, unpublished).

The Mullard Space Science Laboratory experiment on the Copernicus satellite has been used to observe Cen X-3 primarily with a view to refining the position but also to observe eclipse transitions at energies lower than those observed by Uhuru. The instrumentation which has been described elsewhere[7]; contains three co-aligned X-ray detection systems. The first contains a grazing incidence telescope (nonfocusing flux collector) and proportional counter sensitive in the nominal energy range 1.4 to 4.2 keV with circular apertures defining fields of view of 10 arc min (large), 3 arc min (intermediate) and 1 arc min (small); the second is similar and is sensitive in the energy range 0.7 to 2.0 keV with apertures defining fields of view of 10 arc min (large), 6 arc min (intermediate) and 2 arc min (small); the third is a collimated proportional counter sensitive in the energy range 4 to 12 keV with a fixed aperture of 3°.

Cen X-3 was observed by Copernicus between 1900 UT on February 17 and 0200 UT on February 18, 1973. From 1900 UT to 2130 UT on 17 February the package was pointed at α (1950) = 11 h 19 min 1.2 s, δ (1950) = −60° 18′ 41″ using the large apertures on the two telescopes and the X-ray source was observed emerging from an eclipse. The data from the three detectors are shown in Fig. 1; the background has been subtracted in the manner described previously[7]. The effectiveness of this method of background subtraction is well illustrated between 0000 UT and 0200 UT on February 18 when the satellite was passing through the edges of the radiation belts and there are no signs of their effects on the 4 to 12 keV signal.

Figure 1 also shows that the lower energy X rays appear later. This effect was predicted by Schreier *et al.*[2] since they assumed the length of the transition was not due to an extended source but to an extended atmosphere around the occulting star. The present observations confirm this prediction of an extended atmosphere, clearly showing the 1.4 to 4.2 keV signal appearing more than an hour after the 4 to 12 keV signal. There is very little emission at 0.7 to 2.0 keV which reinforces the interpretation of an absorbing atmosphere being present.

Between 2130 UT on February 17 and 0200 UT on February 18 the 2U error box was observed using the intermediate apertures on the two telescopes. Seven points in the error box were chosen for observation with times of typically 20 integration periods (one integration period being 62.5 s). The points were separated by 2 arc min so there was considerable overlap using the intermediate fields of view and significant emission was observed in three of these overlapping fields of view. Simultaneously the high energy system monitored the source and Fig. 1 shows that during this time the intensity of the source varied by less than 10% in the energy range 4 to 12 keV. (The response across the 3° field of view of the high energy detector is approximately triangular and the correction due to the small pointing excursions is less than 2%). So we assume there were no significant variations in the intensity of the source on time scales of minutes during these observations. Since the efficiency profiile of the telescope field of view is well known, an estimate of the most likely position for the source can be made using the ratios of the count rates in the overlapping fields of view for the 1.4 to 4.2 keV system. This best position is at α(1950) = 11 h 19 min 1.8 s and δ(1950) = −60° 20′ 27″.

The error box for this position contains two components. First, there is a component due to the counting statistics giving a rectangular box 40 × 50 arc s and, second, one due to pointing uncertainties, of 10 × 20 arc s. The latter is derived from repeated observations of Sco X-1 which has a position known to better than 1 arc s. Combining these two sources of error gives a 90% confidence error box with corners shown in Table 1.

Table 1 Corners of error box for Cen X-3

	h	min	s		°	′	″
α (1950) =	11	19	1.1	δ (1950) =	−60	19	49
	11	19	7.1		−60	20	11
	11	19	3.7		−60	21	5
	11	18	57.7		−60	20	43

These positions are shown schematically in Fig. 2 with the 90% error box from the 3U catalogue[3]. These error boxes marginally overlap, the Copernicus error box being much smaller (2.4 × 10^{-4} deg²) and more to the south-east.

Several optical candidates have been suggested for Cen X-3 and it is worthwhile examining these in the light of this new X-ray position. Margon and Wray[4] have suggested WRA795, α(1950) = 11 h 18 min 56 s, δ(1950) = −60° 15′ 48″, but this can be definitely eliminated as being over 5 arc min north of this new position. The m_V = 15 blue star suggested by Liller[5], L in Fig. 2, has been questioned by van Genderen[8] who found that the optical period of the star did not fit the X-ray period. This star lies well outside the Copernicus error box and hence can

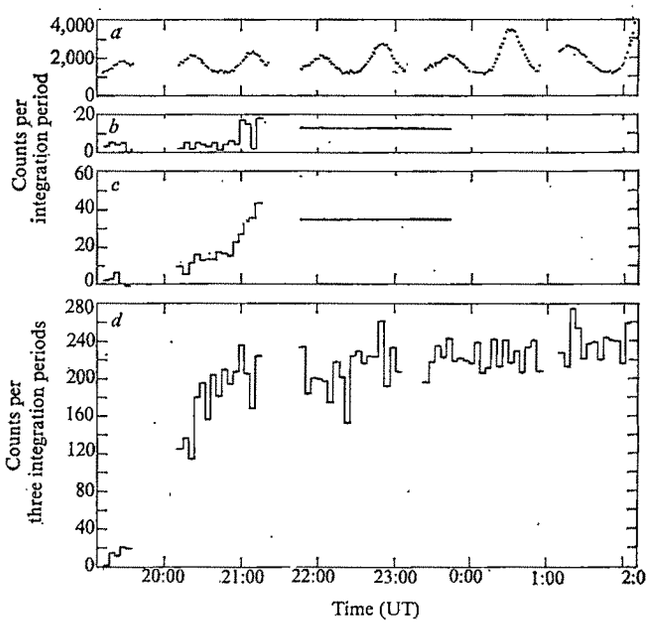


Fig. 1 Copernicus observations of Cen X-3 between 1900 UT on 17 February and 0200 UT on 18 February, 1973. Note the de-eclipse of the X-ray source between 1930 UT and 2100UT. Integration period = 62.5 s, dead time between integration periods = 23 s. *a*, Charged particle background; *b*, 0.7 to 2.0 keV X rays; *c*, 1.4 to 4.2 keV X rays; *d*, 4 to 12 keV X rays.

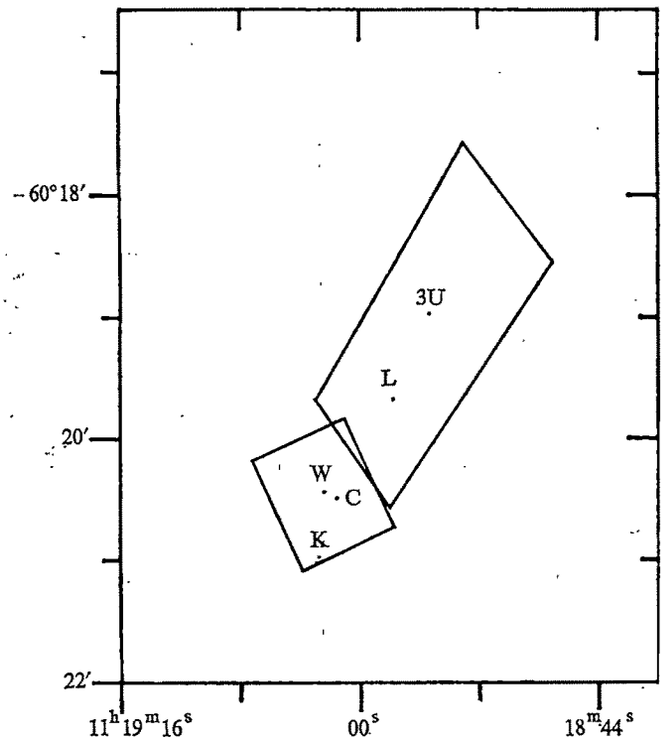


Fig. 2 X-ray error boxes for Cen X-3. C, Copernicus best position; 3U, Uhuru best position[3]; L, W, K, see text. K is the suggested position for Cen X-3.

be eliminated. Webster (unpublished) has suggested CPD −59° 3380 (star 14 in Fig. 1 of ref .9) marked W in Fig. 2. This star is an early B giant, and so may have insufficient mass to be the primary of the system. Krzeminski[6] has found optical intensity variations, which seem to have a period equal to the X-ray period, in the $m_V \approx 13$ red star (K in Fig. 2). This star has been classified as an 09.5—BO.5 Ib star [10,11] and so has sufficient mass to agree with the calculation of Schreier *et al*[2].

Both of these last two stars are inside the Copernicus error box. However, the evidence presently available indicates that the star suggested by Krzeminski at α(1950) = 11 h 19 min 03 s, δ(1950) = −60° 21′ 00″, is the optical counterpart for the primary of the Cen X-3 system.

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1 Giacconi, R., Gursky, H., Kellogg, E., Schreier, E., and Tananbaum, H., *Astrophys. J. Lett.*, **167**, L67–L73 (1971).
2 Schreier, E., Levinson, R., Gursky, H., Kellogg, E., Tananbaum, H., and Giacconi, R., *Astrophys. J. Lett.*, **172**, L79–L89 (1972).
3 Giacconi, R., Murray, S., Gursky, H., Kellogg, E., Schreier, E., Matilsky, T., Koch, D., and Tananbaum, H., *Astrophys. J. Suppl.*, No. 237 (1974).
4 Margon, B., and Wray, J. D., *Astrophys. J. Lett*, **174**, L141–L142 (1972).
5 Liller, W., *IAU Circ.* 2518 (1973).
6 Krzeminski, W., *IAU Circ.* 2612 (1973).
7 Sanford, P. W., *Proc. R. Soc.* (*A*) (in the press).
8 Van Genderen, A. M., *IAU Circ.* 2563 (1973).
9 Brucato, R. J., Kristian, J., and Westphal, J. A., *Astrophys. J. Lett*, **175**, L137–L139 (1972).
10 Wickramasinghe, D. T., Bessell, M. S., and Vidal, N. V., *IAU Circ.* 2631 (1974).
11 Rickard, J. J. *Astrophys J. Lett.*, **189**, L113-L115 (1974).

Interpretation of double structure in the celestial γ-ray bursts

THE essentially isotropic distribution of the Vela satellite γ-ray bursts[1] implies that the sources are either very local and galactic, or very remote and extragalactic. Of the theories for a remote origin there are two schools of thought, either that these γ-rays originate in shock waves during the formation of type II supernovae[2] or that they represent transient pulses of blackbody emission associated with the collapse of a white dwarf to a neutron star[3].

A problem common to both of these theories is that they do not readily account for the fact that most of the observed bursts are double, and that the second pulse is frequently comparable in magnitude to the first.

I here make the suggestion that perhaps the sources of these γ-ray bursts may be identified with the collapse of a rotating magnetised star, following a model of the type computed by LeBlanc and Wilson[4]. In this model, at the later stages in the collapse, axial jets are formed in ~2.5 s which, in their specific example, carry energies $\sim 10^{51}$ erg at a temperature of $\sim 3.5 \times 10^9$ K, comparable to those estimated and required in ref. 3. The main objective of this suggestion is that if acceptable theoretically it could readily account for the double pulses.

Figure 1 represents a replot in polar coordinates of the density profile of Fig. A2a in ref. 4 at $t = 2.67$ s and $\rho = 3 \times 10^6$ g cm^{-3}. Let θ be the angle of tilt between the rotation axis of the source and a perpendicular to the source-Earth line and let φ be the corresponding azimuth angle, φ being zero in Fig. 1.

If now the observed γ-ray bursts originate from such collapsing objects whose axes are randomly distributed, the following predictions are made:

(1) The pulses will in general be double, for radiation will be observable from both polar jets. (2) The delay time T between the two pulses will in general increase with τ, the duration of the individual pulses. (3) Single pulses will arise for all

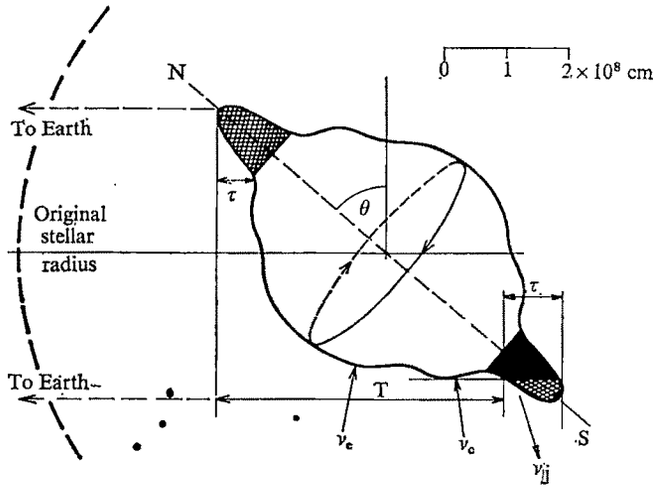


Fig. 1 Diagram to illustrate the active polar regions on a collapsing rotating star with an axial magnetic field, from LeBlanc and Wilson[4].

values of φ when $\theta=0$, for all values of θ when $\varphi=\pi/2$, and if one or other of the jets is totally obscured by the equatorial regions of the collapsing star. (4) The first pulse will always be the stronger—because a larger fraction of the nearer jet is visible to the observer than the farther jet and because Doppler shifts of radiation from matter moving along the jets, resolved along the line of sight, will increase both the flux and the photon energy of the radiation from the nearer jet; and if some or all of the radiation is nonthermal, one might expect it to be directed predominantly along the jet. (5) There should never be more than two pulses.

The duration of the pulses τ will be compounded of the growth time and axial development of the jets, the rate of radiation production and the time difference corresponding to the line of sight path difference between the tip and the base of the 'visible' portion of the jet.

I now look at these predictions in the light of the data. Of the latest list[5] of Vela events I find, in support of prediction (1) above, that of the 19 events for which information on the character of the pulses is presented, 12 are double. Figure 2 shows a plot, for these double events, of the duration τ of the first pulse with respect to the separation T between the pulses (presumed to be measured from the onset of the pulses). The event numbers on the plot correspond to those used in ref. 5. It is at once apparent that τ increases steadily with T, in agreement with prediction (2). I find seven pulses which are single, consistent with (3), five being wide (≥ 1 s) and two narrow (<1 s). The wide pulses may correspond to overlapping of bursts from the individual jets or to relatively slow axial development of the jets. In support of prediction (4) I note that for the double pulses the first component is the larger in all cases, with the possible exception of event 71-2, for which the description is ambiguous. Regarding the multiplicity, prediction (5), the only event which reveals more than two pulses is 72-4, with a third event at $T=28$ s.

Regarding the intensity of the thermal radiation from the poles in the LeBlanc–Wilson model, the quoted mass and temperature of the jets, $\sim 10^{32}$ g and $\sim 3\times 10^{9}$ K respectively, are sufficiently close to the corresponding values in ref. 3 that the γ-ray yields, and hence the distance-range of the sources ($\sim$25 to 300 Mpc) will be comparable on the two models.

A crucial question is whether the jets can penetrate the residual stellar envelope, to densities at which the medium is optically thin to $\sim$100 keV γ rays, and in this connection it would be of interest to know: (a) how the strength, temperature and axial development of the jets in this model change by varying the input parameters; (b) the subsequent history of the jets beyond $t=2.67$ s, at which their calculations were halted; and (c) what effects the axial magnetic field may have on the cooling time. The absolute time scales present a problem. On the specific model in ref. 4, the tips of the jets are $\sim 7\times 10^{8}$ cm apart at $t=2.67$ s, so that for $\theta=60°$, $T\sim 0.02$ s, a pulse spacing less than that measured for the shortest double event, Los Alamos burst No. 69–1. To obtain pulse separations of several seconds, reported in ref. 5, as well as by others[6], we require that the post-collapse dimensions be far larger. We therefore ask whether Colgate's larger Type II $30M_{\odot}$ stars[2], with initial radii 10^{12} to 10^{14} cm can on collapse also develop axial jets, which could then radiate thermally at the required temperatures.

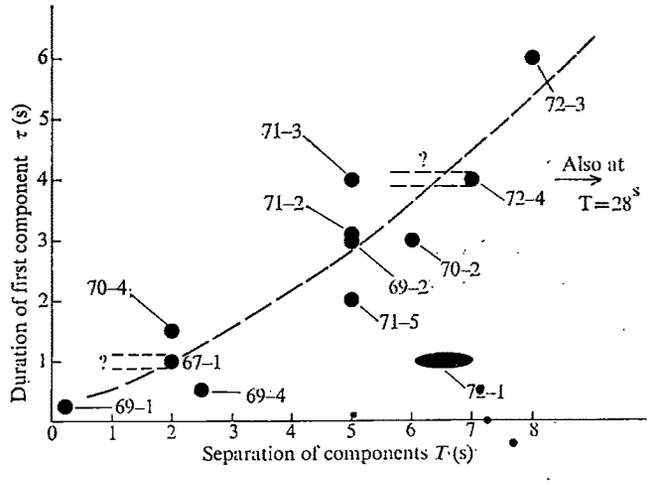


Fig. 2 A plot of the duration τ of the first component of all double pulses listed in ref. 5, with respect to the separation T between the two components.

Finally some remarks on the fine structure. There seems to be no difficulty in explaining quite large intensity fluctuations within the framework of these ideas. There will be temperature fluctuations in the jets and fluctuations in the opacity of the outer regions of the envelope; these fluctuations will appear rapid to the observer, since the velocity vectors v_j and v_e of the jet and envelope respectively (Fig. 1) have opposite sign, see also Figs A6 in ref. 4.

It had previously been suggested[3] that the secondary pulses and the fine structure could be interpreted as due to the rebound of the neutron star and/or its subsequent oscillations. This, however, would seem rather unlikely, as one would expect the primary catastrophic implosion, in view of its vast energy release, to outweigh by far the secondary effects, so that the γ rays from the latter would be expected to be relatively much less intense, by perhaps some orders of magnitude, and hence below the threshold of the existing detectors.

A more thorough check on these ideas will be possible when the structure curves of the individual bursts are available.

While the LeBlanc–Wilson model on which these ideas are based may be rather too specialised, it seems likely that any kind of outward-propagating shock wave in a star which is significantly rotationally flattened, would give rise to two hot spots on the surface and therefore a double burst.

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1 Klebesadel, R. W., Strong, I. B., and Olson, R. A., *Astrophys. J. Lett.*, **182**, L85 (1973).
2 Colgate, S. A., *Early γ rays from supernovae* in *Proc. Los Alamos Conference* (in the press).
3 Ramaty, R., and Cohen, J. M., Goddard Preprint X-660-73-326 (1973).
4 LeBlanc, J. M., and Wilson, J. R., *Astrophys. J.*, **161**, 541 (1970).
5 Strong, I. B., Klebesadel, R. W., and Olson, R. A., *Astrophys. J. Lett.*, **188**, L1 (1974).
6 Cline, T. L., Desai, U. D., Klebesadel, R. W., and Strong, I. B., *Astrophys. J. Lett.*, **185**, L1 (1973).

Fifteenth-order harmonics in the geopotential

THE Earth's gravitational potential is usually expressed as a double infinite series of spherical harmonics. Analysis of thousands of photographic and laser observations of satellites, together with gravity measurements has yielded global models of the geoid[1-3] accurate to about 3 m on average. The harmonic coefficients have been evaluated complete up to degree and order 16 in the Goddard Earth Models (GEM) 4 and 6 (refs 1 and 2), and up to degree and order 18 in the Smithsonian Standard Earth III(SSE)[3]. Models currently being evaluated will extend to degree 25 (D. E. Smith, personal communication). Although these comprehensive global solutions give the overall geoid shape or gravitational field quite accurately, the individual harmonic coefficients of order between 10 and 20 are poorly determined among the matrix of 300 or more coefficients, and accurate methods for evaluating coefficients of a particular order are badly needed.

One of the best methods is to analyse the changes in the orbital elements of Earth satellites which pass slowly through a resonance with the Earth's gravitational field, when the ground track repeats after an integral number of revolutions. The 15th-order resonance, when the successive equator crossings are 24° apart and the track is repeated after 15 revolutions, is particularly useful because 15th-order resonant satellites circulate at heights near 500 km, where air drag is strong enough to ensure that the orbit contracts substantially in the course of a year. Consequently, each year many satellites experience 15th-order resonance as their orbits contract, and some of them pass through the resonance quite slowly, so that the orbital perturbations which result from 15th-order harmonics will build up[4,5]. These perturbations can be analysed to determine 15th-order coefficients in the geopotential.

The longitude-dependent part of the Earth's gravitational potential at an exterior point (r, θ, λ) may be written in normalised form[6] as

$$(\mu/r) \sum_{l=2}^{\infty} \sum_{m=1}^{l} (R/r)^l P_l^m (\cos\theta) \cdot [\bar{C}_{lm} \cos m\lambda + \bar{S}_{lm} \sin m\lambda] N_{lm}$$

where r is distance from the Earth's centre, θ is colatitude, λ is longitude (positive to the east), μ is the gravitational constant for the Earth (398,601 km^3 s^{-2}), R is the Earth's equatorial radius (6,378.1 km), $P_l^m(\cos\theta)$ is the associated Legendre function of order m and degree l, and $\bar{C}_{lm}$ and $\bar{S}_{lm}$ are the normalised tesseral harmonic coefficients which is to be evaluated (for $m = 15$). The normalising factor N_{lm} is given by[6]

$$N_{lm}^2 = 2(2l + 1)\,(l - m)!/(l + m)!$$

For nearly circular orbits passing through 15th-order resonance, the rate of change of orbital inclination, i, is proportional to[5] $(\bar{C}_{15} \sin\Phi - \bar{S}_{15} \cos\Phi)$, where $\bar{C}_{15}$ and $\bar{S}_{15}$ are 'lumped' 15th-order coefficients[7] and Φ is the 'resonance angle' (written as Φ_{15} in ref. 7), which becomes stationary at resonance. A computer program, THROE, has been developed[7] to fit the observed variation of i with a theoretical curve which includes not only the terms in sin Φ and cos Φ, but also terms in sin 2Φ, sin 3Φ, and so on, and terms involving the eccentricity, e, if these are appreciable.

The values $\bar{C}_{15}$ and $\bar{S}_{15}$ found from a particular orbit can be expressed as a linear sum of individual coefficients $\bar{C}_{l,15}$ and $\bar{S}_{l,15}$ of odd degree ($l = 15, 17, 19 \ldots$). To determine these individual coefficients, values of C_{15} and S_{15} need to be found for orbits at a wide variety of inclinations. The first pair of values, determined by Gooding[7] were from analysis of Ariel 3 (1967-42A) at inclination 80°. In 1972 King-Hele utilised the orbits of three further resonant satellites[8] at inclinations of 51°, 62° and 74°, to evaluate the coefficients of order 15 and degree 15, 17, 19 and 21. We have now been able to analyse the orbits of another eight resonant satellites, at inclinations of 31°, 33°, 38°, 51°, 54°, 56°, 74° and 90°. From these and the previous orbits we have obtained values[9] for the 15th-order coefficients of degree 15, 17, 19 . . . 31.

Figure 1 shows the fitting for one of the 11 satellites, Cosmos 395 rocket (1971-13B), which is in a circular orbit at 74° inclination: this satellite is fairly typical, both in the accuracy of the fitting and in the magnitude of the effect. The main resonance oscillation has an amplitude of about 0.015° and the theoretical curve fits well[10], giving values of $\bar{C}_{15}$ and $\bar{S}_{15}$ accurate to 4%.

From the 11 satellites we have derived 11 pairs of linear equations between the coefficients $\bar{C}_{l,15}$ and $\bar{S}_{l,15}$ ($l = 15, 17, 19 \ldots$). To these equations we added a pair derived by Wagner and Klosko[11], and nine pairs of equations specifying constraints on the values of the coefficients given by Kaula's rule-of-thumb[6] that the coefficient of degree l is likely to be of order $10^{-5}\, l^{-2}$. These 21 pairs of equations were then solved by least-squares, taking account of the correlations between $\bar{C}_{15}$ and $\bar{S}_{15}$, to determine nine pairs of values of the individual

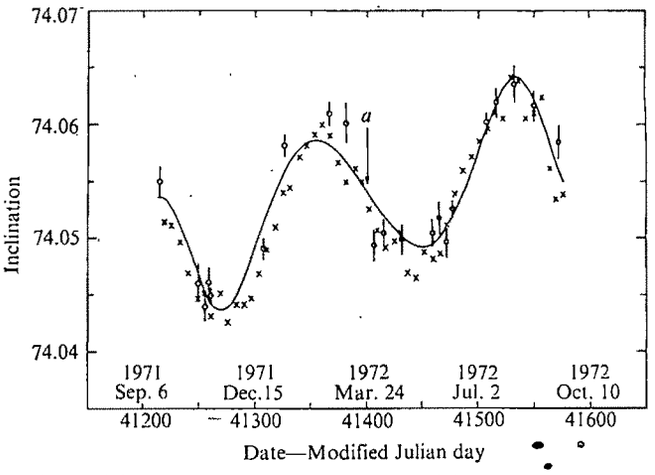


Fig. 1 Values of inclination at resonance of Cosmos 395 rocket (1971-13B), with fitted theoretical curve (—). Crosses, values from United States Navy orbits (s.d. taken as 0.003°); ○, values determined at the Royal Aircraft Establishment from Hewitt camera, visual and United States Navy observations (s.d. shown as error bars); *a*, 15th-order resonance on March 24, 1972.

coefficients of 15th-order, from degree 15 up to degree 31 (Table 1).

Table 1 shows that the numerical values of the C coefficients, of which seven out of nine exceed 10^{-8}, are much larger than the S coefficients, of which only one exceeds 10^{-8}. In a sense this bias is a result of chance, because the choice of reference longitude for defining C and S (the Greenwich meridian) is arbitrary. The effect may, however, be geophysically significant, if the 15th-order S coefficients are small for all degrees.

Table 1 Values of $\bar{C}_{l,15}$ and $\bar{S}_{l,15}$

l	$10^9\bar{C}_{l,15}$	$10^9\bar{S}_{l,15}$
15	−21.5±0.9	−8.4±0.9
17	4.4±1.6	9.0±1.5
19	−15.6±2.6	−14.1±2.7
21	10.4±3.0	7.3±3.5
23	22.5±2.8	1.2±4.4
25	−0.9±4.7	−3.8±5.3
27	−11.2±3.3	9.1±3.2
29	−20.5±5.4	−1.2±6.1
31	17.7±6.6	−1.0±7.1

The last two S values (Table 1) are not significant, and their omission would reduce the standard deviation (s.d.) for the other values. They have to be included because of their correlations with the C values, but it is probable that the real errors in the S coefficients up to degree 27 are smaller than the given s.d.

Another feature of interest (Table 1) is that the C values, though larger than the S values, are still mostly less than the values indicated by the rule-of-thumb $10^{-5}\, l^{-2}$. Thus, the constraint equations used turn out to be quite weak, and the values of the individual coefficients are determined primarily by the 11 pairs of equations from the orbital analysis.

Finally, the rather large values of $\bar{C}_{29,15}$ and $\bar{C}_{31,15}$ indicate that the evaluation ought to be carried to a higher degree. We hope to do this after observation of further satellites passing through 15th-order resonance and analysis of their orbits. There is a particular need for results at orbital inclinations between 40° and 50°, and between 64° and 73°.

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[1] Lerch, F. J., Wagner, C. A., Putney, B. H., Sandson, M. L., Brownd, J. E., Richardson, J. A., and Taylor, W. A., *Goddard*

Space Flight Center Report X-592-72-476 (1972).
[2] Lerch, F. J., *et al.*, Goddard Earth Model 6 (in the press).
[3] Gaposchkin, E. M., Williamson, M. R., Kozai, Y., and Mendes, G., *Smithsonian Astrophysical Observatory Special Report*, 353 (1973).
[4] Allan, R. R., *Planet. Space Sci.*, **15**, 53–76 and 1829–1845 (1967).
[5] Allan, R. R., *Planet. Space Sci.*, **21**, 205–225 (1973).
[6] Kaula, W. M., *Theory of satellite geodesy*, pp. 7, 98 (Blaisdell, Waltham, Massachusetts, 1966).
[7] Gooding, R. H., *Nature phys. Sci.*, **231**, 168–9 (1971); *Royal Aircraft Establishment Technical Report*, 71068 (1971).
[8] King-Hele, D. G., *Nature phys. Sci.*, **238**, 13 (1972); *Space Research* XIII, 21–29 (Akademie Verlag, Berlin, 1973).
[9] King-Hele, D. G., Walker, D. M. C., and Gooding, R. H., *Royal Aircraft Establishment Technical Report*, 74026 (1974).
[10] King-Hele, D. G., and Winterbottom, A. N., *Planet. Space Sci.*, **22** (in the press); *Royal Aircraft Establishment Technical Report*, 73170 (1973).
[11] Wagner, C. A., and Klosko, S. M., *Goddard Space Flight Center Report*, X-592-73-279 (1973).

High resolution α spectrometry

DEFICIENCIES in energy resolution have been an obstacle to the widespread application of dielectric track detectors[1]. The best resolution so far obtained covered a span of only 150 to 200 keV (ref. 2). Now a procedure has been developed which uses the range differences obtained with α particles of diverse emission energies. This procedure provides a substantially improved energy resolution.

The pits, which are etched on to the front of dielectric track detectors, are a result of primary ionisation, and the diameter of each pit therefore provides a measure of energy. Unfortunately, however, the properties of the material used and the etching conditions also influence the diameter of the pits. At the onset of etching, particles have an entrance energy that is higher than the threshold energy. The onset is not very well defined because, near the threshold, the rate of track etching, V_t, differs only slightly from the rate of volume etching, V_b, of the intact material. This explains the low growth of etch pits in the initial phase. If, however, detector sheets are etched from the rear the onset of etching is sharply defined because, as the particle trajectory is approached, the density of radiation damage

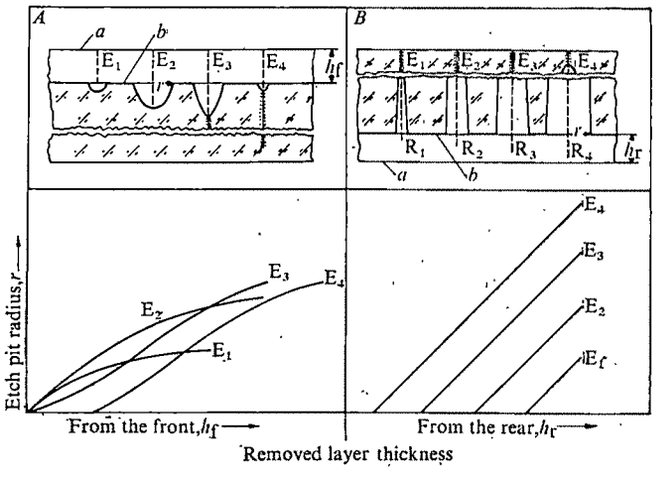


Fig. 1 Two methods of energy determination. The track evolution is shown as a function of the thickness of the removed surface: *A*, etching the frontside of a medium sensitive detector such as cellulose acetate leads to a dependence of the etch pit growth from the particle energy used for energy determination; *B*, etching the rear of a very sensitive detector perpendicularly irradiated with α particles of different energies results in a pit radius kinetic with a slope which is independent of the particle energy, in which the differences of the etch pit radii are equivalent to the range differences of the α particles. *a*, original surface; *b*, etched surface.

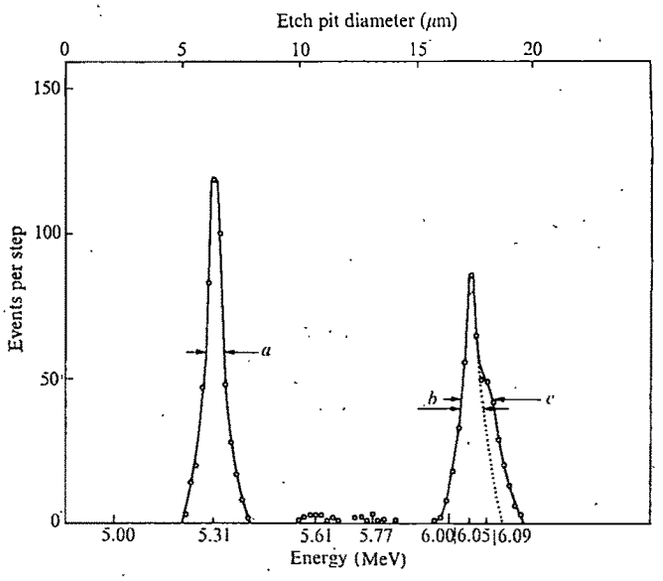


Fig. 2 Distribution of the etch pit diameters measured on the rear of a highly sensitive cellulose nitrate detector irradiated with α particles of ^{210}Po (5.31 MeV) and of ThC (5.61, 5.77, 6.05 and 6.09 MeV). *a*, 54 keV; *b*, 46 keV; *c*, 71 keV.

reaches a maximum. The radius of the etch pit produced then increases proportionally to the thickness of the surface removed from the rear (Fig. 1).

A necessary precondition of this method is that the α particles come to rest in the detector sheet. For maximum resolution, the particles must enter the detector normal to its surface, a condition which can be easily realised. If irradiation occurs under 2π geometry, only round etch pits with an accurate concentric configuration are evaluated. To obtain maximum energy resolution I have used very sensitive cellulose nitrate sheets[3]. In this detector even α particles create a very high track etch rate, so that immediately after the formation of an etch pit, the radius, r, will increase at the same rate as the volume etch rate. Up to a diameter of 25 μm no deviation from this relationship has been observed. Thus, we obtain the optimum condition $\Delta r = \Delta R$ that is, the difference in diameter of the etched tracks corresponds with twice the range difference, ΔR, of the α particles. With one-sided etching of the rear of the detector the particle range corresponds to the sum of the residual thickness and the etch pit radius. If the range – energy characteristic of the particles in the detector material is known, the energy of the particles can be determined.

To demonstrate the high degree of energy resolution of the new method a detector sheet of 38 μm was irradiated with α particles from a ^{210}Po source (5.31 MeV) and a ThC source (5.61 MeV), (1.1%), 5.77 MeV (1.8%), 6.05 MeV (68%) and 6.09 MeV (27%) at a pressure of 5×10^{-2} torr, with a distance of 50 mm between source and detector. The irradiated surface had a diameter of 1.5 mm. The rear of the sheet was then etched, using 30% KOH at 60° C until the thickness was reduced to 25.7 μm. All the emission energies from 5.31 to 6.09 MeV were thus obtained.

The determination of energy was based on the microscopic measurements, at each peak, of the diameters of 500 etch pits. Figure 2 shows the diameter distribution. The step width of the diameter determination was 0.25 μm. The full width at half maximum (FWHM) of the ^{210}Po peak was 54 keV, and the FWHM of the asymmetric peak of ThC was 71 keV. The peaks were at the 6.05 and 6.09 MeV emission energies. The FWHM of the 0.65 major peak was 46 keV. At lower emission energies, a drop in resolution caused by the increase in specific energy loss can be expected.

Using this method the energy resolution is influenced by the specific energy loss, the range straggling, the evenness of the sheet thickness, the etching process, and the microscopic

measurement. Specific energy loss and range straggling depend on the energy of the particle. The evenness of the sheet thickness has to be better than ± 0.2 μm. Under the conditions described here the contribution of the etching process and the microscopic measurement amounts to 40 keV for the ^{210}Po peak and 33 keV for the ThC major peak.

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[1] Fleischer, R. L., Alter, H. W., Furmann, S. C., Price, P. B., and Walker, R. H., *Science*, **178**, 255 (1972).
[2] Somogyi, G., Schlenk, B., Varnagy, M., Mesko, L., and Valek, A., *Nucl. Instrum. Meth.*, **63**, 189 (1968).
[3] Lück, H. B., *Nucl. Instrum. Meth.* (in the press).

Eocene echinoids and the Drake Passage

AUSTRALIA and Antarctica separated about 50 million years (Myr) ago[1]. Echinoid populations indicate a warmer climate at the time of separation than later. A model of oceanic circulation in the Southern Hemisphere, consistent with this observation, leads to a more precise dating of the opening of the Drake Passage between South America and Antarctica.

The oldest echinoid fauna known from the opening seaway between Australia and Antarctica has been preserved at several localities between Albany and Adelaide. The fauna is most abundant and best known in the Late Eocene Tortachilla Limestone, which outcrops along the eastern flank of the St Vincent Basin, south of Adelaide. Eighteen species of echinoids have been described from this formation of small lateral extent and a further five have yet to be described. The marsupiate echinoids, which are intermittently common in the Tertiary of southern Australia, are notably absent. Echinoids are also abundant a little further up the section at a Late Eocene horizon, low in the Port Willunga Beds. Less than 10 species are known at this level, and almost all are of genera different from those in the earlier Tortachilla. They include the earliest of the Australian marsupiate echinoids, the temnopleurid *Paradoxechinus stellatis* Philip and Foster.

A similar situation exists on the opposite flank of the basin. There is a complete change in the echinoid fauna between the Muloowurtie Formation and the overlying Rogue Formation in which *P. stellatis* appears.

By analogy with recent Antarctic brood-protecting echinoids, it has been inferred that the marsupium, developed in the female of several species of Australian Tertiary Temnopleuridae, Clypeasteroida and Spatangoida, confers an advantage in colder water[2]. An unsatisfactory aspect of correlating the presence of marsupiate echinoids with cold water is that the earliest fauna of the discussed region contains no marsupiate echinoids—despite the fact that this fauna probably lived more than 20° further south than its present position. Marsupiate echinoids are, in fact, a conspicuous component of the fauna during the journey of Australia towards the tropics.

Frakes and Kemp[3] proposed a model of early Tertiary climates which may provide the answer. In the Eocene there was no circumpolar cold current in the Southern Ocean because of the presence of a continuous Andean–West Antarctic mountain chain. As Australia was far to the south of South-east Asia, ample room existed for the southern equatorial current of the Pacific to continue into the Indian Ocean, sweep south and return, at least in part, through the new seaway which separated Australia from Antarctica. Because of the long time spent travelling through about 200° of longitude in the tropics, the equatorial current, which left the coast of South America at about 19° C. began its southerly sweep into the Indian Ocean at about 37° C. It was probably still above 20° C as it passed along the southern coast of Australia. A warm water echinoid fauna can therefore be expected. By the mid-Oligocene, the northward drift of Australia had restricted the flow of warm equatorial Pacific water into the Indian Ocean. More important, the breaching of the Andean–West Antarctic mountain barrier allowed a circumpolar cold current to replace the warm current in the widening seaway between Australia and Antarctica. The water temperature probably fell to well below 10° C. A changed echinoid fauna, including brood-protecting species, is consistent with this model.

Dalziel and Elliot[4] estimate the opening of Drake Passage, and thus the beginning of the circumpolar current, to have occurred between the latest Cretaceous (65 Myr) and about 25 Myr ago.

The Tortachilla Limestone was deposited at the beginning of the Late Eocene, probably high in P15 in the planktonic foraminiferal zonation[5]. The Port Willunga Beds have been sampled and dated[6] especially to provide a time framework for the sequence of echinoids, and the horizon containing *P. stellatis* has been placed high in the Late Eocene, corresponding to P17.

Using ages attributed to the planktonic foraminiferal datum planes[5] as a guide, the event which caused the replacement of a warm water by a cold water echinoid fauna in the seaway between Australia and Antarctica can be dated at about 40 Myr—probably 41–36 Myr. This event was probably the inception of the mighty Antarctic circumpolar current, which began with the breaching of the Andean–West Antarctic Cordillera and the concomitant creation of the Drake Passage. Thus, there is indirect evidence which enables the separation of South America from Antarctica to be more precisely dated than before.

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[1] Weissel, J. K., and Hayes, D. E., *Nature*, **231**, 518–522 (1971).
[2] Philip, G. M., and Foster, R. J., *Palaeontology*, **14**, 666–695 (1971).
[3] Frakes, L. A., and Kemp, E. M., *Nature*, **240**, 97–100 (1972).
[4] Dalziel, I. W. D., and Elliot, D. H., *Nature*, **233**, 246–252 (1971).
[5] Berggren, W. A., *Nature*, **224**, 1072–1075 (1969).
[6] Lindsay, J. M., *Q. geol. Not. Geol. Surv. S. Aust.*, **36**, 4–10 (1970).

Closing of the Protocadic Ocean and intraplate basins

THE presence of several long-lived, deeply subsiding basins, autogeosynclines[1], such as those in Michigan, Illinois, North Kansas and Williston suggests that the North American craton or plate remained in a constant position relative to the underlying mantle during the Palaeozoic.

In the Michigan Basin the Precambrian basement surface subsided about 4,000 m between the late Cambrian and the Devonian[1–6]. Throughout this time, there was little deposition of terrigenous sediment and subsidence was largely independent of loading. With a few exceptions, the isopachs of many of the series and stages within the basin are centred to the west and north of Saginaw Bay, a little to the north-east of the centre of the structural basin[7]. However, during the mid-Ordo-

vician there was more subsidence in the south-east[3], than elsewhere and during the early Silurian, the subsidence was suppressed, and carbonate reef belts extended along the northern and southern margins of the basin[8,9]. The rates of subsidence, relative to periods of deformation in the Taconian–Acadian orogenic belt to the east, are not known. Other intraplate basins subsided differentially over long periods of time, but not to such great depths.

The subsidence of the Michigan Basin caused, or resulted in, the displacement of a great volume of crustal material. It is not known whether the Moho was depressed or even if it is still deeper than in surrounding areas. The origin of domes[10] can be attributed to the expansion of crustal rocks above rising convection currents which heat the base of the crust. The formation of downward expanding convection cells is perplexing, but something like that occurred. The fact that the centre of subsidence was more or less fixed geographically seems to indicate that the crust did not move relative to the mantle under the North American plate. Therefore, the deep subsidence must have had a subcrustal cause.

It is believed that the ocean on the eastern margin of the North American craton resulted from the separation of the European and North American plates. It is usually called the 'proto-Atlantic' ocean[11], but I prefer to call it the Protacadic Ocean in reference to its closing during the Taconian–Caledonian–Acadian orogenies[13,17]. It is not the progenitor of the present Atlantic, and the term 'proto-Atlantic' has been applied more logically to the opening of the present South Atlantic Ocean during the early Mesozoic. The Dunnage Melange in north-eastern Newfoundland formed in a subduction zone in which the oceanic plate passed beneath the North American plate to the north-west[12-14]. Island-arc volcanics are represented in the Summerford Group to the north[15]. South of the Dunnage fault-bounded block, the Campbellton sequence of mafic volcanic rocks, manganiferous chert and greywacke flysch may represent ocean floor[16,17] and the better displayed Snooks Arm Group farther west has been attributed to oceanic crust[18,19].

During the early Palaeozoic the European plate does not seem to have had any deep autogeosynclines like those in North America[20]. The total subsidence of a trough through the middle of the European platform is only a tenth of that which occurred in the Michigan Basin over a similar time and there is no specific centre of sinking in this much larger area.

It is suggested that the autogeosynclines in the North American plate were linked to the underlying mantle, as is the case with modern plumes[21]. Both the plate and the mantle retained a fixed geographical position for about 100 Myr during the early Palaeozoic. The European plate moved over the mantle toward the North American plate, closing the Protacadic Ocean. The North American palaeomagnetic pole position for the Ordovician is very close to the Upper Palaeozoic position, which supports this hypothesis; however, it moved to lower latitudes during the Silurian, if the records are correctly interpreted[22].

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Received February 25, 1974.

[1] Kay, M., *Mem. geol. Soc. am.*, **48**, (1951), with earlier references.
[2] Cohee, G. V., *J. Wash. Acad. Sci.*, **55**, 211–223 (1965).
[3] Sanford, B. V., *Geol. Surv. Pap. Can.*, **60**–26 (1961).
[4] Sandford, B. V., *Int. Symp. Devonian System*, **1**, 973–999 (1967).
[5] Fisher, J. H., *Michigan Basin Geological Society Guidebook*, 89–93 (1969).
[6] Alling, H. L., and Briggs, L. I., *Bull. Am. Ass. Petrol. Geol.*, **45**, 515–547 (1961).
[7] Catacosinos, P. A., *Bull. Am. Ass. Petrol. Geol.* **57**, 2404–2424 (1973).
[8] Mesolella, K. J., Robinson, J. D., McCormick, L. M., and Ormiston, A. R., *Bull. Am. Ass. Petrol. Geol.*, **58**, 34–62 (1974).
[9] Lowenstam, H. A., *Illinois geol. Surv. Rept. Invest.* **145** (1949).
[10] Burke, K., and Dewey, J. F., *J. Geol.*, **81**, 406–433 (1973).
[11] Wilson, J. T., *Nature*, **211**, 676 (1966).
[12] Horne, G. S., *Bull. geol. Soc. Am.*, **80**, 2451–2464 (1969).
[13] Kay, M., *Bull. geol. Soc. Am.*, **83**, 13–15, (1972).
[14] Dewey, J. F., *Nature*, **222**, 124 (1969).
[15] Horne, G. S., *Bull. geol. Soc. Am.*, **81**, 1767–1788 (1970).
[16] Kay, M., *Rept. 24th Int. geol. Congr., Montreal*, **3**, 122–133 (1972).
[17] Kay, M., in *Gravity and Tectonics*, 313–326 (Wiley-Interscience, New York, 1973).
[18] Upadhyay, H. D., Dewey, J. F., and Neale, E. R. W., *Proc. geol. Ass. Can.*, **24**, 27–34 (1971).
[19] Dewey, J. F., and Bird, J. M., *J. geophys. Res.*, **76**, 3179–3206 (1971).
[20] *Atlas of lithopaleogeographical maps of the Russian Platform and its geosynclinal framing Part* 1 (edit. by Nalivkin, V. D.), (Moscow-Leningrad, 1960).
[21] Shaw, M. R., *Bull. geol. Soc. Am.*, **84**, 1505–1526 (1973).
[22] McElhinny, M. W., and Opdyke, N. D., *Bull. geol. Soc. Am.*, **84**, 3697–3708 (1973).

Seismic refraction experiment on the Mid-Atlantic Ridge in the FAMOUS area

WE report results from ocean bottom seismographs (OBS)[1] and sonobuoys, in which unreversed refraction lines have been shot along and across the median valley of the Mid-Atlantic Ridge. P-wave arrivals indicate a structure under the median valley with layer 2 overlying 7.3 km s^{-1} mantle. Under the mountains on the ridge crest P and S arrivals suggest that layer 2 overlies layer 3, which in turn overlies 7.3 km s^{-1} mantle.

There are still relatively few determinations of the seismic velocity structure under the Mid-Atlantic Ridge[2-4] and only one has been reported[5] shot within the confines of the median valley. On geological grounds, Cann[6] has proposed the existence of a magma chamber situated at the top of a rising column of the asthenosphere at a depth of 3–4 km beneath the median valley. Francis and Porter[1] suggested that the postulated magma chamber could account for seismological results at 45° N. Seismologists[7-9] working with teleseismic data from earthquakes near the Ridge axis have reported a sharply bounded zone of low Q in the upper mantle beneath the axis, a 'gap in the lithosphere' across which S_n is not propagated.

A straight, but narrow section of the median valley bounded by fracture zones was chosen for this work and three OBS were laid, 10 km apart. Two were in the median valley and one on the mountains on the ridge crest to the east (Fig. 1). The tape recorder in OBS1 jammed, so no records were obtained from it. Three lines of near-surface shots were fired: A and B across the valley, perpendicular to the structure, and C along it. Line A (16 shots) was 37 km long, line B (16 shots) 36 km and line C (14 shots) 30 km. It was anticipated that the structure beneath the median valley would change near the fracture zones, so care was taken not to extend line C as far as them. For line C two recording sonobuoys were laid one mile apart straddling the position of OBS1, and for lines A and B two were laid, also one mile apart, over OBS3. The buoys drifted less than one mile from the laying position and during the shots along line C they stayed over the median valley the whole time.

Topographic correction is clearly necessary although a true scale section along lines A and B shows that the structure is not impossibly rugged. Arrivals have been corrected for a mean depth of 2.64 km (1,445 fathoms), assuming that the rock layering follows the sea floor. The times at the OBS and the sonobuoys have been corrected to the sea surface.

The results obtained by fitting least-squares straight lines to the first arrivals on the time distance diagrams are shown in Table 1. The weighted means of the apparent velocities are 4.91 $\pm$ 0.12 (layer 2), 6.64 $\pm$ 0.08 (layer 3) and 7.26 $\pm$ 0.06 ('mantle').

The data for both lines A and B from OBS3 and the sonobuoys fit together well when plotted on one time–distance

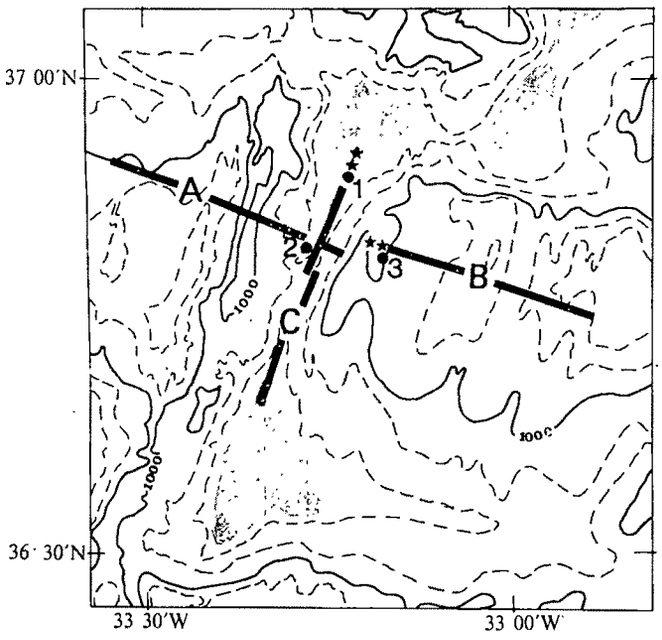


Fig. 1 Bathymetric chart showing the median valley, from an unpublished chart by J. D. Phillips, Woods Hole Oceanographic Institute. Contour interval 200 fathoms. Shaded areas are deeper than 1,500 fathoms. Dots, positions of OBSI, 2 and 3, stars, approximate positions of sonobuoys; Lines A, B and C, approximate track of ship during the firing of surface charges.

diagram, and the velocities and intercepts for the combined data for each line are within the limits of error of those obtained for the receivers treated separately. It is surprising that a similar statement applies to the sonobuoys and OBS2 for line C, considering that the receivers were 10 km apart. This suggests that the gross structure is more level, planar and homogeneous than may have been expected. No more than two refractors were detected on any line and the velocity and intercept of the upper one is not well determined and may be variable. Paths which cross the median valley, line A into OBS3, line B into OBS2, and line C, all detect a velocity of 7.3 km s^{-1} as the deepest refractor; paths which do not cross the median valley, line A into OBS2, and line B into OBS3, detect 6.6 km s^{-1} as the deepest refractor. The structure under the median valley is shown in Table 2*a*; it corresponds very well with the axial structure suggested by Le Pichon *et al.*[2]. The fact that the layer 3 velocity, 6.6 km s^{-1}, was detected under the flanks of the Ridge, should be treated with caution because the lines were not reversed and lateral velocity changes are to be expected. The detection is, however, consistent with the work of Keen and Tramontini[4] who found evidence of the presence of layer 3 within 20 km of the axis at 45° N.

Table 1 P-wave arrivals

Line	Receiver	Velocity* (km s^{-1})	Intercept* (s)	*N*
C	Sonobuoys and OBS2	5.17 ± 0.51	3.72 ± 0.13	11
		7.19 ± 0.09	4.37 ± 0.03	24
C	Sonobuoys	4.21 ± 0.46	3.35 ± 0.21	5
		7.34 ± 0.09	4.45 ± 0.04	19
A	Sonobuoys and OBS3	5.09 ± 0.39	3.60 ± 0.12	12
		7.33 ± 0.17	4.45 ± 0.05	31
B	OBS2	7.42 ± 0.21	4.68 ± 0.10	12
A	OBS2	4.41 ± 1.13	3.50 ± 0.38	6
		6.75 ± 0.26	4.12 ± 0.11	6
B	Sonobuoys and OBS3	5.02 ± 0.36	3.59 ± 0.10	12
		6.57 ± 0.17	3.99 ± 0.07	24

N is the number of points to which the line was fitted. Only the hydrophone traces from the OBS were used.
* Intercepts and velocities are quoted with their standard errors.

Table 2 Structures

	Line	Velocity (km s^{-1})	Thickness (km)
a	C	1.5	2.64
	Sonobuoy and OBS2	[2.8]	0.58
		5.17	2.05
		7.19	
b	A	1.5	2.64
	OBS2	[2.8]	0.35
		4.41	1.34
		6.75	
c	B	1.5	2.64
	Sonobuoy and OBS 3	[2.8]	0.39
		5.02	1.20
		6.57	

Brackets enclose assumed velocities[5].

Whitmarsh concludes, from shots at almost the same location as line C, that layer 3 is present at the ridge axis under the median valley[5]. This may be because the direct water wave arrival on his records has obscured the crossover between the 5.2 km s^{-1} and 7.2 km s^{-1} lines, causing him to fit just one line to these two sets of points. If one line is fitted to the 35 points on our time–distance diagram we obtain a velocity of 6.5 ± 0.12 km s^{-1} and an intercept time of 4.06 ± 0.04 s, which is similar to Whitmarsh's result.

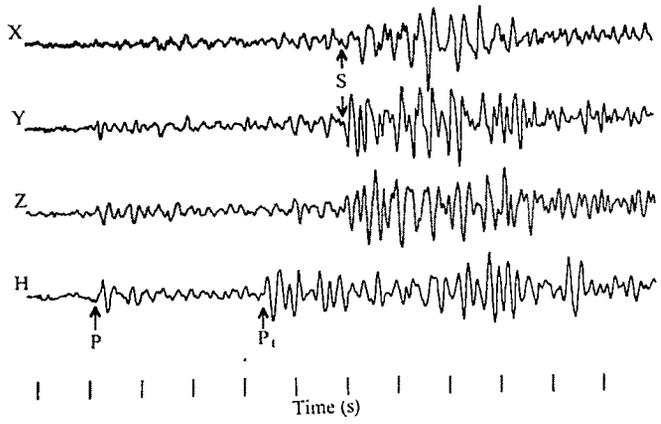


Fig. 2 Record from OBS2 of a shot on line B fired at a range of 36 km.

Table 3 Shear-wave arrivals

Line	Receiver	Velocity* (km s^{-1})	Intercept* (s)	*N*
C	OBS2	3.32 ± 0.63	4.16 ± 0.78	5
A	OBS3	3.96 ± 0.19	5.03 ± 0.31	8
B	PBS2	4.08 ± 0.13	5.49 ± 0.22	9
A	OBS2	4.03 ± 0.12	5.03 ± 0.13	7
B	OBS3	3.82 ± 0.64	5.11 ± 0.92	6

N is the number of points to which the line was fitted.
* Intercepts and velocities are quoted with their standard errors.

A typical record of S waves is shown in Fig. 2. Arrivals are visible on both OBS2 and OBS3 for shots fired at ranges greater than 10 km. The results of fitting least-squares lines to the data are shown in Table 3. It can be seen that there are no significant differences between them. The weighted mean velocity is 4.02 ± 0.05 km s^{-1}. Model calculations using the structures suggested by the P-wave arrivals and assuming a V_p/V_s ratio of $\sqrt{3}$ indicate that the arrivals are consistent with waves transformed to a shear wave at the seafloor and propagated as a head wave in the 7.3 km s^{-1} layer. There is no evidence for a lower velocity on lines A into OBS2 and B into OBS3, on which the highest P-wave velocity was 6.7 km s^{-1}, which could

suggest that the 7.3 km s^{-1} layer underlies layer 3 there. Nor is there any evidence for a velocity of about 2.8 km s^{-1}, corresponding to shear waves propagating in layer 2.

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[1] Francis, T. J. G., and Porter, I. T., *Geophys. Jl R. astr. Soc.*, **34**, 279–311 (1973).
[2] Le Pichon, X., Houtz, R. E., Drake, C. L., and Nafe, J. E., *J. geophys. Res.*, **70**, 319–339 (1965).
[3] Talwani, M., Windisch, C. D., and Langseth, M. G., *J. geophys. Res.*, **76**, 473–517 (1971).
[4] Keen, C., and Tramontini, C., *Geophys. Jl R. astr. Soc.*, **20**, 473–491 (1970).
[5] Whitmarsh, R. B., *Nature*, **246**, 297–299 (1973).
[6] Cann, J. R., *Nature*, **226**, 928–930 (1970).
[7] Molnar, P., and Oliver, J., *J. geophys. Res.*, **74**, 2648–2682 (1969).
[8] Francis, T. J. G., *Geophys. Jl R. astr. Soc.*, **17**, 507–520 (1969).
[9] Solomon, S. C., *J. geophys. Res.*, **78**, 6044–6059 (1973).

Clays and formation of deep-sea chert

CHERT in the Pacific, Atlantic, Indian and Southern Oceans occurs both as nodules and as layers or lenses[1]. Associated sediments include carbonate, biogenous silica, brown clay and volcanic ash. Chert is usually found in Middle Eocene or older sediments; the actual time of chert formation is, however, not known.

Biogenous opal is the principal source of silica for the formation of chert[1,2]. Silica may also be produced by the alteration of volcanic detritus, resulting in the mineral assemblage smectite, zeolite and opal[3,4]. A third possible source, not previously suggested in the literature, is the submarine diagenesis of clay minerals. Alteration of a smectite to hydrous mica or chlorite would release silica. The formation of secondary chert by the subaerial transformation of a ferric-rich smectite to kaolinite has been described by Altschuler *et al.*[5], and several studies have shown that hydrous mica (illite) and chlorite become dominant at the expense of other clay minerals with increasing age[6].

A 9-meter piston core (Scan 16P) taken from a depth of 5,597 m, 16° 25′N 164° 24′W, containing a stiff, homogenous, brown zeolitic clay with a porcellanite layer at 436 cm, has been studied. The age of the sediment was determined from radiolaria to be Middle Eocene. Seismic profiles from the area show an upper, probably chert, reflector at 30–40 m.

The brown clay has a sharp contact with the porcellanite layer and was identified by X-ray diffraction as smectite with minor hydrous mica. Using the X-ray diffraction opal classification of Jones and Segnit[7], the radiolaria are opal-CT with quartz, and the porcellanite is opal-A and opal-CT. Optically, the quartz is chalcedony and forms internal moulds of radiolaria tests.

Scanning electron microscope examination revealed that the outer borders of the porcellanite layer have a botryoidal surface, composed of opal-A and radiating needles of opal-CT. Internally, the porcellanite layer is porous, massive opal-CT, with clay rosettes within the pores (Figs 1 and 2).



Fig. 1 Rosettes of hydrous mica filling the pores in porcellanite (opal–CT). SEM photomicrograph. Scale bar, 10 μm.

Using a transmission electron microscope, an electron diffraction pattern of a well crystallised clay particle was obtained. The *d* spacings of the clay were calculated from several diffraction patterns (4.46 (020,110), 3.30 (022,003), 2.59 (200,131), 2.32 (201,132), 1.97 (005), 1.48 (060,331) ± 0.05 Å) and closely matched those of hydrous mica[8,9].

Evidence which suggests that the mineral is a hydrous mica was also obtained with the electron microprobe. A traverse across a fracture surface of the porcellanite, measuring the presence and relative abundance of aluminium and potassium in the blue luminescent clay gave the results shown in Fig. 3, and the ratio of silicon to aluminium was measured as 1.2. No magnesium, and only a trace of iron, was detected in the clay. A sample of the smectite from the surrounding sediment was also analysed and had a substantially higher silicon to aluminium ratio of 3.4 and a magnesium to iron ratio of 0.8.

The dominant clay mineral in the surrounding sediment is a magnesium–iron smectite and in the porcellanite layer it is a hydrous mica. This distribution, in conjunction with the evidence discussed, suggests a relationship between

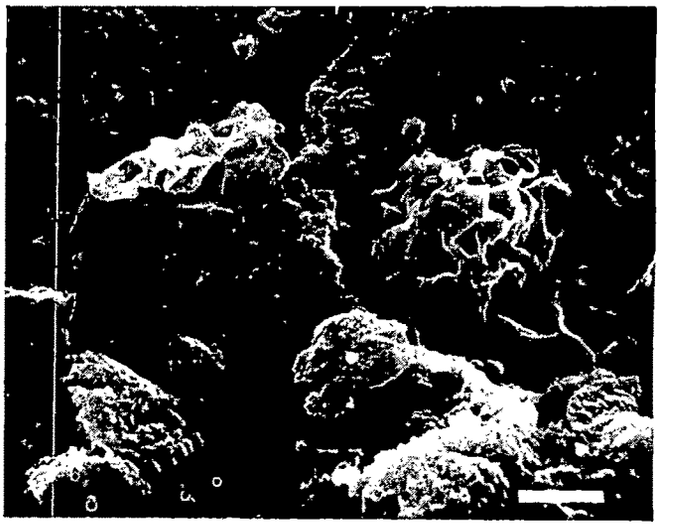


Fig. 2 SEM photomicrograph of porcellanite fracture surface and hydrous mica rosette. Scale bar, 2 μm.

the diagenetic reaction of smectite to hydrous mica and the formation of chert. The habit and distribution of the hydrous mica in the porcellanite layer strongly suggest that it is authigenic, and its formation from a smectite would release large quantities of silica. A release of silica is strongly indicated by the different silicon–aluminium ratio in the smectite and the authigenic hydrous mica. Aluminium is assumed to remain conserved during the reaction.

The actual amount of silica released depends on the chemical composition of the smectite and hydrous mica. For example, it can be calculated that the stoichiometric transformation of 10 g of montmorillonite could produce 6.4 g of hydrous mica and 3.3 g of quartz. These proportions do not, however, have to be represented within the same sedimentary layer. Assuming that all the silica released is precipitated, the amount of pelagic brown clay that would have to alter in the core can be calculated. The brown clay has an approximate porosity of 60%, and smectite composes 60% by weight of the solid phases, and the porcellanite layer has approximately 40% by volume opal-CT (density 2.4 g cm^{-3}). If the smectite has the ideal montmorillonite composition and it all alters, a sediment layer 0.4 cm thick could produce the silica for a 0.1 cm porcellanite layer. The important problems of why the porcellanite layer formed where it did and the mechanism and kinetics of the reaction are still being studied. Seawater is the most probable source of potassium. Based on a 60% porosity and on a conservative diffusion rate of 10^{-7} cm^2 s^{-1},

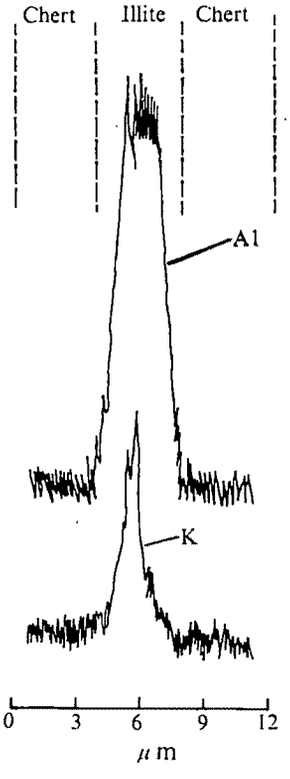


Fig. 3 Relative abundances of aluminium and potassium from an electron microprobe traverse across a fracture surface similar to Fig. 2.

all the potassium necessary to convert a montmorillonite layer of 0.4 cm into hydrous mica in the porcellanite layer, will be supplied in 20 yr. The high mobility of magnesium might account for the lack of a diagenetic phase rich in magnesium. Because of the low mobility of iron, the transformation of montmorillonite rich in iron to hydrous mica poor in iron should result in a higher concentration of iron oxide or hydroxide in the surrounding sediments. The studied core was, however, rich in iron oxide throughout, making it difficult to prove such an enrichment.

The proposed mechanism of chert formation could be further supported by measuring the $^{18}O/^{16}O$ ratios of the coexisting hydrous mica and opal-CT. In addition, a separation of the hydrous mica could be dated to give the actual time of formation of the porcellanite, assuming argon is retained in the structure.

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[1] Heath, G. R., and Moberly, R., in *Init. Repts Deep Sea Drilling Project*, **7**, 991 (edit. by Winterer, E. L., *et al.*), (1971).
[2] Bramlette, M. N., *US Geol. Surv. Prof. Pap.* 212, (1946).
[3] Gibson, T. G., and Towe, K. M., *Science*, **172**, 152 (1971).
[4] Matson, P. H., and Pessagno, E. A., *Science*, **174**, 138 (1971).
[5] Altschuler, Z. S., Dwornik, E. J., and Kramer, H., *Science*, **141**, 148 (1963).
[6] Weaver, C. E., in *Fundamental Aspects of Petroleum Geochemistry* (edit. by Nagy, B., and Colombo, U.), 37 (Elsevier, Amsterdam, 1967).
[7] Jones, J. B., and Segnit, E. R., *J. geol. Soc. Aust.*, **18**, 57 (1971).
[8] Grim, R. E., *Clay Mineralogy* (McGraw Hill), New York, (1968).
[9] Levinson, A. A., *Am. Miner.*, **40**, 41 (1955).

BIOLOGICAL SCIENCES

Terminal deoxynucleotidyltransferase in murine plasmocytomas

FOUR different DNA polymerases have so far been described in animal cells. Two of them are present in all types of cells[1-5]: the low molecular weight nuclear DNA polymerase, and the high molecular weight cytoplasmic DNA polymerase. The concentration of only the latter varies during the cell cycle, suggesting its involvement in DNA replication[6]. The two other DNA polymerases seem to exist only in certain cell types: the first, recently characterised[7], seems to be directed by RNA, although differing from the oncornavirus reverse transcriptase[8]; the second is terminal deoxynucleotidyltransferase which does not exhibit the usual properties of DNA polymerases since it catalyses the polymerisation of deoxyribonucleotides in the absence of template by adding them randomly to the 3'OH end of oligo- or polydeoxyribonucleotides[9,10]. This last enzyme is tissue-specific: it exists only on the thymus of the different species tested[11]. Recently the terminal transferase was also identified in blood lymphoblasts of a child suffering from acute lymphoblastic leukaemia[12].

In addition to the DNA polymerase activities already described in murine plasmocytoma cells[13], we have found in the course of our studies[14] that these tumours also contain terminal deoxynucleotidyltransferase.

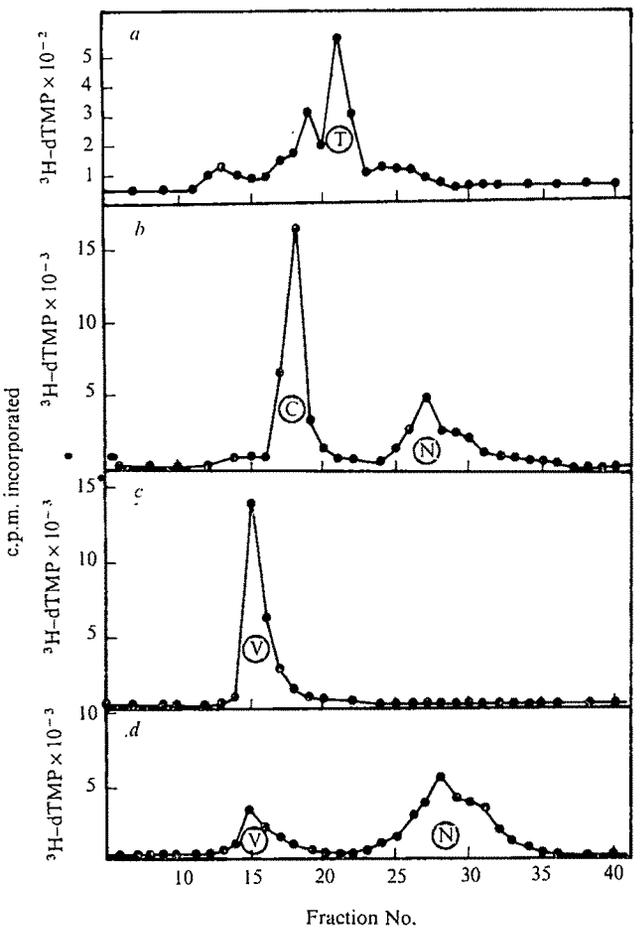


Fig. 1 Phosphocellulose chromatography of DNA polymerase activities from murine plasmocytoma MOPC 173 extracts. Subcutaneous tumours (2 g) were washed repeatedly in phosphate-buffered saline. After three cycles of freeze-thawing, they were homogenised by 10 strokes of a Teflon-glass motor-driven homogeniser in two volumes of TEM buffer (0.05 M Tris-HCl *p*H 7.9, 1 mM EDTA, 2.8 mM 2-mercaptoethanol). The homogenate was diluted in TEM buffer to 20 ml and Nonidet P40 was added (0.5% final). After slow mixing during 15 min at 0° C, the detergent-treated homogenate was centrifuged at 100,000*g* for 1 h. The *p*H of the supernatant was lowered to 5 by dropwise addition of 1 M acetic acid. The *p*H 5 precipitate was collected by centrifugation at 30,000*g* for 10 min, and redissolved in a minimal volume of TEMG buffer (TEM buffer containing 20% glycerol). The protein concentration of the *p*H 5 extract was adjusted to 2 mg ml^{-1} and 10 ml were applied to a P11 phosphocellulose column (10 × 1.2 cm). After a 40 ml wash by TEMG buffer containing 500 μg ml bovine serum albumin (TEMG-BSA), the adsorbed proteins were eluted by an 80 ml gradient of 0–1 M KCl in TEMG-BSA, at a flow rate of 10 ml h^{-1}. Fractions of 2 ml were collected, and assayed for DNA polymerase activities. DNA polymerase assays were performed with 25 μl of each fraction in a final volume of 100 μl for 1 h at 37° C. Depending on the template primer used, the incubation mixture contained: *a*, d(pT)$_4$-primed reaction: 5 μmol of Tris-HCl (*p*H 7.4), 0.2 μmol of dithiothreitol, 0.08 μmol of Mn^{2+} (chloride), 0.3 A_{260} unit of d(pT)$_4$ (Collaborative Research) and 250 pmol of ^{3}H-dTTP (2,000 c.p.m. pmol^{-1}). *b*, Activated DNA-primed reaction: 5 μmol of Tris-HCl (*p*H 7.9), 0.2 μmol of dithiothreitol, 1 μmol of Mg^{2+} (chloride), 0.2 A_{260} unit of activated calf thymus DNA[20] 5 nmol each of dATP, dGTP, dCTP and 200 pmol of ^{3}H-dTTP (4,000 c.p.m. pmol^{-1}). *c*, rA$_n$·dT$_{10}$-primed reaction : 5 μmol of Tris-HCl (*p*H 7.9), 0.2 μmol dithiothreitol, 0.1 μmol Mn^{2+}(chloride), 0.02 A_{260} unit of rA$_n$·dT$_{10}$ (Boehringer) and 1 nmol of ^{3}H-dTTP (500 c.p.m. pmol^{-1}). *d*, rA$_n$·dT$_n$-primed reaction: as *c*, with 0.025 unit of rA$_n$·dT$_n$ (Miles) in place of rA$_n$·dT$_{10}$. The acid-precipitable material was collected by filtration on Millipore filters and the radioactivity was counted in a Nuclear Chicago scintillation spectrometer. Each panel represents the results of the assays of the fractions of the same column, using one of the above template primers.

We used three different murine plasmocytomas: MOPC 173 and its two cell lines MF_2 and ME_2[15], TEPC 15[16] and MOPC 315[17]; as control cells we used normal BALB/c mouse thymi, spleens from BALB/c nude mice, and L cells. The enzyme was detected using a method similar to that of McCaffrey *et al.*[12]: the cell homogenate was treated by a nonionic detergent, centrifuged at 100,000*g* and the pH of the supernatant was lowered to 5. The precipitate was solubilised and fractionated by chromatography on a phosphocellulose column. The elution profile from MOPC 173 is presented in Fig. 1 and from TEPC 15 in Fig. 2; MOPC 315 gave similar results. These profiles were obtained by assaying the protein fractions eluted from the column in conditions which discriminate between the different DNA polymerases by their ability to use specific template primers (Table 1). Plasmocytoma cells produce viral particles (type A and type C), and contain reverse transcriptase.

Table 1 Primer specificity of DNA polymerases from MOPC 173 tumours

Template-primers	DNA polymerases	MOPC 173 tumour enzymes* V (0.28 M)	C (0.35 M)	N (0.55 M)	C-type particles
rA$_n$·dT$_n$ (^{3}H-dTTP)		5·75	1·6	6·7	52
rA$_n$·dT$_n$ (^{3}H-dATP)		0·06	0·70	0·02	0·00
rA$_n$·dT$_{10}$ (^{3}H-dTTP)		12·2	0·47	0·00	65
dA$_n$·dT$_{10}$ (^{3}H-dTTP)		0·02	1·42	7·20	0.00
rC$_n$·dG$_{10}$ (^{3}H-dGTP)		4·60	0·45	0·00	50

*Molarity of elution from phosphocellulose

The assays were performed under the conditions described as in Fig. 1, with 20 μl of each phosphocellulose fraction. Incubations were at 37° C for 30 min. Type C particles were purified from the supernatant of MOPC 173 cells in culture by sucrose gradients as described[14]; they were tested with 50 μg of virus proteins per assay. The 0·55 M phosphocellulose enzyme can use rA$_n$·dT$_{10}$ as template if the temperature is lowered to 30° C, but even under these conditions it cannot use rC$_n$·dG$_{10}$.

For all the cells tested, the polymerases, when present, were eluted from the column as follows: viral reverse transcriptase (0.28–0.3 M KCl: 'V' enzyme) high molecular weight C polymerase (0.35 M), terminal deoxynucleotidyltransferase (0.4 M: 'T' enzyme) and low molecular weight N polymerase (0.55 M.) The chromatogram shows some overlapping mostly between the first two enzymes. The separation between the C enzyme and terminal transferase is not always as satisfactory as in Fig. 1. Good resolutions were obtained when the 0.45 M KCl eluate from the phosphocellulose column (after a 0.25 M wash), was filtered on Sephadex G-150, the elution volume of terminal transferase being consistent with a molecular weight of about 30,000.

The thymus cells gave the same elution profile for terminal transferase as did the MOPC 173 cells. The complete results

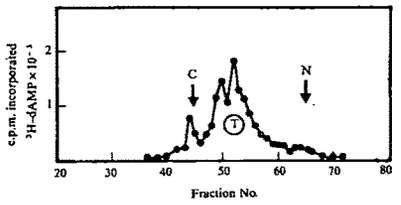


Fig. 2 Terminal deoxynucleotidyl transferase in TEPC 15 plasmocytoma tumours. The experimental procedure was the same as for MOPC 173 tumours (Fig. 1), with the exception of the fraction volumes (1 ml) and of the assay of the terminal transferase which was performed with d(pT)$_{10}$ (Boehringer) and ^{3}H-dATP (250 pmol 2,000 c.p.m. pmol^{-1}). The arrows represent the fractions containing the other DNA polymerases (detected as in Fig. 1).

Table 2 DNA polymerases present in murine tissues

Tissue	Reverse transcriptase V	High molecular weight polymerase C	Terminal-transferase T	Low molecular weight polymerase N
L cells	+	+	—	+
Athymic mouse spleen	—	+	—	+
Thymus	—	+	+	+
MOPC 173 (tumours)	+	+	+	+
MOPC 173 (cells in culture)	+	+	+	+
TEPC 15 (tumours)	+	+	+	+

Results were obtained by the method described in Fig. 1. Relative levels of activity are not shown.

summarised in Table 2 show that the terminal transferase is present only in thymus and plasmocytoma cells.

The properties of the terminal transferase extracted from plasmocytoma MOPC 173 are the same as those of the thymic enzyme: it uses different oligonucleotides as initiators ($d(pT)_4$, $d(pT)_{10}$, $d(pG)_4$ and $d(pA)_{10}$ were tested) and it polymerises separately each of the four deoxynucleoside triphosphates, with a better yield for dATP. A low self initiation takes place in the absence of initiator. Divalent cations are indispensable.

Since plasmocytoma cells are generally considered to be B cells[18], the presence of terminal transferase in these tumours was unexpected. McCaffrey *et al.*[12] pointed out that the presence of this enzyme in leukaemic lymphoblasts would denote a thymic origin. But, neither in the McCaffrey's description nor in our study, have thymus antigens been checked. In most plasmocytomas, θ antigen is lacking[18], whereas B antigen is present (E. Stockert, personal communication). Thus, it would seem plausible to assume that plasmocytoma cells represent an intermediate stage of differentiation between B and T cells, as has been previously proposed for a thymoma cell line [19]. Another hypothesis is that transformation by itself would have led to the derepression of terminal transferase. Further studies are needed before discussing these hypotheses in detail; work is in progress to check the complete set of antigens expressed on plasmocytoma cells; it would also be of interest to isolate the thymic cell population responsible for the terminal deoxynucleotidyltransferase activity.

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1 Weissbach, A., Schlabach, A., Friendlender, B., and Bolden, A., *Nature new Biol.*, **231**, 167–170 (1971).
2 Chang, L. M. S., and Bollum, F. J., *Biochemistry*, **11**, 1264–1272 (1972).
3 Smith, R. G., and Gallo, R. C., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2879–2884 (1972).
4 Brun, G., Rougeon, F., Lauber, M., and Chapeville, F., *Eur. J. Biochem.*, **41**, 241–251 (1974).
5 Rougeon, F., Brun, G., and Chapeville, F., *Eur. J. Biochem.*, **41**, 253–261, (1974).
6 Chang, L. M. S., Brown, M., and Bollum, F. J., *J. molec. Biol.*, **74**, 1–8 (1973).
7 Livingston, D. M., Serxner, L. E., Howk, D. J., Hudson, J., and Todaro, G. J., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 57–69 (1974),
8 Baltimore, D., McCaffrey, R. and Smoler, D. F., in *Virus Research*. 51–59 (Academic Press, New York and London, 1973).
9 Bollum, F. J., *J. biol. Chem.*, **237**, 1945 (1962).
10 Chang, L. M. S., and Bollum, F. J., *Proc. natn. Acad. Sci. U.S.A.*, **65**, 1041–1048 (1970).
11 Chang, L. M. S., *Biochem. biophys. Res. Commun.*, **44**, 124–131 (1971).
12 McCaffrey, R., Smoler, D. F., and Baltimore, D., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 521–525 (1973).
13 Gottlieb, A. A., Smith, A. H., Plescia, O. J., Persico, F. J., and Nicholson, E. D., *Nature*, **245**, 480–482 (1973).
14 Pénit, C., Paraf, A., Rougeon, F., and Chapeville, F. *FEBS Lett.*, **38**, 191–196 (1974)
15 Paraf, A., Moyne, M. A., Duplan, J. F., Scherrer, R., Stanislawski, M., Bettane, M., Leliévre, M., Rouzè, P., and Dubert, J. M., *Proc. natn. Acad. Sci. U.S.A.*, **67**, 983–990 (1970).
16 Potter, M., and Liberman, R., *J. exp. Med.*, **132**, 737–751 (1970).
17 Eisen, H. N., Simms, E. S., and Potter, M., *Biochemistry*, **7**, 4126–4134 (1968).
18 Takamashi, J., Old, L. J., and Boyse, E. A., *J. exp. Med.*, **131**, 1325–1341 (1970).
19 Harris, A. W., Bankhurst, A. D., Mason, S. and Warner, N. L., *J. Immun.*, **110**, 431–438 (1973).
20 Aposhian, H. V., and Kornberg, A., *J. biol. Chem.*, **237**, 519–525 (1962).

Interaction between the antibiotic tyrocidine and DNA *in vitro*

Very little is known about the natural function of the peptide antibiotics. The production of antibiotics may be related to sporulation as only sporulating microorganisms produce antibiotics[1]. In general, the synthesis of antibiotics occurs within a limited time at the end of the logarithmic growth of cell cultures[2], concurrent with a change in the specificity of transcription[3]. Loss, through mutation, of the ability to synthesise the antibiotic leads to asporogeny, in some cases[1]. Specific inhibitors and changes in culture conditions affect both the synthesis of antibiotic and sporulation[4]. Sarkar and Paulus[5] demonstrated that the cyclic peptide antibiotic, tyrocidine, produced by *Bacillus brevis* (ATCC 8185) effects RNA synthesis *in vivo*, whereas DNA synthesis continues. Moreover, these authors give evidence that tyrocidine inhibited an *in vitro* RNA transcriptional system using *B. brevis* DNA and *B. brevis* DNA-dependent RNA polymerase. Complete inhibition of RNA synthesis with a tyrocidine concentration comparable to that in a *B. brevis* cell at the end of the vegetative growing phase was observed. A strong binding of the oligopeptide antibiotics, neotropsin and distamycin A, to double-stranded DNA, resulting in a pronounced heat stabilisation of the double-strands, has been reported[6]. Compared with pure DNA, these peptide-DNA complexes exhibit a low template activity[7].

In this report we describe experiments demonstrating that tyrocidine interacts *in vitro* with DNA. A complex formation between the initiation sites and tyrocidine is suggested as the mode of action.

RNA polymerase was isolated from a logarithmic growing cell culture of *B. brevis* (ATCC 8185) according to the method of Burgess[8] except that the polymerase was eluted from DEAE cellulose by a linear KCl gradient instead of stepwise. The enzyme, finally purified on a linear glycerol gradient, was tested for purity by polyacrylamide gel electrophoresis. The enzyme was at least 90% pure. SDS gel electrophoresis revealed three main components with molecular weights of about 45,000, 55,000 and 160,000, corresponding to the 2 αs, σ and β′β components of the polymerase respectively[9]. In addition there was an unknown component with a molecular weight of about 120,000 which has been already described by Sarkar and Paulus[9]. In contrast to the report of these authors, σ was not released during glycerol gradient centrifugation. This polymerase transcribed both *B. brevis* DNA and calf thymus DNA. In both cases transcription was inhibited proportionally by added tyrocidine. Complete inhibition was achieved when 20 μg tyrocidine was added to a 250 μl transcription assay containing 7 μg *B. brevis* DNA, or when 25 μg tyrocidine was added to the same system containing 2 μg calf thymus DNA. The tyrocidine concentration was thus similar to that which Sarkar and Paulus[5] found to be inhibitory in their transcription assay

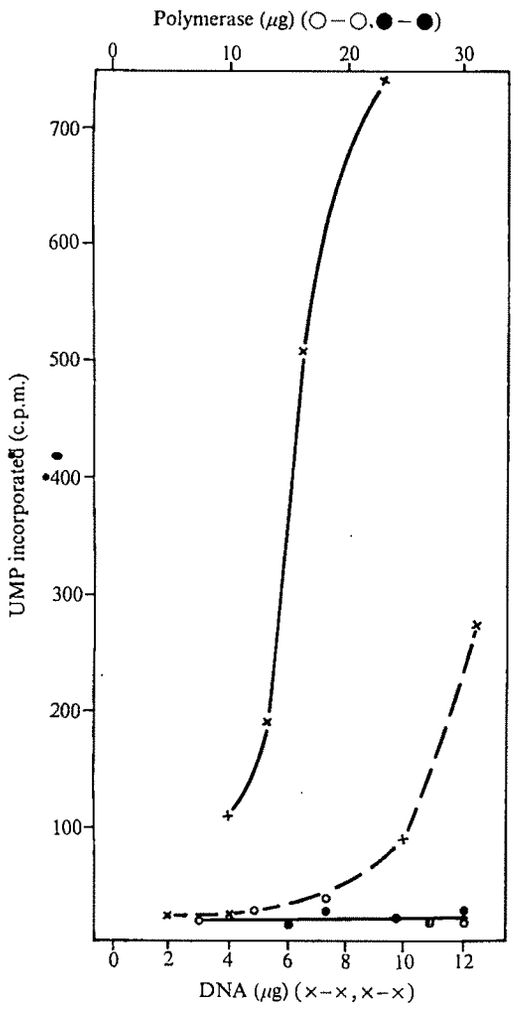


Fig. 1 Effects of DNA and of RNA polymerase on transcriptional system inhibited by tyrocidine. Transcription of 7 μg *B. brevis* DNA was inhibited by tyrocidine to 87%. To this system increasing amounts of *B. brevis* DNA were added (×—×). In parallel transcription was inhibited to 100% and increasing amounts of RNA polymerase were added (●—●). Transcription of 2 μg calf thymus DNA was inhibited by tyrocidine to 100%. To this system increasing amounts of calf thymus DNA (× – – ×) or RNA polymerase (O—O) were added. Polymerase was isolated from cells grown in a rich medium (5 g peptone, 1.5 g yeast extract, 1.5 g beef extract, 3.5 g NaCl, 3.7 g K_2HPO_4, 1.3 g KH_2PO_4, 1.0 glucose in 1,000 ml of water) and collected during exponential growth. Polymerase was purified according to Burgess[8] including the glycerol gradient step. The enzyme contained the σ subunit and was completely dependent on DNA. An incorporation of 16.3 pmol of UMP into RNA in the presence of 3.5 μg *B. brevis* DNA was catalysed by 1 μg of RNA polymerase within 30 min. *B. brevis* DNA was purified by treating cells with lysozyme (10 μg ml^{-1}) for 5 min at 37° C followed by SDS treatment (0.2% sodium dodecyl sulphate in 0.1 M EDTA, 0.4 M NaCl, *p*H 7.8) at room temperature for 15 min. Protein was removed by phenol extraction. RNA was digested by RNase treatment (50 μg ml^{-1}) for 90 min followed by pronase treatment (100 μg ml^{-1}) for 180 min. Pronase was removed by phenol extraction. The DNA was finally purified by CsCl density centrifugation. Calf thymus DNA (Serva) was used without further purification. Transcription assays were carried out at 37° C for 30 min in the presence of 0.03 M Tris-HCl, *p*H 7.9, 0.15 M KCl, 0.005 M $MgCl_2$, 0.1 mM EDTA, 0.001 M 2-mercaptoethanol, 0.02 M each of ATP, GTP and CTP and 0.5 μC ^{3}H-UTP (52 Ci $mmol^{-1}$). For each assay (250 μl) 6 μg polymerase and 7 μg *B. brevis* DNA or 2 μg calf thymus DNA were used. Tyrocidine (Serva) was dissolved in methanol (2 mg ml^{-1}) and added to the assays. Transcription was terminated by adding cold trichloroacetic acid to a final concentration of 7%. The precipitates were collected on glass fibre filters (Whatman GF/C), washed with trichloroacetic acid and ethanol, dried and counted in a Nuclear Chicago liquid scintillation counter.

and was comparable to that in a *B. brevis* cell during the late logarithmic phase. The solvent used for tyrocidine, methanol, had a slightly stimulatory effect on RNA synthesis. Therefore the inhibition was not due to the solvent. When *B. brevis* polymerase was replaced by *Escherichia coli* RNA polymerase a comparable inhibition was obtained. Thus tyrocidine does not act only on the homologous DNA or on the homologous polymerase.

When increasing amounts of DNA were added to an inhibited transcription system, transcription could be restarted. In contrast, RNA polymerase did not affect an inhibited transcription system. This was the case for *B. brevis* DNA as well as for calf thymus DNA (Fig. 1). It is therefore likely that tyrocidine acts on DNA which is no longer accessible to the polymerase and that the polymerase is not affected by tyrocidine.

In the experiments described above, DNA was first mixed with tyrocidine, and the reaction started by the addition of polymerase. In other experiments transcription was allowed to proceed for some time before the addition of tyrocidine. In this case significant transcription ensued for a certain time after the addition of tyrocidine and was only later slowed down or stopped. A possible interpretation of this phenomenon, which was true for the *B. brevis* as well as for the calf thymus system, could be as follows: once transcription has started the transcribing polymerase cannot be stopped by tyrocidine. After terminating, however, no new initiation can occur because the initiation sites are blocked by the antibiotic. Following this interpretation, the target of tyrocidine would be the initiation of the transcription process.

To prove the hypothesis that tyrocidine interacts with DNA, complex formation has to be demonstrated. Sephadex G-25 gel filtration could not be used because tyrocidine adsorbs to the gel due to its high tryptophan content. A DNA-tyrocidine complex was detected by glycerol gradient

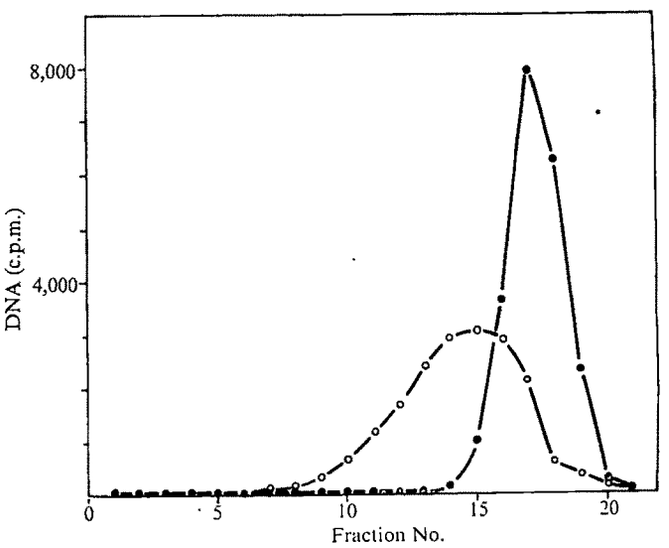


Fig. 2 Complex formation between *B. brevis* ^{3}H-DNA and tyrocidine (O—O) assayed by glycerol gradient centrifugation. ^{3}H-DNA was isolated from *B. brevis* cultures which have been incubated with 1 μC ^{3}H-adenine (specific activity 21 Ci $mmol^{-1}$) per ml for several generations. Cells were broken and DNA purified as described in Fig. 1 except that the CsCl-purification step was omitted. The specific radioactivity of the DNA was 3,100 c.p.m. μg^{-1}. A mixture of 20 μg ^{3}H-DNA and 20 μg tyrocidine in 0.01 M Tris-HCl *p*H 7.8. 0.005 M $MgCl_2$, 0.01 M KCl was incubated for 10 min at 25° C. The complex was then layered on to a 14 ml 10–30% (v/v) glycerol gradient in buffer as above and centrifuged in a SW 27.1 rotor (Beckman, Spinco Ultracentrifuge) at 25° C for 12 h at 26,000 r.p.m. At the end of the run the gradient was fractionated, the DNA precipitated by trichloroacetic acid, and the radioactivity estimated. DNA without tyrocidine (●—●) was run on a parallel gradient as a marker.

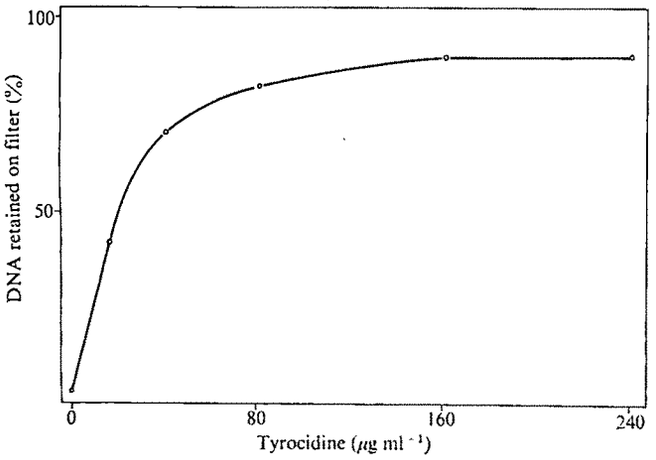


Fig. 3 Complex formation between *B. brevis* DNA and tyrocidine, assayed by the filter binding technique of Riggs *et al.*[10]. ^{3}H-DNA was isolated from *B. brevis* cultures which had been incubated with 1 μC ^{3}H-adenine (specific activity 21 Ci mmol^{-1}) for several generations. In total volumes of 250 μl, 1.98 μg ^{3}H-DNA were incubated with increasing amounts of tyrocidine for 10 min at room temperature in a buffer containing 0.01 M Tris, *p*H 7.8, 0.01 M magnesium acetate, 0.01 M KCl, 0.0001 M EDTA, 5% dimethylsulphoxide and 50 μg bovine serum albumin ml^{-1}. The mixture was then filtered slowly through a nitrocellulose filter (MF 100, Schleicher and Schüll) and was washed once with 0.8 ml of the same buffer but without albumin. The filter was dried and counted in a liquid scintillation counter. Under the conditions described, only 2–4% of the DNA was held back on the filter in the absence of tyrocidine.

centrifugation. ^{3}H-labelled *B. brevis* DNA was incubated with tyrocidine at a concentration of 80 μg ml^{-1} and centrifuged through a glycerol gradient. The majority of the DNA incubated with tyrocidine sedimented more rapidly than the marker DNA (Fig. 2), demonstrating complex formation. At a concentration of 160 μg tyrocidine ml^{-1}, most of the DNA was found to be on the bottom, whereas at a concentration of 40 μg tyrocidine ml^{-1}, most of the DNA moved in the position of the marker DNA.

Another way to demonstrate DNA-tyrocidine complex formation is the nitrocellulose filtration method of Riggs and Bourgeois[10]. In this system double stranded DNA does not adsorb to the nitrocellulose filter. When a complex is formed between DNA and a peptide or protein, the DNA complex is retained on the filter. In these experiments ^{3}H-labelled *B. brevis* DNA was used. The amount of retained ^{3}H-DNA is a measure of the complex formed. Without tyrocidine only about 2–4% of the DNA was bound by the nitrocellulose filter. Figure 3 illustrates the complex formation at a constant ^{3}H-DNA concentration with increasing amounts of tyrocidine.

We tested the stability of the complex. To a complex of labelled DNA and tyrocidine, a 50-fold excess of cold *B. brevis* DNA was added and incubated for 1 h. At various times samples were withdrawn and filtered through nitrocellulose filters. It was found that 50% of the complex was broken down within 5 min, whereas the rest of the complex was almost completely stable for 1 h. It might be that about 50% of the initial complex detected on filters was a non-specific complex and was easily broken down. The remaining 50% stable complex might indicate a specific complex formation.

We conclude from our experiments that there is a complex formation between DNA and the antibiotic tyrocidine *in vitro*. Taking into account the observations from other laboratories, our results suggest that tyrocidine acts *in vivo* at the level of transcription by inhibiting the formation of polymerase-DNA complexes. This might reflect a control mechanism during the transition from the vegetative to the sporulating cell, when many of the genes active during vegetative growth are switched off. Yoshida *et al.*[11] came to similar conclusions working with a different system. They found that vegetative cell cultures of *Streptomyces antibioticus*, which produces actinomycin D, are sensitive to actinomycin D. But the cells are insensitive to the compound during the transition of vegetative growth to sporulation. It is not yet known how genes are transcribed in late logarithmic or stationary cells which contain a high concentration of tyrocidine or actinomycin D. Experiments investigating the behaviour of RNA polymerase from sporulating cells in the presence of tyrocidine are in progress.

The final evidence for a regulatory role of the peptide antibiotic has to come from DNA-tyrocidine complexes isolated from cells and exhibiting a qualitatively different template activity compared with the template from vegetative cells.

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1 Schaeffer, P., *Bact. Rev.*, **33**, 48 (1969).
2 Lipmann, F., *Science*, **173**, 875 (1971).
3 Linn, T. G., Greenleaf, A. L., Shorenstein, R. G., and Losick, R., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1865 (1973).
4 Paulus, H., in *Antibiotics* (edit. by Gottlieb, D., and Shaw, P. D.), **2**, 254 (Springer, Berlin, 1967).
5 Sarkar, N., and Paulus, H., *Nature new Biol.*, **239**, 228 (1972).
6 Zimmer, C., Reinert, K. E., Wähnert, U., Löber, G., and Thrum, H., *J. molec. Biol.*, **58**, 329 (1971).
7 Zimmer, C., Puschendorf, B., Grunicke, H., Chandra, P., and Venner, H., *Eur. J. Biochem.*, **21**, 269 (1971).
8 Burgess, R. R., *J. biol. Chem.*, **244**, 6160 (1969).
9 Sarkar, N., and Paulus, H., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3570 (1972).
10 Riggs, A. D., Suzuki, H., and Bourgeois, S., *J. molec. Biol.*, **48**, 67 (1970).
11 Yoshida, T., Weissbach, H., and Katz, E., *Archs Biochem. Biophys.*, **114**, 252 (1966).

Activation of a C-type virus from the human carcinoma cell line HBT-3 by iododeoxyuridine and testosterone

WE report the activation of a C-type virus from the human carcinoma cell line, HBT-3, with iododeoxyuridine following a testosterone-induced cellular morphology change. We feel that this method will prove useful in activating endogenous C-type virus. Studies are in progress to further characterise the virus and determine the species of origin.

The human breast tumour cell line, HBT-3, has been described in some detail[1]. These cells have been maintained continuously as non-contact-inhibited epithelial cells (HBT-3Ep, Fig. 1*a*). The cells have a high cloning efficiency and an abnormal karyotype with three marker chromosomes. A DNA polymerase resembling the polymerase of RNA tumour viruses has been detected in this cell line[2]. No virus particles have been detected however by electron microscopy (EM) or in tissue culture supernatants banded on sucrose gradients (personal communication from R. Bassin).

The induction of mammary carcinomas in the mouse results from an interaction between the murine mammary tumour virus

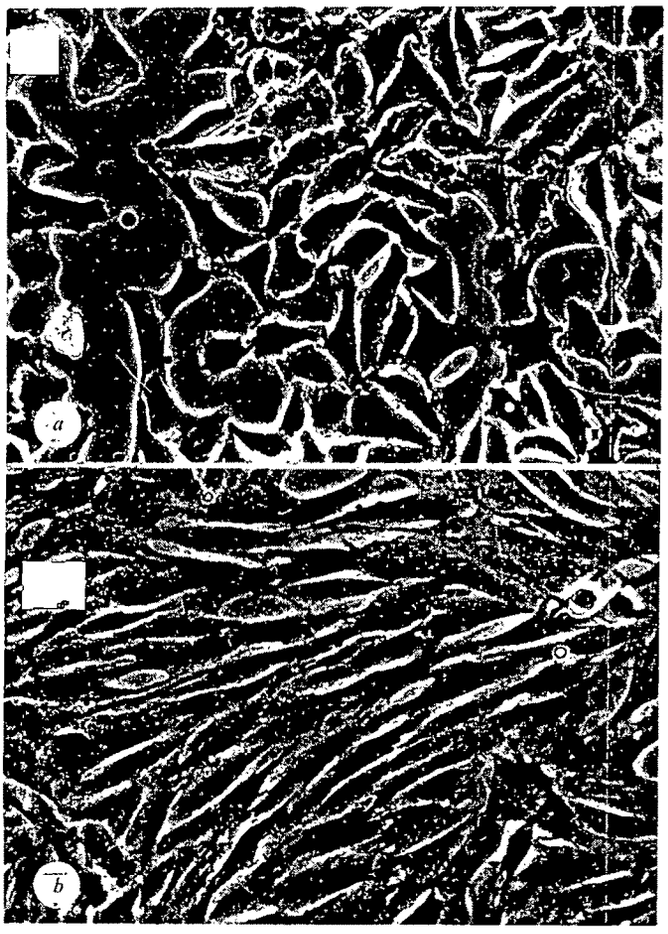


Fig. 1 Phase contrast microscopy of HBT-3 cells in cell culture. *a*, HBT-3Ep cells (×286); *b*, HBT-3 cells after prolonged cultivation with medium containing 5 μg ml^{-1} testosterone acetate (×286). Both cell types were grown at 37° C in 75 cm^2 plastic flasks (Falcon) utilising RPMI-1640 medium containing 15% v/v heat-inactivated foetal calf serum (Gibco), penicillin (100 μg ml^{-1}) and streptomycin (10 μg ml^{-1}). Weekly cell passages were made using 0.25% trypsin.

(MuMTV), the genetic susceptibility of the host and the hormonal environment[3-7]. Although murine mammary carcinoma has been studied extensively, other mammalian systems are less well characterised. Viruses with C-type morphology have been detected by EM in mammary carcinomas of the rat[8], cat[9] and rhesus monkey[10]. EM examination of human breast cancer biopsies has on occasion revealed virus-like particles[11-14], but virus has not been detected in human breast cancer cell lines[1,15-16]. The RNA-dependent DNA polymerase (RDP) assay or the more rigorous 'simultaneous detection' of 60S-70S RNA have been used to detect oncornavirus-like properties in some human milk samples[17-19]. Keydar *et al.* have found particulates with similar properties banding at a density of 1.17 g ml^{-1} in the supernatant fluids of human embryonic cells cocultivated with human breast cancer cells or treated with milk from breast cancer patients known to contain 60S-70S RNA and RDP activity[20]. RNA tumour virus expression in cell culture has been activated or enhanced by growing cells in the presence of halogenated pyrimidines, particularly iododeoxyuridine (IUdR) and bromodeoxyuridine[21-23]. McGrath[4] has shown MuMTV release concurrent with hormone-induced cellular organisation in cultured BALB/cfC$_3$H mammary tumour cells. Moreover, IUdR rescue of C-type virus from mouse fibroblasts is markedly stimulated by the presence of adrenal glucocorticosteroids[24]. We now report that a C-type RNA virus can be activated by IUdR from HBT-3 cells following a testosterone induced morphological alteration.

HBT-3 cells were obtained from Dr Robert Bassin (NCI) at passage level 28. A single clone derived from this cell culture was used for all subsequent studies. Cells grown in the presence and absence of 5 μg ml^{-1} testosterone acetate (Sigma) were observed frequently with a phase contrast microscope. After 8 weeks of continuous cultivation with testosterone, contact-inhibited spindle-shaped cells (HBT-3S) began to appear (Fig. 1*b*). Within 4 d these cells comprised 90% of the population and on subsequent passage no epithelial cells could be discerned by light microscopy. Karyotyping of the spindle-shaped cells revealed two of the original chromosome markers (personal communication from C. S. Stulberg). The lack of any significant change in cell numbers or cell doubling time indicates that the HBT-3Ep cells were morphologically changed to HBT-3S cells. If testosterone is removed within 2 weeks after this change the HBT-3S cells revert to the HBT-3Ep form, taking 2–3 weeks for reversal to occur. The change is not reversible, however, if testosterone is removed later than 2 weeks after the HBT-3S cells appear. These cells have been maintained in culture for up

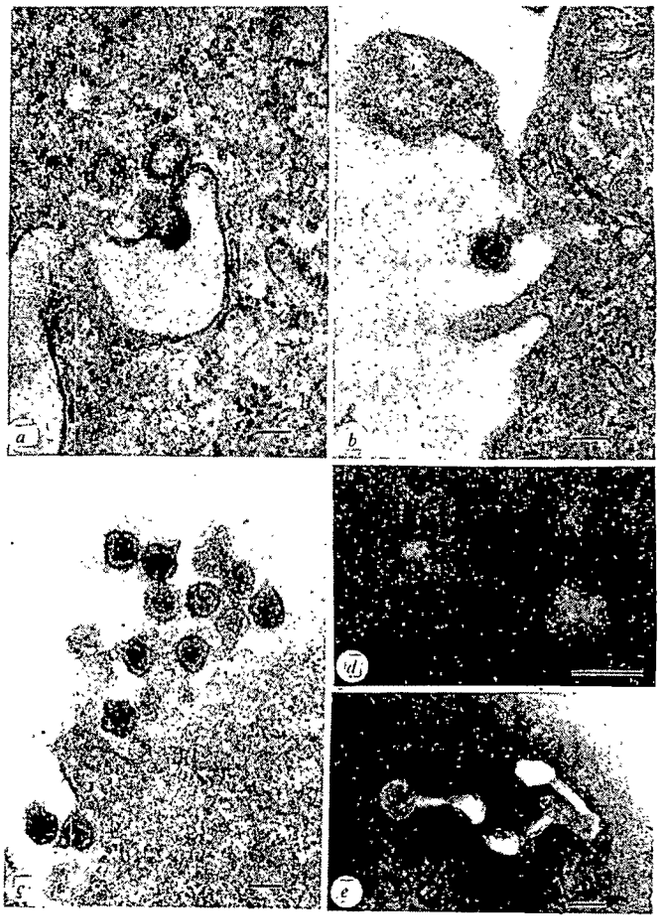


Fig. 2 Virus particles activated from HBT-3S with testosterone acetate and IUdR. *a*, C-type virus budding from cell membrane. The marker represents 100 nm. After IUdR-testosterone activation, cells were fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated and embedded in Epon[26]. Thin sections cut on an LKB Ultratome were stained with 2% aqueous uranyl acetate and lead citrate[27] and examined with a Phillips 300 electron microscope. *b* and *c*, Extracellular C-type virus. The marker represents 100 nm. *d*, Negatively stained preparation of virus using phosphotungstic acid (PTA). Virus containing fluid collected from IUdR-testosterone-activated cells was centrifuged at 4,000 r.p.m. for 15 min and the supernatant subjected to centrifugation at 95,000*g* for 60 min. The resultant pellet was suspended in 0.5 ml of 0.05 M sodium citrate and mixed 1:3 with 2% PTA and examined by EM on formvar-carbon coated grids. The marker represents 100 nm. *e*, Negatively stained virus from 1.15 g ml^{-1} band of isopycnic sucrose density gradient (see Fig. 3*b*). The marker represents 100 nm.

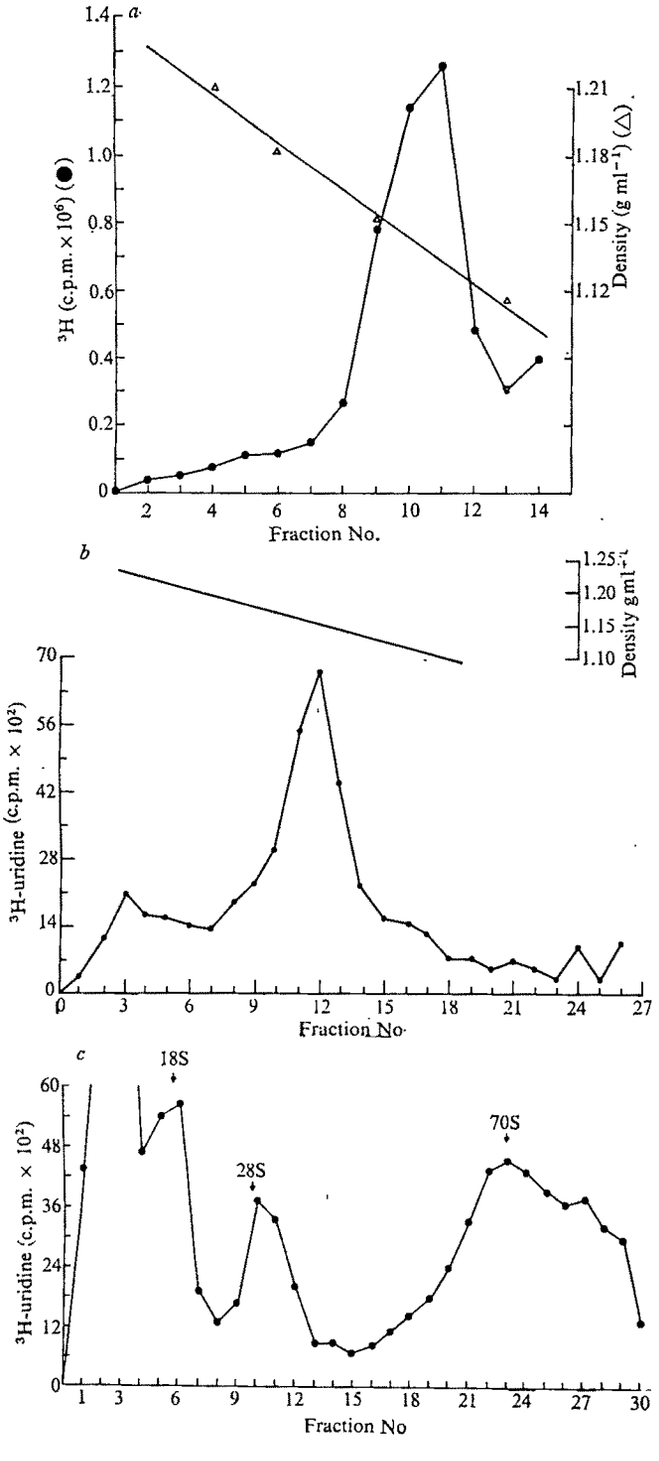


Fig. 3 RNA-dependent DNA polymerase activity and density of HBT-3S IUdR rescued virus and sedimentation rate of its associated RNA. *a*, RDP activity of virus banded in an isopycnic density gradient. IUdR-treated HBT-3S cultures were incubated for 72 h with 100 μg ml^{-1} IUdR. The collected culture fluids were clarified at 10,000*g* for 10 min. The clarified medium was then centrifuged at 80,000*g* for 60 min. The pellet from this centrifugation was resuspended in phosphate buffered saline, layered over a 10–65% linear sucrose gradient in a Beckman SW-40 rotor and centrifuged for 4 h at 200,000*g*. Sedimentation is from right to left. The indicated fractions were diluted to 5 ml with cold growth medium and centrifuged at 80,000*g* for 60 min in a Beckman No. 30 rotor. The tube was drained, 120 μl of a detergent solution was added and two 50 μl aliquots were added immediately to 50 μl reaction mixtures, with (+) and without (−) added polyriboadenylic·oligodeoxythymidylic acid (poly (rA)·poly (dT)$_{(12-18)}$) template. The final reaction in 0.1 ml contained 0.016% Nonidet P-40 (Shell Chemical Co.), 30 mM *tris*-hydroxymethylaminomethane (Tris) hydrochloride (*p*H 8.5), 50 mM dithiothreitol (DTT), 0.5 mM manganese acetate, 30mM sodium chloride, (+) 0.10 A_{260} units poly (rA)·poly (dT)$_{(12-18)}$; 1.0 μM thymidine triphosphate (TTP) methyl-^{3}H (55 Ci mmol^{-1}) and was incubated for 30 min. at 37° C. Trichloracetic acid (TCA) precipitable material was counted on membrane filters (Schleicher and Schuell) in a Packard model 3375 scintillation spectrometer. The data are corrected for (−) poly(rA)·poly(dT)$_{(12-18)}$ (< 200 c.p.m.). *b*, Isopycnic sucrose density gradient centrifugation of ^{3}H-uridine labelled virus from IUdR-activated HBT-3S cells. Conditions were as in (*a*) except that ^{3}H-uridine (50 μCi ml^{-1}) was added simultaneously during IUdR induction and 25 μl aliquots were counted. Sedimentation is from right to left. *c*, Sucrose gradient sedimentation of RNA extracted from 1.15 g ml^{-1} ^{3}H-uridine labelled virus. Fractions between 1.136 and 1.165 g ml^{-1} (from *b*, above) were pooled and diluted to 25 ml with TNE buffer (0.01 M Tris, *p*H 7.5, 0.1M NaCl, and 0.001 M EDTA). The pool was centrifuged for 60 min at 80,000*g* and the virus pellet was resuspended in 200 μl of a solution containing 0.25M Tris, *p*H 7.5, 0.05M EDTA, 2.5% sodium lauroyl sarcosinate, 6% sodium dodecyl sulphate (SDS), 2.5 mM magnesium chloride and 0.1 mM calcium chloride. An equal volume of TNE buffer-saturated phenol was added. The suspension was vortexed and 2.5 volumes of ethanol (−20° C) were added to the aqueous phase. The precipitate was solubilised in 400 μl of TNE buffer (0.1% SDS) and sedimented through a linear sucrose gradient (10–30% w/v sucrose in TNE plus 0.1% SDS) for 3.5 h at 200,000*g* in a Beckman SW-40 rotor. Sedimentation is from left to right. Each fraction was combined with 100 μg yeast carrier RNA, precipitated onto TCA filters and counted as above.

to 3 months. In repeated EM examination no virus was detected in either thin section of cells or in negatively stained preparations of cell-free supernatants in the HBT-3Ep or the HBT-3S cells. Moreover, no significant levels of RDP were detected in the cell-free supernatant of either cell type (Table 1). To determine whether virus expression in either cell type could be induced by IUdR, HBT-3Ep and HBT-3S cells were exposed for 3 d to medium containing IUdR (P-L Biochemicals) at a concentration of 100 μg ml^{-1} (ref. 25). After IUdR treatment only the HBT-3S cells produced C-type virus detectable by both RDP assay (Table 1) and EM (Fig. 2).

The virus is typically 85–100 nm in external diameter with a 40–58 nm core (Fig. 2). It is found extracellularly and budding from the cell membrane in a characteristic C-type formation (Fig. 2*a*, *b* and *c*). It does not resemble MuMTV[3] which has A and B-type particles or the Mason–Pfizer monkey virus (MPMV)[10] which buds as a B-type virus. Phosphotungstic acid (PTA) negatively stained preparations reveal tailed C-type particles without surface spikes with occasional blebs on the tails (Fig. 2*d* and *e*).

Table 1 RNA-dependent DNA polymerase activity of HBT-3Ep and HBT-3S cells treated with IUdR and testosterone

	HBT-3Ep	HBT-3S*
Control	96	152
IUdR†	120	18,190

RDP activity is expressed in c.p.m. For details of assay see legend to Fig. 3*a*.
*HBT-3S cells were derived from HBT-3Ep cells by testosterone as described in the text. Testosterone was maintained in the culture medium for 6 weeks after the appearance of the HBT-3S cells at which time they were used in this study.
†Iododeoxyuridine, 100 μg m^{-1} of medium for 3 d.

RDP analysis of supernatants of the IUdR-induced HBT-3S cells banded in sucrose reveals a single peak of maximum activity at a density of 1.13 to 1.15 g ml^{-1} (Fig. 3*a*). When HBT-3S cells are isotopically labelled with ^{3}H-uridine during IUdR induction, a band of labelled virus is obtained at a density of 1.15 g ml^{-1} (Figs. 2*e* and 3*b*). RNA extracted from this band by the phenol method and sedimented on a sucrose gradient reveals both 70S and 28-30S ^{3}H-uridine labelled regions unique to C-type viruses (Fig. 3*c*). Thus, the biochemical and morphological characterisation identify this as a C-type virus.

Preliminary immunological evaluation of the virus by means of indirect immunofluorescence studies of the IUdR-treated cells reveals a lack of activity with antisera to rat (R-35), murine (Rauscher), feline (feline leukaemia and RD114) and primate (woolly monkey, MPMV, and gibbon ape) viruses. Repeated attempts to cultivate this virus in human and other mammalian cells have not been successful nor does this virus have an observable effect on HBT-3Ep or HBT-3S cells. Intraperitoneal and subcutaneous inoculations of BALB/c mice with 10^7 virus particles have not revealed pathological characteristics for up to 4 months after inoculation.

The requirement for testosterone as a prerequisite to viral activation suggests that it, together with IUdR, has a derepressing function on the viral genetic information within HBT-3S cells. However, demonstration of antigenic determinants and nucleic acid homology to human cancer cells remain to be completed before any definitive statement regarding virus origin can be made. Moreover, the role of this virus, if any, in the aetiology of human cancer remains to be fully elucidated.

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Note added in proof: The presence of chromosomes in HBT-3 cells characteristic of HeLa has been reported[28]. Although the origin of the HBT-3 cells is uncertain, we have established that testosterone and iododeoxyuridine may be used to activate a C-type virus from them and may be a useful technique for virus activation in other cell systems.

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1 Bassin, R. H., Plata, E. J. Gerwin, B. I., Mattern, C. F., Haapala, D. K., and Chu, E. W., *Proc. Soc. Exp. Biol. Med.*, **141**, 673 (1972).
2 Gerwin, B. I., and Bassin, R. H., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2453 (1973).
3 Moore, D. H., *Nature*, **198**, 29 (1963).
4 McGrath, C. M., *J. natn. Cancer Inst.*, **47**, 455 (1971).
5 Bentvelzen, P., Daams, J. H., Hageman, P., Calafat, J., and Timmermans, A., *J. natn. Cancer Inst.*, **48**, 1089 (1972).
6 Nandi, S., *Can. Cancer Conf.*, **6**, 69 (1966)
7 Muhlbock, O., *J. natn. Cancer Inst.*, **48**, 1213 (1972).
8 Engle, G. C., Shirahama, S., and Dutcher, R. M., *Cancer Res.*, **29**, 603 (1969).
9 Feldman, D. G., and Gross, L., *Cancer Res.*, **31**, 1261 (1971).
10 Chopra, H. C., and Mason, M. M., *Cancer Res.*, **30**, 2081 (1970).
11 Tannenbaum, M., Lattimer, J., *J. Virol.*, **103**, 471 (1970).
12 Dmochowski, L., Seman, J. D., and Gallagher, H. S., *Cancer*, **24**, 1241 (1969).
13 Dmochowski, L., Seman, J. D., Myers, B., and Gallagher, H. S., *Med. Rec. Ann.*, **61**, 384 (1968).
14 Moore, D. H., Sarkar, N. H., Kelly, C. E., Pillsbury, N., and Charney, J., *Texas Rep. Biol. Med.*, **27**, 1027 (1969).
15 Plata, E. J., Aoki, T., Robertson, D. D., Chu, E. W., and Gerwin, B. I., *J. natn. Cancer Inst.*, **50**, 849 (1973).
16 Lasfargues, E. Y., and Ozzello, L., *J. natn. Cancer Inst.*, **21**, 1131 (1958).
17 Schlom, J., Spiegelman, S., and Moore, D. H., *Nature*, **231**, 97 (1971).
18 Schlom, J., Spiegelman, S., and Moore, D. H., *Science*, **175**, 542 (1972).
19 Gerwin, B. I., Ebert, P. S., Chopra, H. C., Smith, S. G., Kvedar, J. P., Albert, S., and Brennan, M. J., *Science*, **180**, 198 (1973).
20 Keydar, J., Gilead, Z., Karby, S. and Harel, E., *Nature new Biol.*, **241**, 49 (1973).
21 Stewart, S. E., Kasnic, G., Draycott, C., and Ben, T., *Science*, **175**, 198 (1972).
22 Stewart, S. E., Kasnic, G., Draycott, C., Feller, W., Golden, A., Mitchell, E., and Ben, T., *J. natn. Cancer Inst.*, **48**, 273 (1972).
23 Hampar, B., Berge, J. G., Martos, L. M., and Walker, J. L., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 78 (1972).
24 Paran, M., Gallo, R. C., Richardson, L. S., and Wu, A. M., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2391 (1973).
25 Lowy, D. R., Rowe, W. P., Teich, N., Hartley, J. W., *Science*, **174**, 155 (1971).
26 Luft, J. H., *J. biophys. biochem. Cytol.*, **17**, 208 (1963).
27 Reynolds, E. S., *J. Cell Biol.*, **17**, 208 (1963).
28 Nelson-Rees, W. A., Flandermeyer, R. R., and Hawthorne, P. K., *Science*, **184**, 1093 (1974).

Hepatitis B-specific DNA polymerase activity during post-transfusion hepatitis

HEPATITIS B antigen (HBAg, Australia antigen) has been associated definitively with long incubation human hepatitis[1–4]. It appears before the onset of acute illness[3,4] and decreases during convalescence, with the concomitant development of anti-HBAg[5]. In addition to this classical course of the disease, the discovery of HBAg has revealed subclinical infections, as demonstrated by seroconversion to HBAg (ref. 6), and the carrier state, where antigen is present in blood for long periods with either chronic hepatitis or no apparent disease[7]. Levels of HBAg in blood seem unrelated to the severity of disease, for carriers who generally have high titres of circulating antigen, in most cases, show slight if any liver damage[8,9].

Several structures found in plasma carry HBAg determinants[10–14] including 43 nm spherical particles with 27 nm electron dense cores first described by Dane *et al.*[14]. DNA polymerase activity is associated with the core of a subpopulation of Dane particles in apparently normal carriers[15]. We have now found polymerase-containing particles in blood during acute infection.

We collected serial plasma samples from multiply transfused patients at frequent intervals after surgery, and analysed them for HBAg by a solid phase radioimmunoassay[16], for anti-HBAg by passive haemagglutination[17] and for serum glutamic-pyruvic transaminase (SGPT)[18]. Some samples had been stored at −20° C for up to 3 yr.

Undiluted plasma (25 μl) was used to detect DNA polymerase activity by the procedures described before[15]. When polymerase activity was detected, we investigated whether it was HBAg-specific. Test samples (50 μl) were mixed with 15 μl of either guinea pig anti-HBAg (*ay*) (NIAID Research Resource Reagent V802–501–558) or normal guinea pig serum, and incubated at 37° C for 60 min. Rabbit anti-guinea pig IgG (50 μl) was then added and followed by incubation at 37° C for a further 60 min. The preparation was then centrifuged at 1,300*g* for 15 min and 50 μl of supernatant was removed and assayed for polymerase activity. Polymerase was considered to be specific if antiserum directed against HBAg removed activity from the supernatant while normal serum had no effect.

In the first experiment coded specimens from three patients were tested for the presence of DNA polymerase activity. In a patient who received nineteen 500 ml units of whole blood during open-heart surgery (one of which was positive for HBAg by radioimmunoassay but negative by counterimmu-

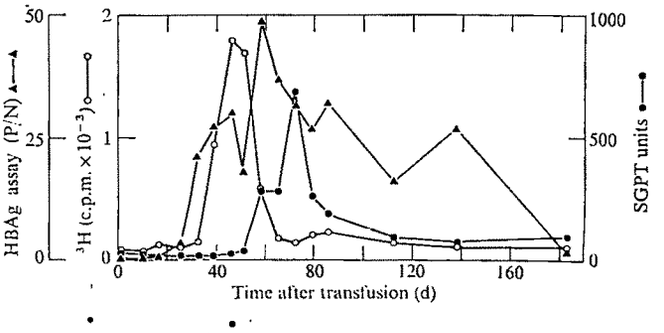


Fig. 1 A case of post-transfusion HBAg-positive hepatitis. Serial plasma samples were tested for HBAg-specific DNA polymerase. (○); HBAg by radioimmunoassay (▲); and SGPT levels (●).

noelectrophoresis) and who subsequently developed post-transfusion type B hepatitis, the first sign of infection was detection of HBAg at day 25 (Fig. 1). Fourteen days later DNA polymerase activity could be detected in unconcentrated serum. Using ten-fold concentrates prepared as described before[10], DNA polymerase could be detected 7 d after the appearance of HBAg (day 32). Polymerase activity reached a peak on day 46, after which it decreased rapidly. The level of HBAg-specific polymerase was low when the peak titre of HBAg was observed (day 58), and was no longer detectable when the peak SGPT was reached (day 72).

Figure 2*A* shows the pattern observed with a patient who received HBAg-negative blood units and appeared normal throughout the study. Serum transaminase levels in this patient were never increased and no HBAg was demonstrated by radioimmunoassay. DNA polymerase activity was not detected in serial samples obtained during the 6 months following transfusion. Figure 2*B* shows the pattern for a patient who developed post-transfusion HBAg-negative hepatitis. Transaminase levels were normal until day 37, when they increased, reaching a maximum of 1,200 on day 44. Throughout the disease no serial samples had detectable DNA polymerase activity.

Table 1 Presence of HBAg-specific polymerase in multiply transfused surgical patients

Group	SGPT*	HBAg†	No. positive‡/No. tested
1	+	+	7/9
2	+	0	0/1
3	0	0	0/4

* Increased to >100 U ml^{-1}.
† Greater than 2.1 times the mean of negative controls by a solid phase radioimmunoassay.
‡ Tested for specificity by immunoprecipitation.

Serial samples from a number of multiply transfused patients were tested under code for polymerase activity and the results obtained, as well as those for the three cases previously described, are summarised in Table 1. Seven of the nine patients who developed post-transfusion hepatitis B as indicated by the presence of HBAg and elevated SGPT (group 1) had detectable HBAg-specific DNA polymerase activity in their plasma during acute infection. In all cases this preceded signs of liver damage as measured by elevated SGPT levels, and appeared slightly after HBAg was first detected. Polymerase activity was not found in the plasma of a patient with HBAg-negative post-transfusion hepatitis (group 2, Fig. 2*B*) nor in four control individuals (group 3). No individual in groups 2 and 3 developed antibody against HBAg. One of the four controls showed polymerase activity, however this activity could not be precipitated with specific antibody and was therefore not considered positive. One of the two individuals with HBAg-positive post-transfusion hepatitis who was considered negative for DNA polymerase also showed this non-specific polymerase activity.

These results show that in most cases HBAg-specific DNA polymerase activity can be detected in plasma from acutely infected individuals. Peak levels of activity detected were higher than those found in the plasma of most carriers (unpublished results). The transient nature of enzyme-containing Dane particles in the blood, and the time at which the peak activity was found resembles the viraemias associated with other viral diseases[19,20]. These data support the hypothesis that the Dane particle is the virus of hepatitis B.

Virus-induced liver damage was not responsible for the presence of the DNA polymerase activity for several reasons. First, activity appeared before liver damage; second, in post-transfusion HBAg-negative hepatitis, where levels of SGPT were increased (Fig 2*B*), no polymerase activity was detected; and third, the enzymatic activity was associated with an antigenic particle, for highly specific anti-HBAg

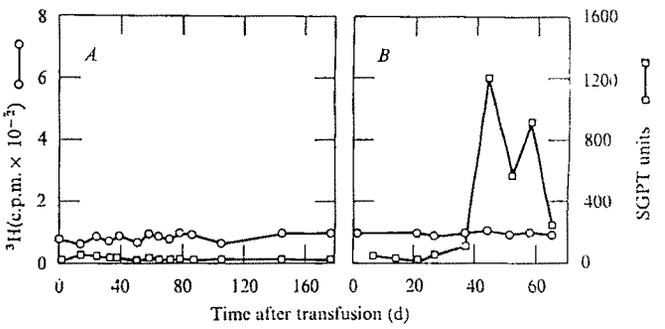


Fig. 2 Two multiply transfused surgical patients, one who showed no clinical disease and was HBAg-negative throughout the course of the study (*A*), and one who developed post-transfusion HBAg-negative hepatitis (*B*). Serial plasma samples were tested for DNA polymerase activity (○) and SGPT levels (□).

precipitated the activity.

Clearly, the radioimmunoassay detects HBAg before the demonstration of DNA polymerase activity, but it detects all antigenic structures in plasma while DNA polymerase activity is much more restricted and is probably only a measure of the presence of circulating Dane particles. If the Dane particle is the virus of hepatitis B, then the actual titre of infectious virus in an HBAg-positive serum may correlate better with the level of specific DNA polymerase than with the titre of HBAg.

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1 London, W., Sutnick, A., and Blumberg, B., *Ann. Inter. Med.*, **70**, 55 (1969).
2 Gocke, D., and Kavey, N., *Lancet*, **i**, 1055 (1968).
3 Prince, A. M., *Proc. natn. Acad. Sci., U.S.A.*, **60**, 814 (1968).
4 Krugman, S., and Giles, J. P., *J. Am. Med. Ass.*, **212**, 1019 (1970).
5 Lander, J. J., Giles, J. P., Purcell, R. H., and Krugman, S., *N. Engl. J. Med.*, **285**, 303 (1970).
6 Lander, J. J., Alter, H. J., and Purcell, R. H., *J. Immun.*, **106**, 1166 (1971).
7 Krugman, S., and Giles, J. P., in *Hepatitis and blood transfusion*, (edit. by Vyas, G. N., Perkins, H. A., and Schmid, R.), 9–18 (Grune and Stratton, New York, 1972).
8 Barker, L. F., and Murray, R., *J. Am. Med. Ass.*, **216**, 1970 (1971).
9 Feinman, S. V., Berris, B., Sinclair, J. G., Wrobel, D. N., Alter, H. J., and Holland, P. V., *Lancet*, **ii**, 867 (1973).
10 Gerin, J. L., Purcell, R. H., Hoggan, M. D., Holland, P. V., and Chanock, R. M., *J. Virol.*, **4**, 763 (1969).
11 Almeida, J. D., *Postgrad. Med. J.*, **47**, 484 (1971).
12 Bayer, M. E., Blumberg, B. S., and Werner, B., *Nature*, **218**, 1057 (1968).
13 Gerin, J. L., in *Hepatitis and blood transfusion*, (edit. by Vyas, G. N., Perkins, H. A., and Schmid, R.), 205–219 (Grune and Stratton, New York, 1972).
14 Dane, D. S., Cameron, C. H., and Biggs, M., *Lancet*, **i**, 695 (1970).
15 Kaplan, P. M., Greenman, R. L., Gerin, J. L., Purcell, R. H., and Robinson, W. S., *J. Virol.*, **12**, 995 (1973).
16 Ling, C. M., and Overby, L. R., *J. Immun.*, **109**, 834 (1972).
17 Vyas, G. N., and Shulman, N. R., *Science*, **170**, 332 (1970).

[18] Wroblewki, F., and LaDue, J. S., *Proc. Soc. exp. Biol. Med.*, **91**, 569 (1956).
[19] Fenner, F., *Lancet*, **ii**, 915 (1948).
[20] Horstman, D. M., McCallum, and Masola, A. B., *J. exp. Med.*, **99**, 355 (1954).

Detergent-solubilised H-2 alloantigen is associated with a small molecular weight polypeptide

THERE have been several reports that purified papain-solubilised HL-A alloantigens contains two polypeptides with molecular weights of approximately 31,000 and 11,500 (refs 1-3). The combined molecular weights of these two polypeptides (42,500) is similar to the molecular weight reported for detergent-solubilised HL-A alloantigens (43,000) (ref. 4). The smaller fragment seems to resemble β_2 microglobulin[3,5], which has an amino acid sequence homologous to that of an immunoglobulin heavy chain constant region domain[6]. This smaller fragment does not seem to contain any alloantigenic activity[5] and is found in association with several different HL-A alloantigenic molecules[1,3]. Further evidence of the close association of these two polypeptides is based on observations that capping or immunoprecipitation of β_2 microglobulin using antisera directed against β_2 microglobulin results in the cocapping[7] or coprecipitation[8] of HL-A molecules. One critical question is whether the HL-A alloantigens and β_2 microglobulin are covalently[5] or noncovalently joined to one another. Our studies on a homologous system, the H-2 complex of the mouse, suggest that mouse H-2 alloantigens are noncovalently associated with a small polypeptide.

We report here that detergent-solubilised H-2 alloantigens from the mouse, purified by immunoprecipitation, contain two polypeptides with molecular weights of 47,000 and 11,500. Since the smaller polypeptide has a molecular weight comparable with that of the β_2 microglobulin molecule associated with HL-A alloantigens, it seems likely that it represents the murine equivalent of β_2 microglobulin. Also, since our solubilisation and purification procedure does not include any known proteolytic action, we propose that the small polypeptide does not represent a covalently-linked component of the H-2 alloantigen, but rather represents a molecule which for unknown reasons has a strong affinity for the major histocompatibility antigen of mice.

Proteins of B10.D2 spleen lymphocytes were labelled with either ^{125}I according to Marchalonis *et al.*[9], or by culturing spleen lymphocytes in Hanks balanced salt solution (Gibco) in the presence of ^{3}H-amino acids for 4–8 h. Briefly, radioiodination was performed by suspending 5×10^7 spleen lymphocytes in 0.5 ml of a phosphate buffered saline (PBS) solution at pH 7.4 which contained 100 μg lactoperoxidase and 5 mCi ^{125}I. Hydrogen peroxide (50 μl of 0.03% solution in PBS) was added and the suspension was incubated for 5 min at room temperature. This was followed by washing of the cells in PBS. Lactoperoxidase-catalysed radioiodination labels cell surface proteins only[9] whereas the short culturing procedure using ^{3}H-amino acids labels all proteins with a rapid turnover rate. Membranes of labelled cells were solubilised by incubation with 0.5% Triton X-100 (Sigma) in a mixture of 0.01 M Tris buffer, pH 7.4 and 0.15 M NaCl for 20 min at 0° C. Labelled immunoglobulin was preprecipitated by the addition of normal mouse serum and rabbit anti-mouse immunoglobulin. H-2 alloantigenic activity was immunoprecipitated as described in Fig. 1. Sodium dodecyl sulphate (SDS) acrylamide gel electrophoresis of the immunoprecipitate of H-2.4 alloantigenic activity in the presence of reducing agent revealed two polypeptide chains with molecular weights of 47,000 and 11,500 respectively, and a third peak of radioactivity which moved with the front, was dialysable, and was present in both the specific and nonspecific immunoprecipitates to the same extent (Fig. 1*a*). When a ^{3}H-amino acid-labelled antigen preparation was used, immunoprecipitates yielded only two radioactive peaks coincident with the ^{125}I-polypeptides (Fig. 1*b*).

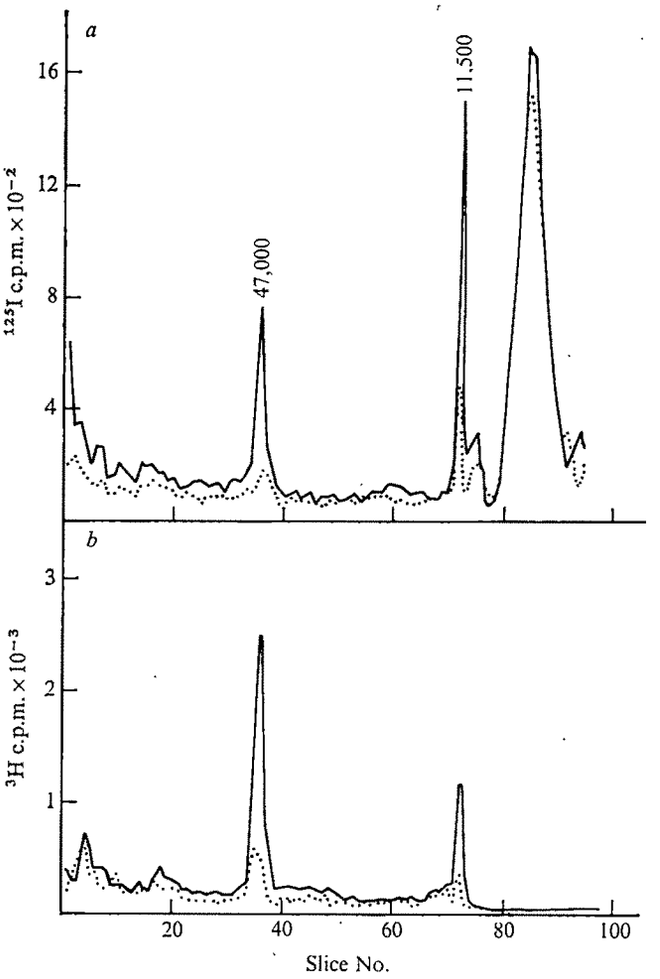


Fig. 1 Antigen preparations labelled with either ^{125}I (*a*) or ^{3}H (*b*) from which labelled immunoglobulins had been removed by preprecipitation were treated with either 4 μl of D-4 H-2 alloantiserum and 16 μl of C57BL/10 normal mouse serum (—) or 20 μl of C57BL/10 normal mouse serum (...). H-2 alloantiserum D-4, which was obtained from the National Institutes of Health, was made in the strain combination (B10. AKM × 129) F_1 anti-B10.A, and is therefore directed against the H-2.4 antigenic specificity of the H-2D region of the H-2^a haplotype. After incubation for 30 min at 0° C, 100 μl of rabbit anti-mouse immunoglobulin was added and incubated at 0° C for 150 min. The resultant precipitate was washed three times, dissolved in 100 μl of Laemmli reducing buffer[10], and electrophoresed on 10% polyacrylamide SDS gels according to Laemmli[10]. Gels were cut into 1 mm thick slices and counted in either a gamma counter (^{125}I) or a liquid scintillation counter (^{3}H). Molecular weights of the major peaks are indicated.

It should be noted that when normal mouse serum was used as a control in the immunoprecipitation method, small radioactive peaks were observed coincident with and in the same relative ratio as those observed using specific anti-H-2 serum. We believe that this is due to a small degree of reactivity of the small polypeptide (presumably the mouse analogue of β_2 microglobulin) with rabbit anti-mouse im-

munoglobulin, and that partial precipitation of this polypeptide by this reagent also results in partial coprecipitation of the H-2 alloantigenic molecule which is associated with it. SDS polyacrylamide gel electrophoresis of immunoprecipitates in the absence of reducing agent also revealed the smaller polypeptide peak, indicating that it was not bound by disulphide bonds to the larger polypeptide.

Our data indicate that the smaller polypeptide present in our immunoprecipitates is strongly associated with H-2 alloantigens, is present on the surface of lymphocytes, is rapidly synthesised, and has a molecular weight of 11,500. All of these properties are consistent with those of the β_2 microglobulin-like molecule associated with papain-solubilised HL-A alloantigens. Sequence analysis of the smaller polypeptide molecule is in progress in our laboratory to determine whether it is indeed the murine analogue of β_2 microglobulin. We feel that further speculation concerning the role of β_2 microglobulin as a component of HL-A alloantigens will require detailed chemical analyses of detergent-solubilised alloantigens.

Our investigations reveal that H-2 alloantigens are closely although noncovalently associated with a smaller polypeptide molecule. The strong affinity which these two membrane proteins exhibit for each other suggests that they may function as complementary receptors on the surfaces of lymphocytes and, accordingly, play a part in cellular recognition and(or) interactions.

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[1] Cresswell, P., Turner, M. J., and Strominger, J. L., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1603–1607 (1973).
[2] Tanigaki, N., Katagiri, M., Nakamuro, K., Kreiter, V. P., and Pressman, D., *Fedn Proc.*, **32**, 1017 (1973).
[3] Peterson P. A., Rask, L., and Lindblom, J. B., *Proc. natn. Acad. Sci. U.S.A.* (in the press).
[4] Springer, T., and Strominger, J. L., *Fedn Proc.*, **32**, 1018 (1973).
[5] Nakamuro, K., Tanigaki, N., and Pressman, D., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2863–2865 (1973).
[6] Peterson, P. A., Cunningham, B. A., Berggard, I., and Edelman, G. M., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1697–1701 (1972).
[7] Poulik, M. D., Bernoco, M., Bernoco, D., and Ceppellini, R., *Science*, **182**, 1352–1355 (1973).
[8] Grey, H. M., Kubo, R. T., Colon, S. M., Poulik, M. D., Cresswell, P., Springer, T., Turner, M., and Strominger, J. L., *J. exp. Med.*, **138**, 1608–1612 (1973).
[9] Marchalonis, J. J., Cone, R. E., and Santer, V., *Biochem. J.*, **124**, 921–927 (1971).
[10] Laemmli, U. K., *Nature*, **227**, 680–685 (1970).

Demonstration of acetylcholine-sensitive adenyl cyclase in malignant neuroblastoma cells in culture

WE have recently reported[1] the existence of dopamine-sensitive adenyl cyclase (AC) in mouse adrenergic neuroblastoma cells which contain tyrosine hydroxylase (TH) but no choline acetyltransferase (ChA); whereas, noradrenaline-sensitive AC at low noradrenaline concentrations becomes apparent in cyclic AMP induced 'differentiated' neuroblastoma cells. The presence of acetylcholine receptors in neuroblastoma cells has been demonstrated by Harris and Dennis[2]. These acetylcholine receptors are blocked by nicotinic and muscarinic inhibitors[3]. We now report the presence of acetylcholine-sensitive AC in addition to dopamine- and noradrenaline-sensitive AC in inactive neuroblastoma cells which contain neither TH nor ChA, and in cholinergic neuroblastoma cells which contain ChA but no TH. Acetylcholine, dopamine and noradrenaline produced an additive stimulatory effect on AC activity, suggesting that these neurotransmitters act on different receptor sites all of which are linked with AC.

The procedures for culturing and maintaining the mouse neuroblastoma cells were previously described[4]. Cells of previously defined[5] clones NBDB$^-$ and NBE^-_A were used. The effects of dopamine, noradrenaline and acetylcholine on the AC activity in homogenates of malignant neuroblastoma cells were measured. Cells (0.5×10^6) were plated in large Falcon plastic flasks (75 cm^2). The medium was changed first at day 2 and then every day thereafter. The samples for enzyme assay were prepared 4 d after plating. The cells were washed twice with phosphate buffer solution (*p*H 7.0), 5 ml of Tris-HCl (*p*H 7.5, 0.05 M) was added to each flask, and then the cells were removed from the flask surface using a Pasteur pipette. The contents of four flasks were pooled, and then the cells were frozen, thawed and homogenised. It is essential that cells are gently homogenised immediately after thawing as a longer interval between thawing and homogenisation decreases the enzyme activity. In addition, the stability of AC under frozen conditions varies from one clone to another. The stability of AC activity in frozen adrenergic cells could be maintained for more than a week; however, in cholinergic cells enzyme activity decreases by about 40% within a week. Therefore, the AC activity was assayed in samples which were frozen for a maximum of 3 d. The AC activity was determined by a modification[6] of the procedure described by Krishna *et al.*[7] Essentially, the formation of ^{3}H-cyclic AMP from ^{3}H-ATP is measured. The assay system was modified to include phosphoenolpyruvate and pyruvate kinase to recycle ADP formed by ATPase activity back to ATP. Also, cyclic AMP (0.8 mM) was included in the assay as a trap for the ^{3}H-cyclic AMP formed during the incuba-

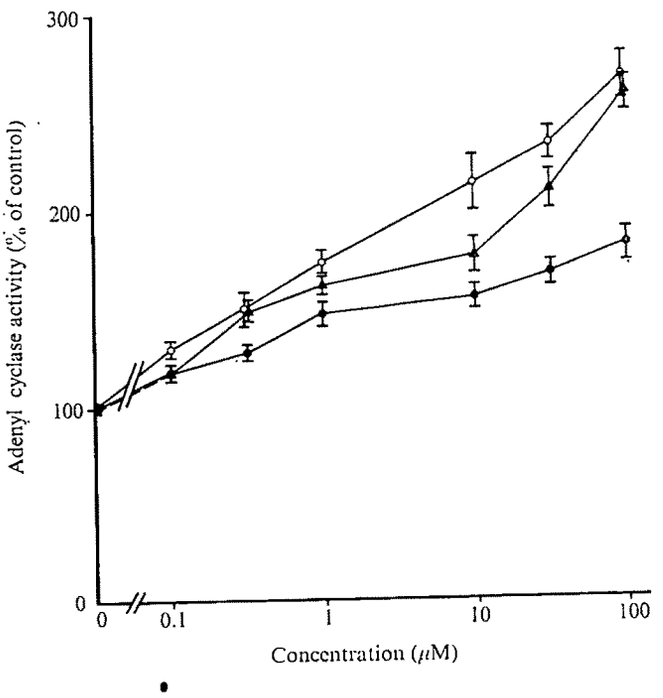


Fig. 1 Changes in adenyl cyclase activity (AC) in homogenates of inactive neuroblastoma cells. The basal activity of AC (22±2 pmol per mg protein per min) was considered 100% control values and the AC values of treated cells were expressed as percent of control. Each value represents an average of four to six samples. The bar at each point is standard deviation. Treatment with: ○, dopamine; ▲, acetylcholine; ●, noradrenaline.

tion. The incubation period for each sample was 15 min. The absorbance at 260 nm of the purified cyclic AMP fraction serves as a measure of recovery of ^{3}H-cyclic AMP. The formation ^{3}H-cyclic AMP was linear with time up to 30 min at 37° C and linear with protein concentration over the range 0.05–1.0 mg ml^{-1}. The total volume of the incubation mixture was 1.0 ml. The protein was determined by the method of Lowry *et al.*[8]. Data were expressed as pmol cyclicAMP formed per mg protein per min.

The procedures for making solutions of dopamine, noradrenaline and prostaglandin (PG)E$_1$ were previously described[1]. To study the nature of receptors, propranolol (blocks β-receptors), phentolamine (blocks α-receptors), haloperidol (blocks dopamine-receptors), atropine (blocks acetylcholine-muscarinic receptors), hexamethonium and nicotine (blocks acetylcholine-nicotinic-receptors) were used. These agents were dissolved in 0.01 N HCl. Nicotine was dissolved in 70% ethyl alcohol. In addition, analogues of acetylcholine such as bethanechol and carbachol were used, since they are virtually resistant to acetylcholinesterase or non-specific cholinesterase. Carbachol stimulates both muscarinic and nicotinic receptors, whereas bethanechol has primarily nicotinic activity.

Figure 1 shows that low concentrations of acetylcholine, dopamine and noradrenaline stimulated AC activity in homogenates of inactive neuroblastoma cells. Noradrenaline was much less active than either dopamine or acetylcholine. A maximal stimulation of AC activity was achieved by 100 μM of any of these neurotransmitters; however, noradrenaline produced only 80% stimulation, whereas acetylcholine and dopamine produced about 160% stimulation in AC activity. None of the above neurotransmitters at a higher concentration (1,000 μM) produced any significant effect on the basal activity of AC.

Acetylcholine (100 μM) also stimulated AC activity in the cholinergic neuroblastoma cells to about the same extent as that produced in the inactive neuroblastoma cells (data not shown).

Table 1 shows that PGE$_1$ and serotonin also stimulated AC activity; however, adrenaline and histamine had no significant effect. Bethanechol and carbachol also stimulated AC activity, but less than did acetylcholine. The reasons for acetylcholine being more effective than bethanechol and carbachol are not known. When these agents were combined individually with acetylcholine, they produced an additive stimulatory effect on AC activity. The combination acetylcholine with dopamine or noradrenaline or both produced an additive stimulatory effect on AC activity, indicating that these agents act on the different receptors. Acetylcholine-stimulated AC activity was markedly reduced by atropine, nicotine, and hexamethonium; but it was not significantly affected by haloperidol, phentolamine and propranolol (Fig. 2). Dopamine and noradrenaline stimulated AC activity was markedly inhibited by 10 μM of haloperidol and propranolol, respectively (data not shown). Atropine also reduced dopamine-stimulated AC activity; however, it reduced noradrenaline-stimulated AC activity only slightly (Fig. 3). Atropine, hexamethonium, nicotine, haloperidol, propranolol or phentolamine alone produced no significant effect on the basal activity of AC.

Table 1 Effect of combination of various neurotransmitters on adenyl cyclase activity in homogenates of neuroblastoma cells (NBDB$^-$)

Treatment	Adenyl cyclase activity (pmol per mg protein per min)
Control	22 ± 2*
Dopamine (100 μM)	58 ± 3
Noradrenaline (100 μM)	40 ± 1
Adrenaline (100 μM)	26 ± 2
Acetylcholine (100 μM)	57 ± 4
Bethanechol (100 μM)	36 ± 2
Carbachol (100 μM)	31 ± 2
Acetylcholine + bethanechol	88 ± 3
Acetylcholine + carbachol	77 ± 2
Dopamine + acetylcholine	100 ± 2
Noradrenaline + acetylcholine	92 ± 5
Dopamine + noradrenaline + acetylcholine	133 ± 3
Serotonin (100μM)	24 ± 2
Histamine (100μM)	24 ± 2
Prostaglandin E$_1$ (10 μM)	52 ± 3

*Standard deviation.
Change in adenyl cyclase activity after treatment with various neurotransmitters in homogenates of inactive neuroblastoma cells. Each value represents an average of four to six samples.

The reason for the marked reduction in dopamine-stimulated AC activity by atropine is unknown. Many blocking agents do not seem to be as specific as they have been presumed to be. For example, AC activity of neuroblastoma cells (unpublished observation) and of caudate nucleus[9] is blocked by chloropramazine in a non-specific way. Haloperidol which is supposed to be specific inhibitor for dopamine-receptors also prevents noradrenaline-stimulated cyclic AMP level in rat brain slices[10].

The fact that the muscarinic and nicotinic inhibitors of acetylcholine-receptors block acetylcholine-stimulated AC activity indicates that AC may be linked with both types of receptors. It has been suggested[11] that nicotinic receptors are linked with AC, whereas muscarinic receptors are linked with guanyl cyclase. This concept does not appear to be applicable to this neuroblastoma clone.

Since dopamine and noradrenaline do not increase the cyclic AMP level until the phosphodiesterase activity is inhibited[12], these neurotransmitters may not mimic the effect of cyclic AMP in causing differentiation of neuroblastoma cells. Indeed, when the cells were exposed to dopamine and noradrenaline for 1 h, no differentiation occurred[13], although dopamine induced reversible inhibition of cell division. A long drug-exposure was not feasible because these agents are auto-oxidised in solution. Acetylcholine (100 μM) did not produce any significant change on growth or differentiation (data not shown).

Our results demonstrate the presence of acetylcholine-sensitive AC in addition to dopamine- and noradrenaline-sensitive AC in inactive and cholinergic neuroblastoma cells. These cells thus differ from adrenergic cells in which acetylcholine-sensitive AC activity is not demonstrable and noradrenaline-sensitive AC activity does not become

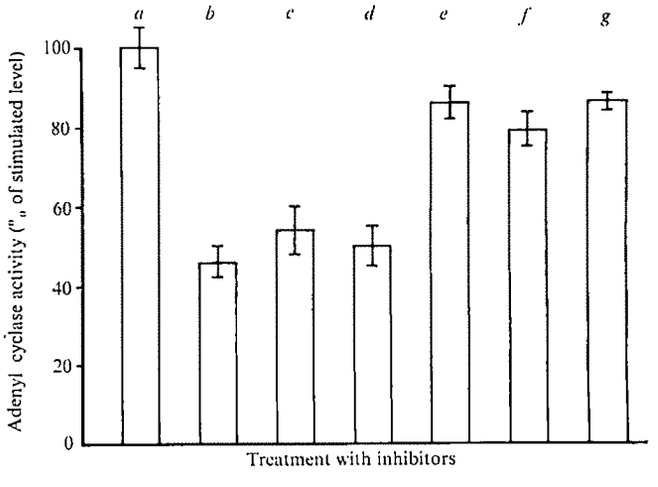


Fig. 2 Effect of various inhibitors of receptors on acetylcholine-stimulated adenyl cyclase activity in homogenates of inactive neuroblastoma cells. A concentration of 30 μM of *b*, atropine; *c*, hexamethonium; *d*, nicotine; *e*, haloperidol; *f*, phentolamine or *g*, propranolol was added immediately before the addition of ACh (100 μM). *a*, The acetylcholine-stimulated activity of adenyl cyclase (57±4 pmol per mg protein per min) was considered as 100%, and the values of AC in the presence of inhibitors were expressed as a percentage of the stimulated value. Each value represents an average of four to six samples. The bar at each point is standard deviation.

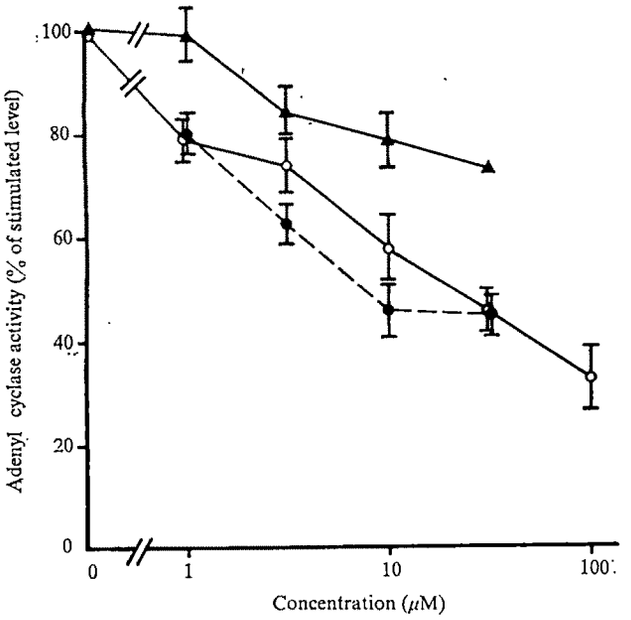


Fig. 3 Effect of atropine on acetylcholine (○)-, dopamine (●)- and noradrenaline (▲)-stimulated activity of adenyl cyclase (AC) in homogenates of inactive neuroblastoma cells. Atropine was added at various concentrations immediately before the addition of acetylcholine (100 μM) dopamine (100 μM) or noradrenaline (100 μM). The stimulated value of AC for dopamine (58±3 pmol per mg protein per min), for acetylcholine (57±4 pmol per mg protein per min) and for noradrenaline (40±1 pmol per mg protein per min) was used as 100%, and the values of AC activities in atropine-treated homogenates were expressed as a percentage of the stimulated value. Each value represents an average of four to six samples. The bar at each point is standard deviation.

apparent until the cells are differentiated as a result of raised cyclic AMP levels[4,12,14,15]. It is interesting that the application of dibutyryl cyclic AMP or acetylcholine into the lateral hypothalamic area of rat greatly increased water intake[16]. Atropine completely inhibited food and water intake induced by dibutyryl cyclic AMP[16].

The fact that the adenyl cyclase retains its sensitivity to low concentrations of acetylcholine and dopamine in homogenates of neuroblastoma cells suggests that this system may provide a sensitive method in search of new agents that can mimic or antagonise the effect of catecholamines and acetylcholine on mammalian neurones.

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1 Prasad, K. N., and Gilmer, K. N., *Proc. natn. Acad. Sci. U.S.A.* (in the press).
2 Harris, A. J., and Dennis, M. J., *Science*, **167**, 1253 (1970).
3 Simantov, R., and Sachs, L., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2902 (1973).
4 Prasad, K. N., and Hsie, A. W., *Nature new biol.*, **233**, 141 (1971).
5 Prasad, K. N., Mandal, B., Waymire, J. C., Lees, G. J., Vernadakis, A., and Weiner, N., *Nature new Biol.*, **241**, 117 (1973).
6 Perkins, J. P., and Moore, M. M., *J. biol. Chem.*, **246**, 62 (1971).
7 Krishna, G., Weiss, B., and Brodie, B. B., *J. Pharm. exp. Ther.*, **163**, 379 (1968).
8 Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., *J. biol. Chem.*, **193**, 265 (1951).
9 Kebabian, J. W., Petzold, G. L., and Greengard, P., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2145 (1972).
10 Uzunov, P., and Weiss, B., in *Advances in Cyclic Nucleotide Research* (edit. by Greengard, P., Robison, G. A. and Paoletti, R.), **1**, 435 (Raven Press, New York, 1972).
11 Guidotti, A., Mao, C. C., and Costa, E., in *Proc. third int. Catecholamines Symposium, Strasbourg, France* (1973).
12 Prasad, K. N., Sahu, S. K., and Kumar, S., in *Differentiation and Control of Malignancy of Tumor Cells* (Gann Monograph on Cancer Research, Tokyo, in the press).
13 Prasad, K. N., *Cancer Res.*, **31**, 1457 (1971).
14 Prasad, K. N., in *The Role of Cyclic Nucleotides in Carcinogenesis* (edit. by Gratzner, H., and Schultz, J.), **6**, 207 (Academic Press, New York, 1973).
15 Prasad, K. N., and Kumar, S., in *Control of Proliferation in Animal Cells* (edit. by Clarkson, B., and Baserga, R.), (Cold Spring Harbor Laboratory, Cold Spring Harbor, in the press).
16 Sciorelli, G., Poloni, M., and Rindi, G., *Brain Res.*, **48**, 427 (1972).

Effect of cyclic AMP, caffeine and theophylline on differentiation of lens epithelial cells

Papaconstantinou has clearly shown that differentiation of lens epithelial cells into fibre cells involves a complex series of morphological and biochemical events[1]; however, at the molecular level the factors involved in the initiation of this differentiation are almost entirely unknown. The purification and characterisation of the mRNA species specifying bovine lens proteins[2] have made desirable the development of a system in which details of lens differentiation could be studied. Such a system for the study of lens epithelial cell differentiation in tissue culture should have the following characteristics: (1) The differentiation should demonstrate characteristic morphological changes which can be correlated with those expected of the process *in vivo;* (2) specific proteins characteristic of the differentiated tissue should be absent in epithelial cells inoculated into the culture and should appear during the course of the morphological changes; (3) it should be possible to maintain the epithelial cells in culture in an undifferentiated state by the criteria of unaltered morphology and the absence of specific proteins), and to induce them to differentiate in response to a specific set of conditions; (4) clones of epithelial cells should show characteristics similar to freshly explanted epithelial cells. In addition to these criteria, a useful characteristic of the system would be an accelerated or inhibited differentiation in response to hormones of cyclic nucleotides.

In establishing such a system, both the choice of protein to be monitored as an indication of differentiation and the tissue to be cultured may be critical. We observed in a previous study[3] by immunofluorescent staining that epithelial cells at early stages of lens development in the rat do not contain γ-crystallins. In recent (unpublished) work we have found that when epithelial cells from embryonic or newborn rat lenses are cultured, no γ-crystallins can be found initially when fixed monolayer cultures of freshly explanted epithelial cells are stained by indirect immunofluorescence, but that subsequently such cells grow and differentiate morphologically, and in 5 d may be stained positively for γ-crystallins.

One of the most marked features of the monolayer cultures is the stimulation of cell growth and acceleration of the appearance of the γ-crystallins by dibutyryl cyclic AMP. At 24 h, in contrast to controls (Fig. 1*a*), monolayers treated with dibutyryl cyclic AMP (Fig. 1*b*) or caffeine (over the concentration range 10^{-6} to 10^{-3} M) exhibited patches of confluent growth and all the single cells were elongated in patterns similar to those observed for control cells only after three

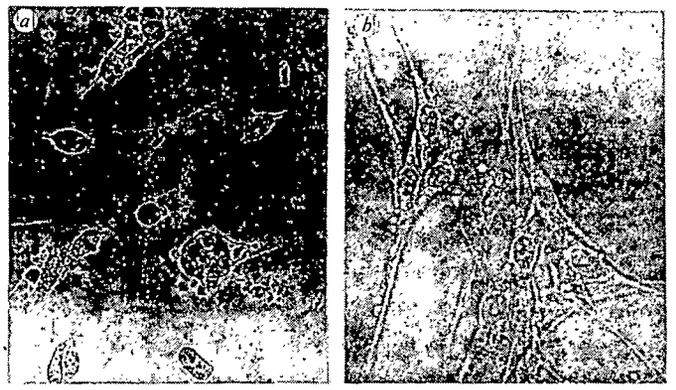


Fig. 1 Lens epithelial cells were removed from 1-d-old rat lens by 30 min treatment in medium 199 containing 0.08% trypsin at 37° C. A suspension culture of medium washed cells were incubated at 37° C in screw-capped shaker culture flasks. The cells were grown in suspension culture medium 199 containing 20% foetal calf serum, 0.1% D-L glutamine, penicillin G-sodium 100 U ml^{-1} and dihydrostreptomycin sulphate 100 μg ml^{-1}. The *p*H was adjusted to 7.1 with sodium bicarbonate. After 5 d growth cells were inoculated in fresh medium into tissue culture chamber slides (Lab-Tek) at a density of 3.3×10^8 ml^{-1}. This high cell concentration was necessary since only 10–12% of the cells will attach to the glass. After 6 h the unattached cells were removed by sterile aspiration of the media and fresh media added. Monolayer cultures were incubated at 37° C, 100% humidity in an atmosphere of 5% CO_2–95% air. Medium (0.8 ml per chamber) was changed after 2 d and subsequently every 2 d. All test substances were present from the time of monolayer inoculation. Phase contrast micrographs were taken under white light 24 h after inoculation (×370.50). *a*, Normal cultures of lens epithelial cells; *b*, culture treated with dibutyryl cyclic AMP (1×10^{-5} M).

days of growth. After 48 h in such treated monolayers strong γ-crystallin immunofluorescence was detected. No cell elongation or areas of confluent growth were found in monolayers incubated with butyric acid, cyclic GMP or theophylline (also over the concentration range 10^{-6} to 10^{-3} M), but caffeine potentiated the effect of cyclic AMP.

This effect is in marked contrast to the usual inhibitory effect of high concentrations of cyclic AMP on the growth of a number of cell lines[4–8], and on the differentiation of an established myogenic cell line[4]. It occurs at much lower concentrations of cyclic nucleotide than used to stimulate differentiation of the mouse neuroblastoma cell line (5 mM) (ref. 9), or the temporary stellate configuration in adult newt iris cells[10]. It is likely that a growth response to cyclic nucleotides is typical of embryonic or neonatal cells[11], but the additional possibility of inducing differentiation in rat lens epithelial cells is expected to be valuable in exploring differentiation at the molecular level.

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1 Papaconstantinou, J., *Science*, **156**, 338–346 (1967).
2 Berns, T. J. M., Schreurs, V. A. M., van Kraaikamp, M. W. G., and Bloemendal, H., *Eur. J. Biochem.*, **33**, 551–557 (1973).
3 Shubert, E. E., Trevithick, J. R., and Hollenberg, M. J., *Can. J. Ophthamol.*, **5**, 353–365 (1971).
4 Wahrmann, J. P., Winand, R., and Luzatti, D., *Nature new Biol.*, **245**, 112–113 (1973).
5 Daniel, V., Litwack, G., and Tomkins, G. M., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 76–79 (1973).
6 Ryan, W. L., and Hudreck, M. L., *Science*, **162**, 1484–1485 (1968).
7 Johnson, G. S., and Pastan, I., *J. natn. Cancer Inst.*, **48**, 1377–1387 (1972).
8 Bombik, B. M., and Burger, M. M., *Expl Cell Res.*, **80**, 88–94 (1973).
9 Prasad, K. N., and Hsie, A. W., *Nature new Biol.*, **233**, 141–143 (1971).
10 Ortiz, J. R., Yamada, T., and Hsie, A. W., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2286–2290 (1973).
11 Hovi, T., and Vaheri, A., *Nature new Biol.*, **245**, 175–176 (1973).

New unstable haemoglobin (Hb Moscva, β24 (B4) Gly→Asp) found in the USSR

THE unstable haemoglobins form a well-characterised group of human mutants which have been observed in many parts of the world[1]. We have recently cooperated in the study of two such haemoglobins seen in Moscow. Haematological and clinical aspects have been published in Russia[2,3]. One of the haemoglobins, discovered in a mildly anaemic patient who had pancreatitis (and in his son), was found to be Hb Tacoma (β30 (B12) Arg → Ser), previously described in three members of a United States Caucasian family[4]. As this haemoglobin has already been fully characterised[5] it will not be discussed further here.

The second is a new variant, Hb Moscva, with the amino acid substitution β24 (B6) Gly → Asp. It was found in a patient who suffered from myeloid leukaemia and who has since died, but it was possible to send her blood to Cambridge for further study. Incubation of the blood with brilliant cresyl blue[6] resulted in the appearance of numerous Heinz bodies. The heat instability test[7] and incubation with isopropanol[8] both caused the formation of a red precipitate confirming the presence of an unstable haemoglobin. Subsequently an unstable haemoglobin was also found in the patient's half sister (by the maternal line), and the sister's son.

Hb Moscva migrated more rapidly towards the anode than Hb A on starch gel electrophoresis at *p*H 8.6 (ref. 9) and could be removed from the haemolysate, before electrophoresis, by heat precipitation[7]. There was only a single Hb A_2 fraction indicating that the abnormality was probably in the β, rather than the α chains. The proportion of Hb A_2 (4.1%) was slightly above the normal range (2.5 – 3.5%). The abnormal component, which represented 17% of the total haemoglobin, was isolated in the carbonmonoxy form on DEAE Sephadex[10] and further purified, for structural studies, by paper electrophoresis[11].

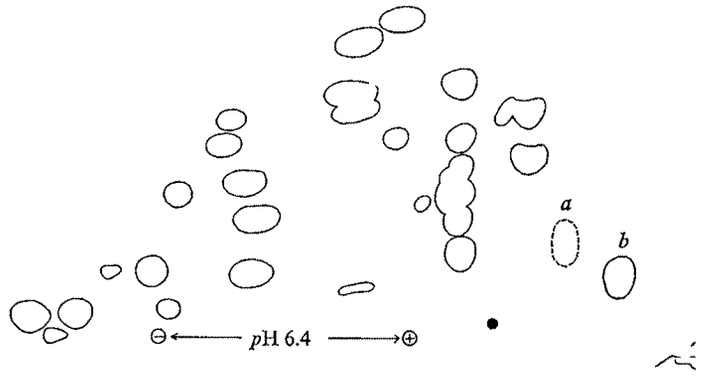


Fig. 1 Fingerprint of the soluble tryptic peptides of the globin from Hb Moscva. Electrophoresis at *p*H 6.4, 53 Vcm^{-1} for 1 h; ascending chromatography in pyridine–isoamyl alcohol–water (6:6:7, by volume) for 18 h. Peptides were located with 0.2% (w/v) ninhydrin containing 1% (v/v) pyridine and with the specific staining reagent (Sakaguchi) for arginine-containing peptides. ●, Origin; *a*, position of β^{A}TpIII; *b* = β^{Moscva}TpXIII. The variant βTpIII was isolated by paper electrophoresis at *p*H 6.4 (61 V cm^{-1}, 1 h).

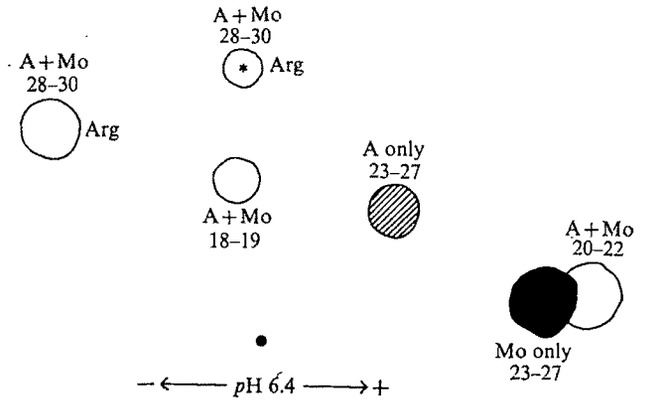


Fig. 2 Fingerprint of the thermolysin peptides of βᴬTpIII (A) and βTpIII from Hb Moscva (Mo). Electrophoresis at *p*H 6.4, 53 V cm^{-1} for 45 min; ascending chromatography and location of peptides as in Fig. 1. The peptide β^{Moscva}23–27 was purified from β20–22, for sequence studies, by paper electrophoresis at *p*H 9 (1% (w/v) $(NH_4)_2CO_3$, 44 V cm^{-1}, 1 h). ●, Origin.

*An electrically neutral peptide with an amino acid composition similar to that of the positively-charged peptide β28–30 (Leu-Gly-Arg); it amounted to 6% of total β28–30 and was thought to represent a minor reaction product, produced between ninhydrin and the guanidinium side chain of arginine β30, during location of βTpIII after its preparation on paper.

On the fingerprint of the tryptic digest of Hb Moscva, prepared by standard techniques[12], peptide βᴬTpIII (β18–30) (*a* in Fig. 1) was replaced by a new, more negatively charged peptide (*b* in Fig. 1). The amino acid composition of the new peptide (Table 1) resembled that of βᴬTpIII except for a substitution of one of the three glycine residues β24, 25 or 29, (Table 2) by an aspartic acid residue. Asparagine is converted to aspartic acid during the hydrolysis which precedes amino acid analysis; a Gly → Asn substitution could, however, be excluded because of the increased negative charge of the variant peptide.

To determine which of the three residues had been replaced, the variant βTpIII was digested with thermolysin and the digest fingerprinted at *p*H 6.4. Comparison of the fingerprint with that from βᴬTpIII (Fig. 2) showed that the peptide βᴬ23–27 (Val-Gly-Gly-Glu-Ala) had been replaced by another, more negatively charged peptide, the amino acid composition of which (Table 1) indicated that one residue of glycine (β24 or β25) had been replaced by one of aspartic acid. Dansyl-Edman degradation[13] showed the sequence β23–25 to be Val-Asp-Gly and the amino acid substitution in Hb Moscva, therefore, to be β24 (B6) Gly → Asp.

Table 1 Amino acid composition of the tryptic peptides β18-30 and the thermolysin peptides β23–27 from Hb A and Hb Moscva.

Amino acid	Peptide			
	βTpIII	(β18–30)	β23–27	
	A	Moscva	A	Moscva
Asp	2	3.0	—	0.9
Glu	2	2.0	1	1.0
Gly	3	2.0	2	1.0
Ala	1	1.2	1	1.0
Val	3	3.0	1	1.0
Leu	1	1.2		
Arg	1	1.1		

Peptides were hydrolysed with constant-boiling HCl for 18 h at 108° C in sealed evacuated tubes. After removal of the excess HCl in a rotary evaporator, the hydrolysates were analysed using a Locarte amino acid analyser.

Measurements of the oxygen affinity of isolated Hb Moscva showed it to have a slightly lower oxygen affinity than Hb A (Fig. 3), but with a near normal Bohr effect and haem–haem interaction.

All known normal globin chains have a Gly in position B6, with four exceptions (α-chains of mouse NB and grey kangaroo, one of the midge and one of the plant haemoglobins)[14,15]. M.F Perutz has examined, with us, the possible consequences of the substitution in Hb Moscva using his atomic model of human haemoglobin. B6 is in a densely packed region of the molecule where a side chain could not be easily accommodated without forcing apart the B and E helices. In the three unstable haemoglobins Riverdale-Bronx[16] Savannah[17] and Moscva, the glycine residue β24 (B6) is replaced by one of arginine, valine and aspartic acid, respectively. It is probable that these substitutions disturb the spatial relationships of the haem with the adjacent polypeptide chain; for example, J. Peisach observed that the EPR spectrum of met–Hb Riverdale-Bronx differs from that of met–Hb A (cited in ref. 16). Hb Savannah shows an increased dissociation into αβ dimers[17], suggesting that the α1β2 contact may be indirectly affected.

It is not possible to predict how these substitutions may affect the oxygen affinity. Hb Riverdale-Bronx shows, in contrast to Hb Moscva, a higher oxygen affinity than HbA (P. A. L., un-

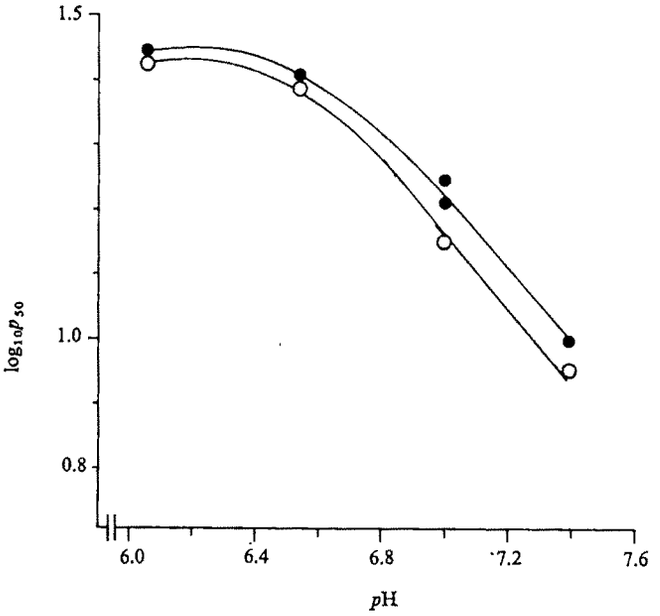


Fig. 3 Values of $\log_{10}p_{50}$ for, ○, Hb A and, ●, Hb Moscva at various *p*H values; p_{50} is the pressure of O_2 in mm Hg required to attain half saturation of the haemoglobin. The CO-Hb was concentrated to about 5% (w/v) by ultrafiltration at 4° C and then equilibrated with 0.05 M potassium phosphate buffer *p*H 8.6, by gel filtration on a 1.0 × 9.0 cm column of Sephadex G25 (coarse). The CO-Hb was converted to the oxy-form[18] and oxygen dissociation curves were recorded by the automatic method of Imai *et al.*[19] using 0.1% (w/v) haemoglobin solutions in 0.1 M potassium phosphate buffers of various *p*H values containing 0.5 mM EDTA. The measurements were made at a temperature of 23° C using monochromatic light of wavelength 474 nm. The values of p_{50} were determined from the dissociation curves and the values of *n*, the exponent of the Hill equation, were determined from the slope of the Hill plots. The curves were nearly parallel showing that the Bohr effect, which is represented by the slope of the curve $d(\log_{10}p_{50})/d(pH)$, is the same for Hb Moscva and Hb A.

Table 2 Amino acid sequence of the peptide βTpIII from Hb A and Hb Moscva

Residue No.	18	19	20	21	22	23	24	25	26	27	28	29	30
Helical No.	A15	B 1	B 2	B 3	B 4	B 5	B 6	B 7	B 8	B 9	B10	B11	B12
Residues													
Hb A	Val	Asn	↓Val	Asp	Glu	↓Val	Gly	Gly	Glu	Ala	↓Leu	Gly	Arg
Hb Moscva	Val	Asn	↓Val	Asp	Glu	↓Val	Asp	Gly	Glu	Ala	↓Leu	Gly	Arg

The arrows indicate the positions of hydrolysis by thermolysin (1 mg μmol^{-1} peptide); the bond between Glu 22 and Val 23 was only 52% hydrolysed in 5 h at 37° C but was completely split in 8 h at 50° C.

published) but both have a near-normal Bohr effect and haem–haem interaction.

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Note added in proof: After this manuscript was submitted, we identified a third unstable haemoglobin from Russia. Its substitution was β106 (G8) Leu–Pro. This variant has been found independently in the United States in two unrelated persons as Hb Casper[20] and in the United Kingdom in one individual as Hb Southampton[21].

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1 Lehmann, H., and Carrell, R. W., *Br. Med. Bull.*, **25**, 14–23 (1969).
2 Idelson, L. I., Didkovsky, N. A., Volkova, M. A., Fleischman, E. V., Troitskaya, O. V., Margulis, M. I., and Filippova, A. V., *Problemy Gemat. Pereliv. Krovi*, **15**, 29–31 (1971).
3 Didkovsky, N. A., Filippova, A. V., and Idelson, L. I., *Problemy. Gemat. Pereliv. Krovi*, **17**, 38–45 (1972).
4 Baur, E. W., and Motulsky, A. G., *Humangenetik*, **1**, 621–634 (1965).
5 Brimhall, B., Jones, R. T., Baur, E. W., and Motulsky, A. G., *Biochemistry*, **8**, 2125–2129 (1969).
6 Rigas, D. A., and Koler, R. D., *Blood*, **18**, 1–17 (1961).
7 Dacie, J. V., Grimes, A. J., Meisler, A., Steingold, L., Hemsted, E. H., Beaven, G. H., and White, J. C., *Br. J. Haemat.*, **10**, 338–402 (1964).
8 Carrell, R. W., and Kay, R., *Br. J. Haemat.*, **23**, 615–619 (1972)
9 Poulik, M. D., *Nature*, **180**, 1477–1479 (1957).
10 Huisman, T. H. J., and Dozy, A. M., *J. Chromat.* **19**, 160–169 (1965).
11 Cradock-Watson, J. E., Fenton, J. C. B., and Lehmann, H., *J. clin. Path.*, **12**, 372–373 (1959).
12 Sick, K., Beale, D., Irvine, D., Lehmann, H., Goodall, P. T., and MacDougall, S., *Biochim. biophys. Acta*, **140**, 231–242 (1966).
13 Gray, W. R., *Meth. Enzymol.*, **11**, 469–475 (1967).
14 Dayhoff, M. O., *Atlas of protein sequence and structure*, **5**, (The National Biomedical Research Foundation, Silversprings, Maryland, 1972).
15 Romero-Herrera, A. E., Lehmann, H., Joysey, K. A., and Friday, A. E., *Nature*, **246**, 389–395 (1973).
16 Ranney, H. M., Jacobs, A. S., Uden, L., and Zalusky, R., *Biochem. biophys. Res. Commun.*, **33**, 1004–1011 (1968).
17 Huisman, T. H. J., Brown, A. K., Efremov, G. D., Wilson, J. B., Reynolds, C. A., Uy, R., and Smith, L. L., *J. clin. Invest.*, **50**, 650–659 (1971).
18 Kilmartin, J. V., and Rossi-Bernardi, L., *Biochem. J.*, **124**, 31–45 (1971).
19 Imai, K., Morimoto, H., Kotani, M., Watari, H., Hirati, W., and Kuroda, M., *Biochim. biophys. Acta*, **200**, 189–196 (1970).
20 Jones, R. T., Koler, R. D., Duerst, M., and Stocklen, Z., *Hemoglobin and Red Cell Structure and Function* (edit. by Brewer, G.), 79 (Plenum, New York, 1972).
21 Hyde, R. D., Hall, M. D., Wiltshire, B. G., and Lehmann, H., *Lancet*, ii, 1170–1172 (1972).

Endocytotic formation of rat brain synaptic vesicles

THE present knowledge and theories regarding the fate of synaptic vesicles was reviewed by Holtzman *et al.*[1]. Using electron microscopy it has been found that a marker protein (horseradish peroxidase) was incorporated into vesicles of frog neuromuscular junctions by microinvagination of the synaptic plasma membrane[2–5] and also into brain synapses[6]. Heuser and Reese[2] have put forward a model postulating recirculation of synaptic vesicle structural materials. Thus, during release of neurotransmitter into the synaptic cleft the vesicle membrane should fuse with the presynaptic membrane, and then vesicles could be reformed by invaginations of the synaptic plasma membrane distant from the synaptic cleft.

We have investigated whether synaptic vesicles could be labelled after intracerebral injection of a marker protein. Rats were injected intracisternally with bovine serum albumin (BSA) and after isolation of the synaptic vesicle fraction and specific quantitative assay of its BSA content, the synaptic vesicles were found to be enriched with the label in an endocytotic process dependent on time and affected by barbiturates.

Thirty-five-day-old rats in groups of eight were injected with 2.2 mg BSA in 10 μl 0.9% NaCl, into the cisternae magna by the suboccipital route. The rats were anaesthetised with equal volumes of oxygen and carbon dioxide during the injection. They recovered less than half a minute after removal of the anaesthetic. After 5, 120 or 240 min the rats were decapitated. Their brains were homogenised in isotonic sucrose: and by sedimentation rate separations crude synaptosomal–mitochondrial subfractions (cmf) were produced. These were osmolysed in water and separated on a density gradient by ultracentrifugation[7,8]. The 0.2/0.4 M sucrose density gradient interface and the 0.4/0.6 M interface were removed, diluted with water and centrifuged to give synaptic vesicles (ves) and synaptic vesicles heavily contaminated with microsomes (m-ves), respectively. The ves and m-ves layers have been identified by electron microscopy[9] and by analysis of an antigen characteristic of vesicles[7]. No differences were found between the three labelling periods with respect to the amount of vesicle antigen. The brain homogenate (hom) containing both free and bound BSA, and the subcellular fractions cmf, ves and m-ves containing only bound BSA were solubilised in Triton X-100 and analysed for BSA content by rocket immunoelectrophoresis.

Figure 1 shows that the synaptic vesicle subcellular fractions are enriched in BSA both compared with the parent cmf fraction and the microsome-contaminated m-ves fraction. The enrichment of BSA in the synaptic vesicle fraction was greatest at 120 min labelling time, but the decrease with 240 min labelling time paralleled the decrease in hom and cmf. When BSA was added directly to prepared, until then unlabelled, hom and cmf fractions, the specific BSA content in subsequent prepared ves fractions was less than 0.08 μg BSA per mg protein (lower detection limit), ruling out unspecific adhesion to subcellular particles.

We have compared the specific BSA content in the synaptic vesicle fraction between control groups, and groups of rats

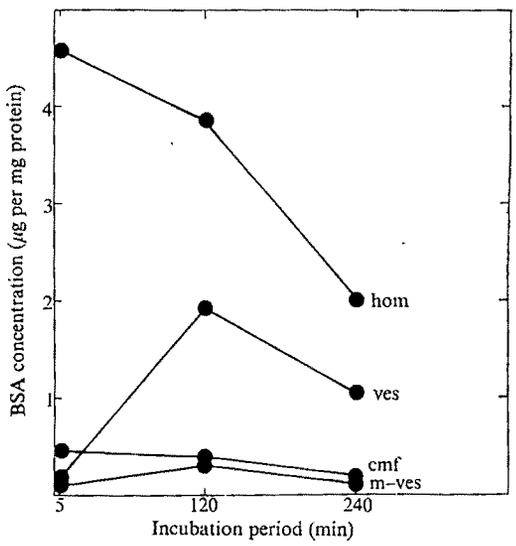


Fig. 1 Specific concentration of suboccipital injected BSA in fractions of rat brains at different incubation periods. Subcellular particles were solubilised in 2% Triton X-100, 10 mM phosphate, 1 mM EDTA and 1% Trasylol (a protease inhibitor), adjusted to *p*H 8.5. After 2 h at 4° C the supernatant from an ultracentrifugation at 6,000,000*g* × min was used for rocket immunoelectrophoresis. Here we used 0.17 μl rabbit anti-BSA cm^{-2}, detecting between 5 and 400 ng BSA in samples of 20 μl solubilised material[10].

anaesthetised with barbiturate during a labelling period of 120 min. The specific contents of BSA in ves from rats treated with barbiturate (Nembutal) were only 66% of that of the controls.

As the vesicles were enriched compared with both parent (cmf) and sister (m-ves) fractions assayed, the BSA-containing particles must be an integral part of the ves fraction. The BSA uptake may be explained by pinocytotic *de novo* formation of the vesicles from the synaptic plasma membrane as well as by transitory fusion of preformed vesicles with the synaptic membrane during release of transmitter into the synaptic cleft[11]. But taken together with the electron microscopic observations of micropinocytosis in the central nervous sytem, these results indicate that at least a part of the synaptic vesicles have been formed or reformed from the synaptic plasma membrane in an endocytotic process. Anaesthesia decreased the BSA content of the vesicles to 66% possibly by impeded vesicle formation. It has also been shown that electrical stimulation of peripherial nerves increased the endocytotic uptake of horseradish peroxidase[3]. Thus, it should be possible to include the quantitative measurement of endocytotic formation of brain synaptic vesicles in the armament of the neuropharmacologist.

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1 Holtzman, E., Teichberg, S., Abrahams, S. J., Citkowitz, E., Crain, S. M., Kawai, N., and Peterson, E. R., *J. Histochem. Cytochem.*, **21**, 349-385 (1973).
2 Heuser, J. E., and Reese, T. S., *J. Cell Biol.*, **57**, 315-344 (1973).
3 Ceccarelli, B., Hurlbut, W. P., and Mauro, A., *J. Cell Biol.*, **57**, 499-524 (1973)
4 Ceccarelli, B., Hurlbut, W. P., and Mauro, A., *J. Cell Biol.*, **54**, 30-38 (1972).
5 Clark, A. W., Hurlbut, W. P., and Mauro, A., *J. Cell Biol.*, **52**, 1-14 (1972).
6 Turner, P. T., and Harris, A. B., *Nature*, **242**, 57-58 (1973).
7 Bock, E., Jorgensen, O. S., and Morris, S. J., *J. Neurochem* (in the press).
8 Whittaker, V. P., and Sheridan, M. N., *J. Neurochem.*, **12**, 363-372 (1965).
9 Formby, B., Hvidt, Aa., Morris, S. J., and Vive-Larsen, J., *Biochim. biophys. Acta*, **279**, 407-415 (1972).
10 Weeke, B., *Scand. J. Immun.*, **2**, suppl. 1, 37-46 (1973).
11 Whittaker, V. P., Dowdall, M. J., and Boyne, A. F., *Biochem. Soc. Symp.*, **36**, 49-68 (1972).

Chemical transfer of learned colour discrimination in goldfish

THE literature describing chemical transfer of learned information has been characterised by contradictory findings during the past ten years[1-3].

We have, however, recently managed to obtain reliable and repeatable results with goldfish, and we report below two experiments which were designed to test the specificity of chemical information transfer.

The reliability and direction of transfer has been reported to be influenced by the amount and type of extract injected, the amount of training of the donor and recipient animals and the time between injection and test[4-7]. These same variables have been important in our own work with goldfish[8]. We have shown, for example, that very small concentrations (1 μg) of nucleic acid extracts from trained donors produce stronger transfer effects than higher concentrations, when compared with injections of the same concentrations from untrained animals. Overtraining of the donor animals also has been shown to have marked effects on the degree of transfer in recipient animals[9].

The avoidance learning task in our experiments was similar to the task required of fish in the shuttle-box described by Horner *et al.*[10] except that two coloured lamps served as the conditioned stimuli. The four shuttle-boxes were rectangular plastic aquaria (78×15×15 cm) equipped with trapezoidal-shaped barriers dividing the boxes into two compartments. Each box had graphite electrodes along the sides to deliver an electric shock. Photo cells at each end of the barrier monitored the response and registered the side of the shuttle-box containing the fish for each trial. The task for the fish was to learn to anticipate a mild (5 V a.c.) electric shock by swimming over the barrier to the other compartment whenever one of two coloured signals (red or green) was presented. All stimulus and response contingencies were controlled and recorded by automated circuitry.

The two experiments below differed in the consequences of responding to the wrong signal, the amount of training given the donor animals and the number of groups of recipient animals. In the first experiment, fish which would later become recipients of the nucleic acid extract from donor animals were trained in a red-green avoidance discrimination. The red signal was presented for 20 s, after which a pulsed electric shock was delivered. Whenever the green signal was presented, it remained on for 30 s and no shock was delivered, regardless of the fish's reaction to it. We designate this as a R+G$_0$ discrimination to differentiate it from a R+G− task where response to green was punished. All fish in both experiments were obtained from local suppliers and were 7–8 cm long. They were housed for one week in a 400 l aquarium and later transferred to individual 6 l aquaria which were supplied with filters. Water temperature was maintained at 20° C.

In the first experiment 74 donor fish (*Carrasius auratus*) were trained in a R+G− discrimination and seven to a G+R− task for 3 d (100 trials each day, 50 red, 50 green,

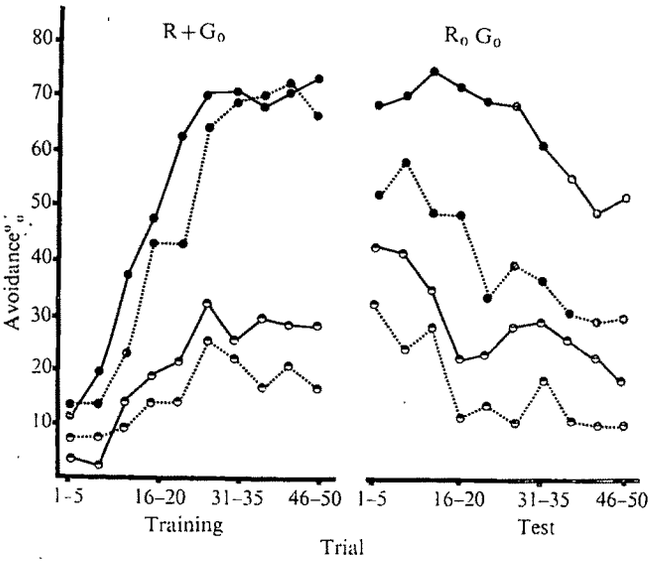


Fig. 1 Action of nucleic acid extracts on two groups of goldfish trained in a red-green avoidance learning task (R+G$_0$). The upper curves on the left (solid black dots) show the percentage avoidance to the positive red stimulus while the bottom two curves on the left (black and white dots) show the response to the incorrect green stimulus. The effect of the dissimilar injection is shown by the set of curves on the right when the same fish were tested under extinction conditions (R$_0$G$_0$). The solid line fish were injected with extract from R+G− trained donors while the broken line fish were injected with G+R− trained extract.

randomly presented). A large number of donor animals were trained in order to have enough extract for dose-response studies. Each trial consisted of the onset of the red or green stimulus lamp on the side of the box containing the fish. When the positive stimulus (R+ or G+) was presented, a 5 V a.c. pulsed shock was delivered (1 s^{-1}, 80 ms duration) 20 s later, unless the fish swam over the barrier into the other compartment. When the negative stimulus (R− or G−) was presented, the animal could avoid shock by staying in its compartment. If the animal responded during the negative stimulus presentation, it was shocked until it returned to the original side. In the R+ or G+ trials, the shock was limited to 10 s when the fish failed to escape. The intertrial interval was 30 s.

Twenty-four hours after this 3 d training period, the fish were decapitated, their brains extracted and placed in liquid nitrogen. The hot-phenol procedure[8] was used to extract nucleic acids from the brain tissue. This method yielded a RNA/DNA mixture of about 300 to 400 μg per fish brain. Only fish that had reached at least the 50% avoidance level to the positive stimulus were used in the extraction procedure.

Two groups of 23 fish each, designated to become recipients, were trained to the R+G_o discrimination described above for one day or 100 trials (50 red, 50 green). Immediately after this training, each fish was injected with 1 μg of extract in 10 μl of saline. The injection was intracranial into the large cranial cavity over the brain. Using a blind procedure, one group was injected with extract from the R+G− trained donors and the other with G+R− extract. They were tested 24 h later for 100 trials under extinction conditions (R_oG_o).

Both groups reached about the 70% avoidance level in responding to the positive stimulus (R+) at the end of training (Fig. 1). Although there was some generalisation to the G_o stimulus (about 25% responding), discrimination is obvious. The effect of the intracranial injection from differentially trained donors was apparent in the test trials on the next day. Fish receiving extracts from similarly trained donors remained at a relatively high level during the extinction trials while fish receiving extract from the G+R+ or dissimilarly trained donors showed a much lower level of performance. This difference is statistically significant at the $P<0.01$ level.

Although the results of the first experiment show a dramatic injection effect, they can only be regarded as suggestive evidence for specific information transfer because of a difference in performance level of the donor animals in the R+G− and G+R− tasks. Since the R+G− discrimination is easier to learn, it can be argued that the observed negative transfer may be due to the different number of shocks received by the G+R− donor animals. The complete factorial design of the second experiment makes this possibility highly unlikely.

In the second experiment, two groups of donor animals were trained as before except that an overtraining procedure was used. Overtraining of the donors has been shown to maximise transfer in a single-stimulus avoidance training situation. The advantage of overtraining is that it equalises asymptotic performance of the R+G− and G+R− donor groups although it does not ensure that both groups receive the same number of shocks since the R+G− animals reach their asymptotic level (90–95%) faster than the G+R− donors. They were trained either on a R+G− or a G+R− task for 5 d (100 trials d^{-1}), rested for 2 d and further trained for 2 d. Immediately after this training their brains were extracted and processed as before. All donor fish had reached at least the 50% avoidance level.

Four groups of fish, designated as recipients, were trained for a 1 d, 100 trial session. There were two R+G− groups and two G+R− groups, each with 15 fish. Immediately after this training, each animal was intracranially injected with 1 μg of extract from donor animals that were similarly or dissimilarly trained. Twenty-four hours after this injection all animals were given 100 test trials under extinction conditions (R_oG_o).

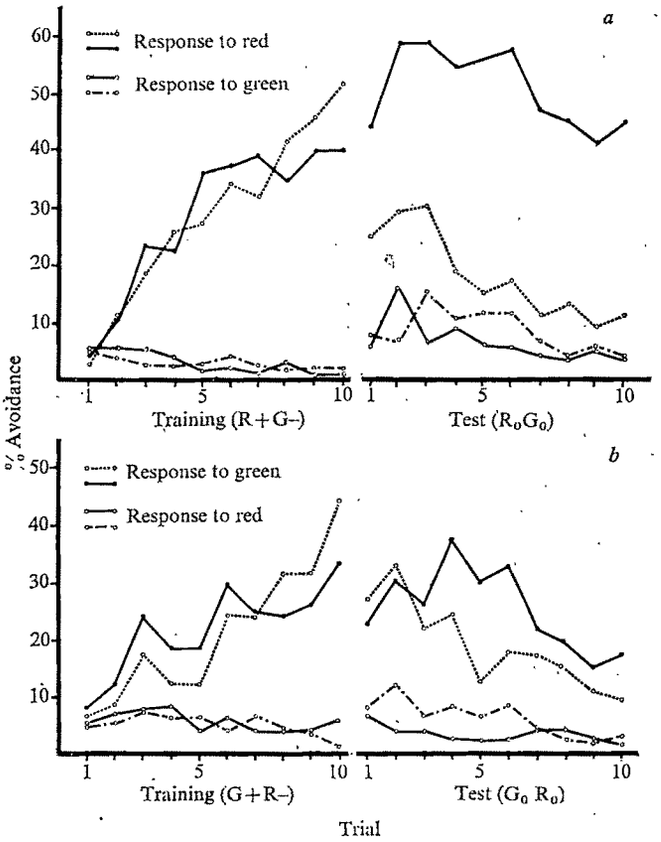


Fig. 2 *a*, Action of nucleic acid extracts on two groups of goldfish trained in a red-green avoidance learning task (R+G−): the upper two curves on the left show the percentage avoidance to the positive red stimulus while the bottom two curves on the left show response to the incorrect green stimulus. The effect of the dissimilar injection is shown in the right set of curves where the same fish were tested under extinction conditions (R_oG_o). The solid line fish were injected with extract from R+G− trained donors while the broken line fish were injected with extract from G+R− trained donors. *b*, Action of nucleic acid extracts on two groups of goldfish trained in a green-red avoidance learning task (G+R−). The upper two curves on the left show the percentage avoidance to the positive green stimulus while the bottom two curves on the left show the response to the red stimulus. The effect of the dissimilar injection is shown in the set of curves to the right when the same fish were tested under extinction conditions (G_oR_o). The solid line fish were injected with G+R− trained extract while the broken line fish were injected with extract from R+G− trained fish.

Figure 2 shows the training and test performance of the four recipient groups. The divergent effects of the injections are apparent for both red-trained and green-trained groups but are particularly striking for the R+G− groups (Fig. 2*a*). Fish receiving extract from animals trained in the identical task improved in performance, while those receiving extract from the opposite trained donors show a marked decline.

Figure 2*a* shows that the difference between the groups in the test trials was due to both the positive transfer on the R+G− trained/R+G− injected group and a negative transfer on the R+G− trained/G+R− injected group. This distinction is not observed in the G+R− recipients where the major part of the difference after injection is caused by the decline in performance of the G+R− trained/R+G− injected group.

The statistical significance of these findings was confirmed

by a five-way analysis of variance with repeated measures on three factors. The difference in performance to the positive stimulus between groups receiving extract from similarly or dissimilarly trained donors is significant at the $P<0.001$ level. Not only is response to the positive stimulus affected by the type of extract, but there is also a tendency for the response to the negative stimulus to be affected as well, although to a much lesser degree.

We are puzzled by the apparent interaction with colour *per se*. The major effect is seen with the red-trained groups. It is possible that this difference is associated with the relatively greater difficulty fish have in learning the avoidance response in the G+R− task. This colour-sensitivity difference between red and green performance has been observed in all studies of the red-green discrimination in this laboratory. The difference persists even when the two stimuli are equated for intensity. An additional control group with extracts from donors trained on an avoidance task with an entirely different set of stimuli might have added to our understanding and assessment of the differences in each pair of subgroups.

The results of both these experiments are consistent with the hypothesis that the extract contains chemically coded specific information which facilitates or inhibits performance. These results are also consistent with other data from this laboratory where similar designs were used in a single stimulus task[9]. Two groups of fish were trained to make avoidance responses to a light-onset signal. They were then injected with extract from donors that were similarly (light-onset) or dissimilarly (light-offset) trained. The test trial performance showed the same divergence in performance as observed here.

In another complete factorial study we conducted, where the recipient animals were overtrained for 3 d (also a red-green task), the striking difference observed in the above two experiments failed to appear. Although a positive transfer is more difficult to achieve in animals already performing at the 80–85% level after 3 d of training, a negative transfer, as was found in the first experiment, is possible but does not appear when the recipients are overtrained. It is possible that well-consolidated information is not as susceptible to chemical interference as has been observed in the puromycin consolidation studies of memory[11–12].

We are planning experiments to further characterise the nucleic acid extracts responsible for the information transfer.

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1 Ádám, G., and Faiszt, J., *Nature*, **216**, 198 (1967).
2 Byrne, W. L. *et al.*, *Science*, **153**, 658–59 (1966).
3 Dyal, J. A., in *Chemical Transfer of Learned Information* (edit. by Fjerdinstad, E. J.), (North-Holland, Amsterdam and London, 1971).
4 Rosenblatt, F., in *Molecular Approaches to Learning and Memory* (edit. by Byrne, W. L.), (Academic Press, New York and London, 1970).
5 Rucker, W., and Halsted, W. C., in *Molecular Approaches to Learning and Memory* (edit. by Byrne, W. L.), (Academic Press, New York, 1970).
6 McConnel, J . V., *J. biol. Psychol.*, **9**, 40–48 (1967).
7 Ungar, G., *Molecular Mechanisms in Learning and Memory* (Plenum Press, New York, 1970).
8 Bisping, R., Zahl-Begnum, O. H., Longo, N., and Niemoller, U., *J. biol. Psychol.*, **13**, 32–35 (1972).
9 Zahl-Begnum, O. H., thesis, University of Dusseldorf (1973).
10 Horner, J. L., Longo, N., and Bitterman, M. E., *Am. J. Psychol.*, **74**, 114–120 (1961).
11 Agranoff, B. W., and Klinger, P. D., *Science*, **146**, 952 (1964).
12 Flexner, J. B., Flexner, L. B., and Stellar, E., *Science*, **141**, 57–59 (1963).

Influence of local hydrophobic environment on acid dissociation constants of coordinated water molecules

The hydration of carbon dioxide by Zn(II) carbonic anhydrase is thought[1,2] to occur through a mechanism in which a hydroxyl group coordinated to the zinc atom has an important function. As a consequence, the *p*K of the conjugate acid coordinated water molecule has been the subject of some discussion[2,3]. In Zn(II) (ref. 4) and Co(II) (ref. 5) carbonic anhydrase, the water molecule bound to the metal ion, apparently has a *p*K in the range 6.9–7.9, which is two units lower than the *p*K range quoted[6] for the first protolysis of $[Zn(H_2O)_6]^{2+}$ and $[Co(H_2O)_6]^{2+}$.(The *p*K values quoted for $[M(H_2O)_6]^{2+}$ exhibit a considerable variance and have been determined under a variety of experimental conditions. When M = Co, Ni, Cu and Zn mean *p*K values of 9.8, 10.3, 7.8 and 9.5, respectively, are derived from the range of values quoted at zero ionic strength and at 298 K (*p*K values for all four metal ions in 1 M $NaClO_4$ are not available).)

Structural studies[7] show that the zinc atom in Zn(II) carbonic anhydrase is situated at the bottom of a cleft, lined with hydrophobic groups, in the protein molecule, and also that it is tetrahedrally coordinated by three imidazole groups and one water molecule. It has been postulated that this local hydrophobic environment is largely responsible for the decreased *p*K of the coordinated water molecule[2,3]. Although the variation of the *p*K of coordinated water with the metal ion character and environment is well known[8,9], there seems to be no report of a study in which this postulate may be tested.

Here we report a study in which the environment of a water molecule is changed from hydrophilic to hydrophobic for a range of metal ions, whilst the ligand donor atoms and metal ion coordination number remain constant and the coordination geometry almost so.

Divalent cobalt, nickel, copper and zinc all form stable 1 : 1 complexes with both 2, 2′, 2″-triaminotriethylamine (tren) (ref. 10) and 2, 2′, 2″-tri (N, N-dimethylamine) triethylamine (Me_6tren) (ref. 11) in aqueous solution. The complexes are five coordinate[12–14] with the general formula $[MtrenH_2O]^{2+}$ and $[MMe_6trenH_2O]^{2+}$ with the exception of the species $[Ni\ tren(H_2O)_2]^{2+}$. An examination of space filling models based on the known solid state structures of the[15] $[M\ trenNCS]^+$ and [11] $[MMe_6trenBr]^+$ ions reveals that the coordinated water molecule in $[M\ trenH_2O]^{2+}$ is closely adjacent to the hydrophilic primary amine groups of the tren ligand, whereas the coordinated water molecule of $[MMe_6trenH_2O]^{2+}$ is sterically crowded by the hydrophobic methyl groups of the Me_6tren ligand (Fig. 1). Thus, these complexes provide an opportunity for the examination of both the effects of the metal ion and of the change of environment on the *p*K of the coordinated water molecule.

The complexes undergo protolysis as follows:

$$[M\ trenH_2O]^{2+} + H_2O \rightleftharpoons [M\ trenOH]^+ + H_3O^+$$

$$[MMe_6trenH_2O]^{2+} + H_2O \rightleftharpoons [MMe_6trenOH]^+ + H_3O^+$$

Apparent *p*K values were determined by sodium hydroxide titration, under nitrogen, of 5×10^{-3} M complex solutions in aqueous 1M sodium perchlorate media, using a Radiometer combined glass and calomel electrode, and a Radiometer

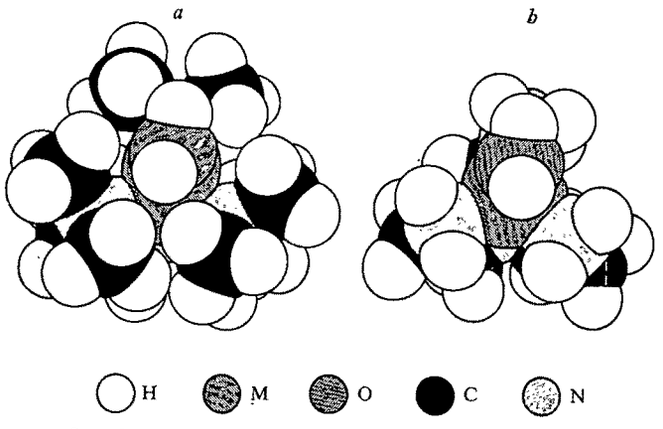


Fig. 1 Space filling models of $[MMe_6trenH_2O]^{2+}$ (*a*) and $[MtrenH_2O]^{2+}$ (*b*).

PHM4 or PHM26 *p*H meter. Sodium hydroxide was added with an Agla microsyringe. Complex solutions were prepared in carbon dioxide-free water by the addition of equimolar amounts of metal perchlorate salt and polyamine ligand solution; except when the solid complex $[MMe_6trenC10_4]C10_4$ (M = Ni, Co, Cu) was used directly. (The titration data were consistent with rapid dissociation of the coordinated perchlorate to produce $[MMe_6trenH_2O]^{2+}$ upon dissolution.) Titration curves were corrected for the concentration of uncombined hydroxyl ion by reference to a titration of 1M sodium perchlorate alone. Apparent *p*K values were calculated from plots of *p*H against log ([salt]/[acid]) by a least squares regression analysis. Each complex was titrated in duplicate at 288 K, 298 K and 308 K thus facilitating the computation of ΔH^0 and ΔS^0 for the protolysis reaction. The results obtained are summarised in Table 1.

In Fig. 2 the *p*K (298 K) values for the $[MtrenH_2O]^{2+}$ and $[MMe_6trenH_2O]^{2+}$ series show similar dependences upon the nature of the central metal ion, the general trends in which persist for each series over most of the solution liquid range as indicated by the thermodynamic data. The *p*K for six coordinate $[Ni\ tren(H_2O)_2]^{2+}$ cannot be strictly compared with those of its five coordinate cobalt, copper and zinc analogues. The titration data, however, show the first *p*K for this species to be greater than 11.0, and this datum is shown by the broken box in Fig. 2. The M–Br bond distances[13] for $[MMe_6trenBr]^+$ are also plotted in Fig. 2 and there exists a strong similarity between the dependence of these distances upon the metal ion (which has been explained in terms of d orbital occupancy[13]) and the variation in *p*K values exhibited by the $[MMe_6trenH_2O]^{2+}$ series. On the assumption that the M–OH_2 distances exhibit a similar variation it would seem that increasing metal ion attraction for the coordinated water molecule results in a decrease in *p*K. It is noteworthy that there is no correlation between *p*K value and any of the M–N distances[13] in the $[MMe_6trenBr]^+$ series.

The *p*K variation between the $[MtrenH_2O]^{2+}$ and $[MMe_6trenH_2O]^{2+}$ series might arise either because of differences in the environment of the coordinated water molecule in each case, or because of differences in electron donation to the central metal atom between the $-NH_2$ groups of tren and the $-N(CH_3)_2$ groups of Me_6tren, or a combination of these two factors. The crystal field splitting for $[Co\ trenH_2O]^{2+}$ has been shown to be greater than that of its methylated analogue[12], and on this basis the greater electron donation to the metal ion in the $[MtrenH_2O]^{2+}$ series might result in a higher *p*K for these complexes than for the $[MMe_6trenH_2O]^{2+}$ series. This is the trend observed and consequently it is evident that the *p*K variation cannot be attributed solely to variation of the local environment of the coordinated water molecule in the two series, although it seems that this last factor is of major importance.

A reasonable linear free energy relationship (LFER) characterised by an isoequilibrium temperature of 269 ± 16 K, exists (Fig. 3) for the thermodynamic data of Table 1. This LFER indicates that a single major factor accounts for the difference between the entropy dominated $[MtrenH_2O]^{2+}$ *p*K values and the enthalpy dominated $[MMe_6trenH_2O]^{2+}$ *p*K values. The systematic displacement of the data about the LFER regression line is a consequence of ΔH° and ΔS° becoming increasingly positive in the order Cu < Co < Zn for both series of complexes, and indicates the specific metal ion perturbation of the major LFER relationship. The isoequilibrium temperatures for the isometallic $[MtrenH_2O]^{2+}$ and $[MMe_6trenH_2O]^{2+}$ pairs are 239 K (Co), 261 (Cu) and 250K (Zn).

The thermodynamic differences between the two series of complexes are summarised in Table 2. If, following the method of Hepler[17], ΔH° and ΔS° are considered as sums of internal contributions, arising from interactions within the metal complexes, and external contributions arising from interactions between the complex and the solvent, one may write:

$$\Delta H^\circ = \Delta H^\circ_{int} + \Delta H^\circ_{ext}$$
$$\Delta S^\circ = \Delta S^\circ_{int} + \Delta S^\circ_{ext}$$

Table 1 Apparent thermodynamic data at 298 K for the acid dissociation of a single water molecule coordinated to tren and Me_6tren complexes

Complex	*p*K ± (s.e.)	ΔH^0 ± (s.e.) kJ mol^{-1}	ΔS^0 ± (s.e.) J K^{-1} mol^{-1}
$[Co\ trenH_2O]^{2+}$	10.22 ± 0.01	+ 0.9 ± 2.7	−192.7 ± 9.1
$[Cu\ trenH_2O]^{2+}$	9.37 ± 0.01	−13.6 ± 1.9	−225.0 ± 6.4
$[Zn\ trenH_2O]^{2+}$	10.26 ± 0.02	+11.9 ± 1.9	−156.5 ± 6.4
$[CoMe_6trenH_2O]^{2+}$	8.80 ± 0.04	+33.9 ± 1.9	− 54.5 ± 6.4
$[NiMe_6trenH_2O]^{2+}$	9.53 ± 0.01	+33.9 ± 1.9	− 68.7 ± 6.4
$[CuMe_6trenH_2O]^{2+}$	8.52 ± 0.01	+28.8 ± 1.9	− 66.5 ± 6.4
$[ZnMe_6trenH_2O]^{2+}$	9.00 ± 0.01	+49.2 ± 1.9	− 7.2 ± 6.4

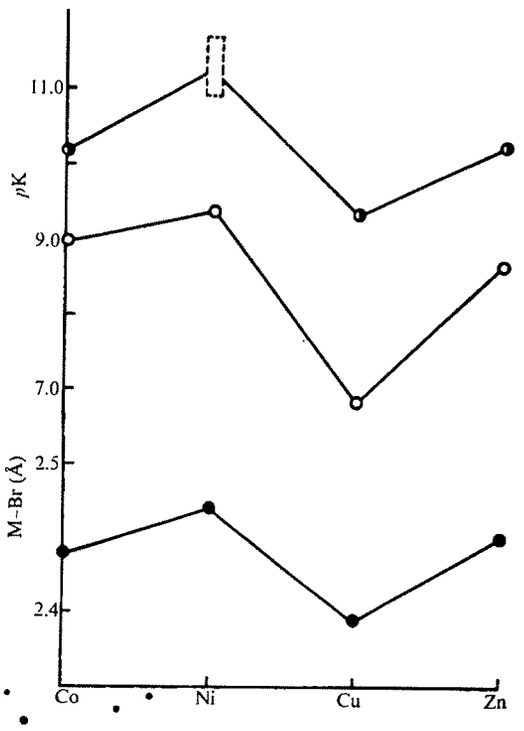


Fig. 2 Apparent *p*K values of $[MtrenH_2O]^{2+}$ (◑) and $[MMe_6trenH_2O]^{2+}$ (○); and the M–Br distances in $[MMe_6trenBr]^+$ (●) when M = Co, Ni, Cu and Zn.

Further, Pitzer[18] has shown that for the dissociation of a weak acid $\Delta S°_{int} \approx 0$. Thus, the differences in $\Delta S°$ between equivalent members of the two series of complexes are most probably entirely due to differences in the external contributions to the standard entropies of protolysis. Examination of Table 2 shows that the values of $\Delta S°_{Me_6tren} - \Delta S°_{tren}$ are very similar for Co, Cu and Zn; indicating that the differences between $[MtrenH_2O]^{2+}$ and $[MMe_6trenH_2O]^{2+}$ are caused by a similar change in environment for each metal.

Table 2 Differences in apparent thermodynamic quantities between Me_6tren and tren complexes

Metal	$pK_{Me_6tren} - pK_{tren}$	$\Delta H°_{Me_6tren} - \Delta H°_{tren}$	$\Delta S°_{Me_6tren} - \Delta S°_{tren}$
Co	−1.42	33.0 (± 4.6)	138.2 (± 15.5)
Cu	−0.85	41.4 (± 3.8)	158.5 (± 12.8)
Zn	−1.26	37.3 (± 3.8)	149.3 (± 12.8)

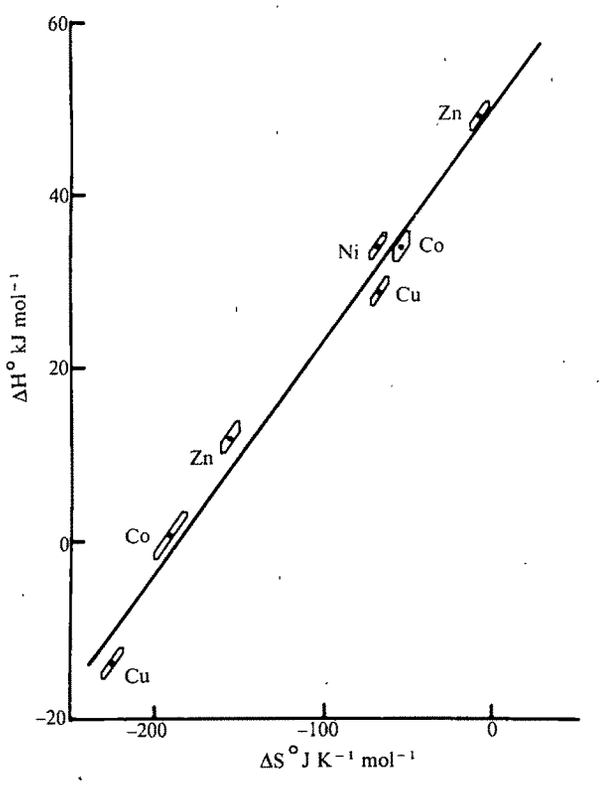


Fig. 3 The linear free energy relationship for the protolysis of $[MMe_6trenH_2O]^{2+}$ (upper set of points) and $[MtrenH_2O]^{2+}$ (lower set of points) when M = Co, Ni, Cu and Zn. The error estimates were calculated by the method of Mandel and Linnig[22].

The most probable environmental difference between $[MtrenH_2O]^{2+}$ and $[MMe_6trenH_2O]^{2+}$ is in the degree of ordering of solvent water molecules close to these complexes. It seems from two partly related considerations that the degree of solvent water ordering about $[MMe_6trenH_2O]^{2+}$ is likely to be the greater. First, the surface of $[MMe_6trenH_2O]^{2+}$, with the exception of the coordinated water molecule, is composed of hydrophobic alkyl groups—a situation generally considered to engender the formation of an ordered 'ice-like' structure in solvent water adjacent to such a surface[19,20] as is observed in the vicinity of the zinc atom in carbonic anhydrase[7]. By contrast the hydrophilic amine groups of $[MtrenH_2O]^{2+}$ seem unlikely to affect the adjacent water structure significantly[19]. Second, the charge density on the surface of the two complexes, particularly in the vicinity of the coordinated water molecule seems likely to be greater in the case of $[MtrenH_2O]^{2+}$ as this species is smaller (Fig. 1) and also because of the polarity of the amine groups. According to Frank and Wen[19], large ions with a low surface charge density are surrounded by a layer of water, disordered to an extent approximately proportional to the charge density (region B in the terminology of Frank and Wen[19]) as a consequence of two competing influences; the ionic charge solvent water dipole interaction, and the structural orientating influence of neighbouring bulk water molecules acting upon solvent water molecules adjacent to the ion. It seems that as a result of its greater surface charge density and because of the hydrophilic nature of the amine groups, solvent water adjacent to $[MtrenH_2O]^{2+}$ will be less ordered and the decrease in entropy greater upon protolysis than will be the case for $[MMe_6trenH_2O]^{2+}$ consistent with the observed trend in $\Delta S°$.

These conclusions lend some support to the suggestion that the low pK values for the coordinated water molecule in carbonic anhydrase[2] and also for other weak acid groups conjugated to proteins[21] are, in part, a consequence of their hydrophobic environment. It should be noted, however, that the systems studied here are structurally simple and that the relative importance of hydrophobic effects in these systems may differ considerably from the importance of such effects in the protein systems.

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1 Lindskog, S., and Coleman, J. E., *Proc. natn., Acad. Sci. U.S.A.*, **70**, 2505 (1973).
2 Prince, R. H., and Woolley, P. R., *Angew. Chem.*, Int. Ed. **11**, 408 (1972).
3 Prince, R. H., and Woolley, P. R., *Nature phys. Sci.*, **240**, 117 (1972).
4 Bradbury, S. L., *J. biol. Chem.*, **244**, 2002, 2010 (1969).
5 Ward, R. L., and Fritz, K. J., *Biochem. biophys. Res. Commun.*, **39**, 707 (1970).
6 Sillen, L. G., and Martell, A. E., in *Stability Constants of Metal Ion Complexes, Chemical Society Special Publication No. 17*, 1964, and *supplement No.* 1, 1971.
7 Liljas, A., *et al.*, *Nature new Biol.*, **235**, 131 (1972).
8 Beaumont, R. C., *Inorg. Chem.*, **8**, 1805 (1969).
9 Couldwell, M. C., House, D. A., and Powell, H. K. J., *Aust. J. Chem.*, **26**, 425 (1973).
10 Paoletti, P., Ciampolini, M., and Sacconi, L., *J. chem. Soc.*, 3589 (1963).
11 Paoletti, P., and Ciampolini, M., *Inorg. Chem.*, **6**, 64 (1967).
12 Ciampolini, M., and Paoletti, P., *Inorg. Chem.*, **6**, 1261 (1967).
13 Orioli, P. L., *Coordn. Chem. Rev.*, **6**, 285 (1971).
14 Criotini, A., and Ponticelli, G., *J. inorg. nucl. Chem.*, **35**, 2691 (1973).
15 Jain, P. C., and Lingafelter, E. C., *J. Am. chem. Soc.*, **89**, 6131 (1967).
16 Wells, P. R., in *Linear Free Energy Relationships* (Academic Press, London, 1968).
17 Hepler, L. G., *J. Am. chem. Soc.*, **85**, 3089 (1963).
18 Pitzer, K. S., *J. Am. chem. Soc.*, **59**, 2365 (1937).
19 Frank, H. S., and Wen, W. Y., *Discuss. Faraday Soc.*, **24**, 133 (1957).
20 Klotz, I. M., *Brookhaven Symp. quant. Biol.*, **13**, 25 (1960).
21 Klotz, I. M., *Science*, **128**, 815 (1958).
22 Mandel, J., and Linnig, F. J., *Analyt. Chem.*, **29**, 743 (1957).

Prolongation of skin allografts after oral application of ALS in rats

THE potency of anti-lymphocytic serum (ALS) in prolonging skin allografts has been well investigated. Four different routes of administration are being used: intravenous[1], intraperitoneal[2], intramuscular[3] and subcutaneous[4]. Every route has its own advantages and disadvantages from the immunological and clinical point of view and these have been discussed elsewhere[5,6]. Oral application of ALS was never tried because it was assumed that intact proteins like gamma globulins are enzymatically metabolised before they pass through the intestinal wall. But we shall demonstrate, that even oral application of ALS can prolong the survival time of skin allografts.

Two different series of experiments were performed, using altogether 65 white male colony-bred Sprague-Dawley rats, weighing 250 g. All animals were grafted with skin from black inbred BD-6 rats. In the first series, animals were fed 1 ml ALS daily, until graft rejection was completed and controls were fed with normal rabbit serum (NRS). In the second series animals were treated orally with 1 ml ALS or 1 ml NRS on days 1, 3 and 5, only. Feeding was by a polyethylene tube placed into the stomach. The effect of oral ALS application was compared with the effect of intraperitoneal administration of ALS or NRS. One group of rats remained entirely untreated. For statistical analysis mean values, standard error of the mean (s.e.m.), and P values according to student's t test were calculated.

ALS was produced in rabbits by 'two pulse' injection of lymphocytes from the thoracic duct and thymus of rats. The first pulse was applied together with complete Freund's adjuvant. The lymphocytotoxic titre of ALS was 1 : 64, and the haemagglutination titre 1 : 4, after one absorption with rat erythrocytes. ALS and NRS both had a protein concentration of 50 mg ml^{-1}.

The results are set out in Table 1. The mean survival time (MST) of skin grafts in rats without any treatment was about 10 d. Intraperitoneal as well as oral application of NRS did not prolong the MST of skin grafts significantly ($P < 0.1$). As expected, i.p. administration of ALS either every day or just on days 1, 3 and 5 increased the MST of skin allografts significantly ($P < 0.001$). But the daily oral application of ALS also prolonged the MST to 26.5 ± 1.9 d; this was 13 d longer than that for control animals treated in the same manner with NRS. When the high dosage of ALS of 1 ml daily was reduced to 1 ml on days 1, 3 and 5, this caused a decrease of the MST from 26.5 ± 1.9 d to 23.4 ± 1.8 d. Nevertheless, skin allografts survived significantly longer ($P < 0.001$) after oral application of ALS then after oral application of NRS, the difference being 10 d.

Table 1 Prolongation of skin allografts after oral and i.p. application of ALS

Group	No.	Treatment	Route	Dose (1 ml)	MST* ± s.e.m. (d)	Median ST† (d)
Series 1						
1	9	ALS	oral	Daily	26.5 ± 1.9	23.8 ± 1.4
2	6	NRS	oral	Daily	13.6 ± 1.7	10.3 ± 0.3
Series 2						
3	10	ALS	oral	Days 1, 3, 5	23.4 ± 1.8	19.2 ± 1.9
4	7	NRS	oral	Days 1, 3, 5	13.1 ± 0.6	12.2 ± 0.75
Controls						
5	9	ALS	i.p.	Daily	31.7 ± 1.2	28.5 ± 1.2
6	5	NRS	i.p.	Daily	12.8 ± 1.1	11.0 ± 0.5
7	10	ALS	i.p.	Days 1, 3, 5	18.6 ± 0.3	17.8 ± 0.2
8	5	NRS	i.p.	Days 1, 3, 5	10.6 ± 0.3	9.6 ± 0.3
9	14	—	—	—	10.1 ± 0.2	9.6 ± 0.2

* Mean survival time.
† Median survival time.

The oral route of application seems to be less effective than the i.p. route when ALS is given daily. The i.p. application of ALS on days 1, 3 and 5 prolonged the MST of skin allografts only to 18.6 ± 0.3 d, whereas after oral treatment, allografts were rejected after 23.4 ± 1.8 d. A statistical analysis, however, does not show significant differences between the groups.

The prolongation of skin allografts after oral application of ALS contradicts the generally held opinion that gamma globulins are metabolised on their way through the gut by digestive enzymes[7,8]. Providing the effect of ALS depends on an interaction with lymphocytes or with the graft, the significant prolongation of the MST after oral application can be explained by a partial absorption of intact protein molecules. Since BSA (refs 9 and 10), horseradish peroxidase[10] or kallikrein[11] are known to be absorbed without biochemical or immunological alterations the absorption of intact gamma globulin is perhaps not surprising. The alternative hypothesis, that orally administered ALS causes lesions in the intestinal wall which allow protein molecules to leak through, is made highly improbable by histological and macroscopic observations on the intestine. It is, nevertheless, conceivable that ultrastructural changes in the gut wall facilitate the penetration of unaltered gamma globulins. It is also possible that lymphocytes normally invading the lumen of the gut[12] are exposed to ALS there. By this means ALS might reduce the absolute number of lymphocytes without penetrating the intestinal wall.

Preliminary studies in rats and dogs have shown that it is possible to detect xenogeneic globulins in the blood of recipients using Ouchterlony's technique and Sephadex radiochromatography, following oral application of ALS. It is therefore possible that a proportion of unaltered gamma globulins does pass through the intestinal wall. But rats have less proteinase in their gut than dogs and man; this means that ALS is metabolised less in the small intestine of rats than in that of humans. Consequently, more ALS might be absorbed in rats and this may explain the success in prolonging skin allografts after oral treatment.

Apart from this theoretical interest, the oral route may become important for the clinical application of ALS, because the antibody production of orally treated animals seems to be markedly reduced. Whereas the i.p. application of ALS causes the formation of precipitating anti-horse serum protein antibodies in all recipients, oral administration causes precipitating antibodies in only 10% of rats after 14 d of treatment.

We thank Professor L. Brent for criticism.

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1 Abaza, H. M., Nolan, B., Watt, J. B., and Woodruff, M. F. A., *Transplantation*, **4**, 618 (1966).
2 Woodruff, M. F. A., and Anderson, N.F., *Ann. N.Y. Acad. Sci.*, **120**, 110 (1964).
3 Starzl, T. E., Marchiero, T. L., Porter, K. A., Ywasaki, Y., and Cerilli, G. J., *Surg. Gynec. Obstet.*, **124**, 301 (1967).
4 Levy, R. H., and Medawar, P. B., *Ann. N.Y. Acad. Sci.*, **129**, 164 (1966).
5 Berenbaum, M. C., Brent, L., and Kilshaw, P. J., *Transplantation*, **12**, 18 (1971).
6 Dalton, R. G., Touraine, J. L., Anderson, N. F., and Woodruff, M. F. A., *Nature*, **225**, 1140 (1970).
7 Brambell, F. W. R., *Lancet*, **ii**, 7473 (1966).
8 Wilson, T. H., and Wiseman, G., *J. Physiol.*, **123a**, 116 (1954).
9 Thomas, H. C., and Parrot, D. M. V., *Immunology* (in the press).
10 Walker, W. A., Isse Bacher, K. J., and Bloch, K. J., *Science*, **177**, 608 (1972).
11 Moriya, H., Moriwaki, C., and Akimoto, S., *Chem. Pharm. Bull.*, **15**, 403 (1967).
12 Meader, R. D., and Landers, D. F., *Am. J. Anat.*, **121**, 763 (1967).

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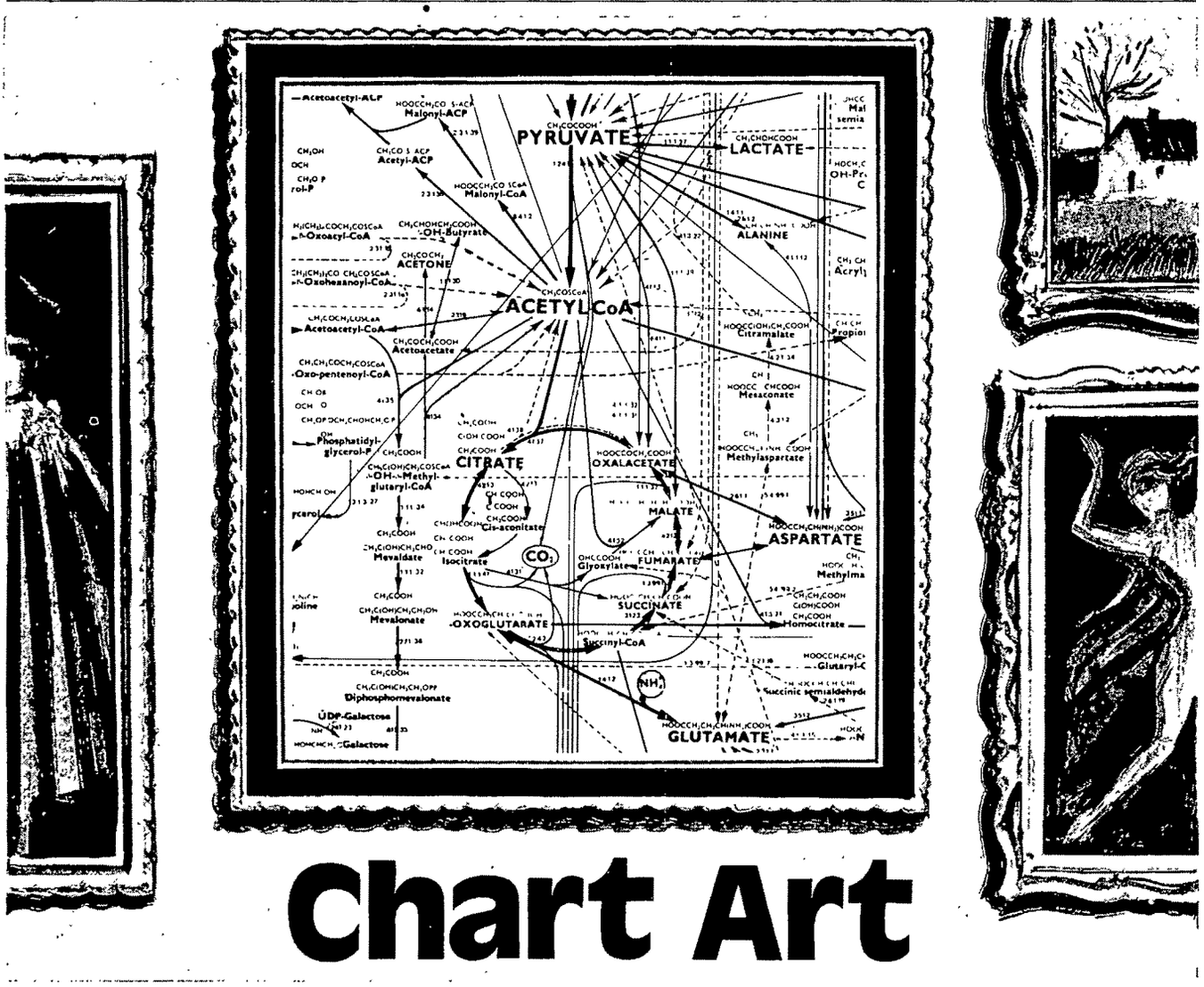


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matters arising

Nonradioactive silver

SIR,—Lindner *et al.*[1] described the detection of small amounts of radioactive silver isotopes (^{108m}Ag and ^{110m}Ag) in silver bars from an eastern European source. They suggested that the activity may have originated from any one of several causes: nuclear mining, that is, the breaking up of rock before the removal of the ore, using nuclear explosions; neutron irradiation of the ore after it was mined; the introduction of silver isotopes into the melted silver during processing; or irradiation of the silver bars.

Another possibility has been suggested by Boyle[2]. An intimate contact of silver, uranium and light elements such as boron, might produce, through (α, n) reactions, enough neutrons to give measurable events of ^{108m}Ag ^{110m}Ag. This phenomenon could occur in uranium deposits of the vein type, which are found in eastern Europe.

We have checked this speculation by counting specimens of silver ore collected from the Echo Bay Mine (near Port Radium), Great Bear Lake, Canada[3]. Ore samples from this vein-type uranium deposit included two pieces of massive native silver mined from uraninite-rich areas of the 302 Stope, and a third sample from an occurrence of pitchblende, 206A W Drift in the mine, containing veinlets of native silver. The first two samples were counted directly but in the latter case the silver was separated from the ore by dissolution in nitric acid. The silver was therefore in intimate contact with the pitchblende until a few hours before it was counted.

For counting, a 70 cm^3 Ge(Li) γ-ray detector and multichannel analyser were used. The sensitivity was 10 nCi, the same as that of the NaI(Tl) detector used by the Dutch workers. Since we did not possess an anticoincidence array, we were compelled to count for several days in order to achieve this. In all three cases there was no measurable ^{108m}Ag or ^{110m}Ag in the samples. On the basis of the Dutch work, we would have expected to have detected as much as 3 μCi.

We conclude that, at least for our samples, uranium does not produce significant activity in the silver associated with it. In view of the calculated low neutron flux[2], this is not surprising. Our conclusion can probably be applied to similar ore bodies.

R. W. Boyle (personal communication) cannot find any reported nuclear detonations which correlate with the time of origin of the activity as calculated by the Dutch workers. We agree with their current view (personal communication) that the most likely source of the reported activity is the addition of silver tracer to the silver during some stage of the refining process.

Yours faithfully,

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2 Boyle, R. W., *Nature*, **243**, 460 (1973).
3 Robinson, B. W., thesis, Univ. Alberta (1971).

Great Glen Fault

SIR,—Detailed mapping of the regional metamorphic pattern of the central and northern Highlands has led to the conclusion that a post-metamorphic sinistral shift of 160 km must have occurred along the Great Glen Fault[1]. A reappraisal of the considered data suggests, however, that the proposed shift probably represents a minimum estimate; a displacement of 200 km would, for example, fit the data equally well but even this figure cannot be taken as a reasonable upper limit of possible strike-slip movement.

The most likely northward continuation of the Great Glen Fault is through the Walls Boundary Fault in Shetland[2]. This provides a slight curvature in the fault line which seems to have an important geophysical bearing on the displacement problem. Thus, it can be shown[3,4] that a certain discordance in Devonian palaeomagnetism across the Great Glen Fault can be fully explained by incorporating a late or post-Devonian sinistral shift of at least about 200 km. At present, palaeomagnetic results cannot be used to set an upper limit of the actual movement but a displacement of at least 250 km is possible. What is really important at this stage, however, is to stress that a geophysical and a geological method have both led to practically identical palaeogeographic reconstruction of Scotland, which involve a transcurrent movement, at least twice as large as previously suspected along the Great Glen Fault. Consideration of the lower age limit of this movement (provided by palaeomagnetic results) seems to indicate that the Strontian and Foyers granites (K/Ar ages of around 400 Myr, ref. 5) never formed part of the same intrusive complex.

Yours faithfully,

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1 Winchester, J. A., *Nature phys. Sci.*, **246**, 81 (1973).
2 Flinn, D., *Geol. J.*, **6**, 279 (1969).
3 Storetvedt, K. M., *Geol. Mag.*, **111**, 23 (1974).
4 Waage, H. L., and Storetvedt, K. M., *Z. Geophys.*, **39**, 937 (1973).
5 Miller, J. A., and Brown, P. E., *Geol. Mag.*, **102**, 106 (1965).

DR WINCHESTER replies: Storetvedt's comments are very welcome. The suggested displacement of 160 km was chosen as the optimum fit of the metamorphic patterns in the opposing blocks. A precise metamorphic zonal correlation across the fault is not possible because on opposite sides of the fault, Moinian rocks are concealed beneath Devonian sediments east of Inverness, and beneath Tertiary volcanics in Mull. The zonal pattern may therefore be extrapolated across these areas to fit a post-metamorphic displacement of between 140 and 200 km along the Great Glen Fault, before it becomes impossible to match without requiring an unjustified warping of the metamorphic pattern adjacent to the fault. A displacement of 250 km is therefore not supported by the metamorphic evidence. It is significant, however, that the maximum displacement allowed by the metamorphic pattern, and the minimum shift indicated by Storetvedt's palaeomagnetic work should agree so well.

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McCollough colour after effect

SIR,—McCollough[1] demonstrated a striking, long lasting colour after effect. Observers adapted (2–4 min) to a vertical grating of black and orange stripes alternating with a horizontal grating of black and blue stripes. Thereafter a pattern made up of a black and white vertical grating and an adjacent horizontal grating appeared tinted with colours complementary to the adapting colours. The

vertical grating appeared blue-green and the horizontal grating appeared orange.

Piggins and Leppmann[2] repeated the McCollough-type experiment with the adapting pattern stabilised on the retina by the conventional contact lens method. They were unable to build up the after effect with a stabilised image. They concluded that the eye must scan the adapting pattern to generate the after effect, and explanations of the after effect must include 'image motion mechanisms'.

Our experiments show that the after effect requires no retinal image motion. Four observers adapted to a high contrast sine-wave grating of two cycles per degree flashed on an oscilloscope field that was 12° in diameter. The observer carefully fixated a tiny light in the centre of the field and then pressed a button which presented a stationary grating for one 9 ms sweep. The luminance of the P4 white phosphor, as measured with a 1P22 phototube, fell to $1/e$ of the peak value in 1 ms. The field was dark between flashes. The grating was presented in a different, random position on each flash and could be vertical or horizontal. When vertical, it was viewed through a Wratten 30 magenta filter (or a Wratten 11 yellow-green filter) and when horizontal, through the yellow-green filter (or magenta filter). The mean luminance of the gratings through the filter was about 4 cd m^{-2}. The observer presented himself with a grating about every second for a total of 2,000 times. Every 50 flashes, the grating changed orientation and colour. After effects were built up to counter any initial colour bias that the observer reported while viewing black and white test gratings before adaptation.

All observers saw weak McCollough after effects of pink and green on a test pattern consisting of adjacent vertical and horizontal square-wave gratings of two cycles per degree. The pattern was viewed in fluorescent room light. The colours were more pronounced when the mean luminance was reduced considerably—from 100 to 0.1 cd m^{-2}.

Retinal image motion during adaptation cannot be completely ruled out in this experiment. It has been shown,[3] however, that movements greater than 1 min of arc are very unlikely during attempted fixation of a target which is exposed for less than 20 ms, and the retinal image is virtually stationary for exposures less than 10 ms. The second experiment eliminates even these tiny retinal image movements.

High contrast vertical and horizontal square-wave gratings of two cycles per degree were placed on a diffusing screen to form two fields 9° in diameter. These patterns were illuminated from the rear by Grass photo-stimulators rated at about 10^6 candles. Measurements showed that the flash lasted less than 60 μs. The lights did not appear bright, and the after image they produced lasted 1 or 2 s. Four observers viewed the vertical grating through the magenta filter and the horizontal grating through the yellow-green filter. To spread the adaptation evenly over the retina, four tiny lighted fixation points were placed on each grating, separated by phase angles of 90° with respect to the fundamental period of each grating. The observer triggered the flash about every 2 s while fixating one of these lights. Each fixation light was fixated equally often. Total adaptation was 200 flashes, with alternation between the vertical and horizontal grating every 20 flashes.

All observers reported seeing the McCollough effect, which was very evident. The colours were still seen 30 min after adaptation. This experiment shows that no retinal image motion is required to build up the McCollough effect. Our method of stabilising the image has the advantage over the contact lens method in that the image does not fade over time, and is thus a potent stimulus.

Retinal image motion is thus not required for the McCollough effect. Flashed gratings and gratings stabilised by the contact lens method might well cause movement mechanisms to fire and thus adapt. But the optimal stimulus for these mechanisms is a pattern moving in a specific direction. Thus, these experiments tell us nothing about movement mechanisms. It is possible, however, to generate McCollough effects selective to the direction of motion[4,5].

We thank Professor Karl Pribram and Professor Leo Ganz for support.

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2 Piggins, D. J., and Leppmann, P. K., *Nature new Biol.*, **245**, 255 (1973).
3 Riggs, L. A., Armington, J. C., and Ratliff, F., *J. opt. Soc. Am.*, **44**, 315 (1954).
4 Hepler, N., *Science*, **162**, 376 (1968).
5 Stromeyer, C. F., III, and Mansfield R. J. W., *Percept. Psychophys.*, **7**, 108 (1970).

Mouse granulocyte precursors and multiple sclerosis

Sir,—Brown and Gajdusek[1] have recently reported failure to reproduce the suppression of peripheral blood granulocytes which was described[2] in mice following inoculation of material from patients with multiple sclerosis (MS).

We have also studied the effect on groups of ten weaning C57BL and CBA mice of intraperitoneal inoculation of 0.2 ml serum or brain extract (prepared as in ref. 2) from patients with MS. In agreement with Brown and Gajdusek, we found no differences in peripheral blood granulocyte levels up to 35 d after inoculation between mice inoculated with MS material, normal human serum, normal human brain or saline.

We used the agar culture technique for granulocyte-macrophage colonies[3] to enumerate granulocyte-macrophage precursor cells (colony-forming cells, CFC) in the marrow and spleen of inoculated mice, and in addition measured serum levels of the myelopoietic colony-stimulating (CS) factor[4]. Table 1 shows that 34 d after inoculation of MS brain extract into groups of 5 weanling C57BL mice there was no depression of granulopoietic potential compared with similar mice given normal brain extract or the medium used to prepare the extracts.

Table 1 Colony-forming cells after inoculation of normal and MS brain.

Material inoculated	CFC per femur ($\times 10^{-3}$)	CFC per 10^6 spleen leukocytes	Serum CS activity
Medium	42±17	25±15	10± 7
Normal brain	45±10	19± 8	8± 5
MS brain	61± 6	16± 7	15± 7

In this experiment the mice inoculated with MS brain actually showed a statistically significant increase in CFC per femur (but not in spleen which is usually a more sensitive indicator of changes in CFC populations[5]) compared with the mice given normal brain ($0.01 > P > 0.005$). No consistent differences in any of these parameters were found, however, between MS serum-inoculated mice and controls when tested in both C57BL and CBA strains.

We thank Dr J. H. D. Millar for sera from his MS patients, Dr Margaret Haire for specimens of normal and MS brain and the Medical Research Council for financial support.

Yours faithfully,

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1 Brown, P., and Gajdusek, D. C., *Nature*, **247**, 217 (1974).
2 Carp, R. I., Licursi, P. C., Merz, P. A., and Merz, G. S., *J. exp. Med.*, **136**, 618 (1972).
3 Metcalfe, D., and Moore, M. A. S., *Haemopoietic cells* (North Holland, Amsterdam, 1971).
4 Metcalfe, D., *Aust. J. exp. Biol. med. Sci.*, **50**, 547 (1972).
5 McNeill, T. A., Fleming, W. A., McClure, S. F., and Killen, M., *Immunology*, **25**, 91 (1973).

book reviews

General relativity

Gravitation. By C. W. Misner, K. S. Thorne, and J. A. Wheeler. Pp. xxvi+1279. (Freeman: San Fransisco and Reading, December 1973.) £19.20 boards; £8.60 paper.

IN most cases it is probable that, if a scientific discovery had not been made by the person normally credited with it, it would have been made by someone else within a few years. There is one notable exception to this general rule: had Einstein died before 1916, the world would have probably had to wait another 50 years or so for the General Theory of Relativity. In fact Einstein was ahead of his time: after an initial burst of activity in the 1920s, there was very little work on the theory until about 1960. This neglect was caused partly by the belief that the theory was too complicated to allow predictions to be made in any but the simplest cases and partly by the fact that there did not seem any prospect of comparing the predictions with observation apart from the three famous Solar System tests which Einstein himself proposed.

During the period 1920–1960 the most exciting field of physics seemed to be quantum theory. The success of quantum electrodynamics led most physicists to feel that physics was Feynman diagrams. They distrusted general relativity because of its classical and geometric nature. This dislike of geometry is shown, for example, by the particle physicist Steven Weinberg in the introduction to his otherwise excellent book *Gravitation and Cosmology* (John Wiley, New York, 1972): ". . . too great an emphasis on geometry can only obscure the deep conncetions between gravitation and the rest of physics. . . . I believe that the geometrical approach has driven a wedge between general relativity and the theory of elementary particles".

But geometric insights have played an important part in many of the outstanding recent theoretical developments such as black holes, space–time singularities and gravitational radiation. The answer therefore seems to be not to free general relativity from geometry but to teach geometry to physicists brought up on the normal ideas of special relativity; that is, to teach them to regard space–time as a differentiable manifold and not as a flat vector space.

This is what Misner, Thorne and Wheeler have set out to do in their book. They have also endeavoured to give at least an introduction to those fields such as cosmology, gravitational waves, post-Newtonian effects in the Solar System, relativistic stars and black holes where general relativity is now, or is on the point of, making contact with the recent great advances in observation. All this combined with fairly large print and wide margins has produced a book weighing 6 pounds. Because this is too long for most postgraduate courses on general relativity, the authors have divided the material into two tracks which are indicated on the top corners of the pages. Track 1, which is designed to accompany a one-semester course, gives an impressionistic overview of the theory and its applications but to perform any calculation one would really need to master most of the Track 2 sections on the basic theory.

The style is typical of the Princeton school (Misner and Thorne are former students of Wheeler at Princeton). It is aimed more at giving the reader an intuitive feeling for the concepts involved than giving a mathematically rigorous axiomatic account. Important results and other material of interest are displayed in boxes. These are generally helpful though they sometimes rather interrupt the text. The book is also well provided with exercises ranging from easy to major research problems. These should be very helpful to students learning the subject.

The authors' research interests have obviously influenced the coverage of the book. There are very good sections on relativistic stars, black holes and experimental tests of relativity. On the other hand the section on cosmology is less detailed than that in Weinberg's book and suffers from the ideological insistence that the Universe is spatially closed. The section on gravitational waves gives a good account of the generation and detection of gravitational radiation but does not mention the work of Bondi, Sachs, Newman and Penrose on the 'peeling off' of the curvature tensor, the Bondi mass loss formula and the Bondi–Metzner–Sachs group. These and the comparative neglect of spinors are the main gaps in this book, which does not aim to describe in detail global results such as singularity theorems. Despite these omissions the book is a major landmark in the field and is likely to become the most widely used textbook and reference work on the subject. It is well printed and bound and the paperback version is cheap for a book of this size.

S. W. HAWKING

Radial parameters

Ligand-field Parameters. By M. Gerloch and R. C. Slade. Ppxii+235. (Cambridge University: London, August 1973.) £5; $15.

ALTHOUGH many books exist dealing with the theory of transition-metal complexes, this one is welcome, since it treats the subject in a novel way. Other texts approach ligand-field theory through symmetry and group theory, being concerned with aspects of the subject determined by molecular symmetry alone. This is a valid approach, but it is sometimes necessary to stress those parts of ligand-field theory where the molecular symmetry is less significant, and this is done here.

For all systems, group theory can only give information on quantities dependent upon the angular properties of wave functions. Radial properties appear only as proportionality constants. Furthermore, complexes of lower than cubic symmetry are less amenable to group-theoretical treatment, needing large numbers of parameters to describe the system.

In this book stress is laid on calculations of radial properties of regular octahedral and tetrahedral complexes, and the ligand-field parameters of non-cubic systems, thus rationalising pertinent data on such complexes. A summary of the role of symmetry in ligand-field theory is followed by a development of the crystal-field formalism, with a discussion of inter-electron repulsion parameters in cubic systems. Treatment of the radial parameters in various complexes of lower symmetry follows, with emphasis on trends in the parameters Cp and Dq.

The second half of the book seeks to interpret these radial parameters, covering calculations of $10Dq$, and use of the point charge model and semi-empirical molecular orbital methods (especially the angular overlap model) in rationalising trends in Dq and Cp. The final chapter contains a discussion of the Nephelauxetic effect.

The authors hope that the book will be useful to advanced undergraduates and young researchers; but although the treatment is sound and logical, the level of difficulty will probably prove to be too great for all save very few

undergraduates. Nevertheless, this is a very worthwhile contribution to a little-known aspect of an important area of chemical theory, which should be read by anyone with a serious interest in ligand-field theory. The book is well produced, with extremely few typographical errors.

G. DAVIDSON

Bioengineer's dreams

Phloem Translocation. By M. J. Canny. Pp. x+301. (Cambridge University: London, November 1973.) £7.50; $22.50.

THE question of how cells in phloem tissue, transport organic foodstuffs, viruses and other material from one end of a plant to the other, remains, tantalisingly, open. The nature of the movement and the force which drives it are still unknown. It is not even agreed about where in the pipelines any forces might be applied.

The reasons for this ripe state of affairs, and suggestions for improving it, are set out in this lively book by Martin J. Canny, Professor of Botany at Monash University.

It is now ten years since Professor Canny first proposed his own hypothesis or model to explain translocation. In the very first sentence of the preface he warns "This book is less a review of a field of plant physiology than a lengthy statement of a point of view about that field which, in the course of the argument involves the review of most of the work". His point of view is that translocation behaves like diffusion but is faster.

The angle of view in part one of the book ("The Experimental Facts", 211 pages) is wide. It gives an interesting, though sometimes closely argued account of the ups and downs of research into translocation; of the quantities translocated; of how the channel of movement was identified; of how patterns and rates of movement and gradients of concentration were studied; of the effects of temperature; of the nature of phloem exudates; of why functioning sieve elements are so difficult to examine and experiment with; and of what is known or controversial about their structure. In particular Professor Canny discusses very fully how the distribution of radioisotopic tracers and their concentration profiles in the transport channels are related to various patterns of flow proposed to occur through sieve tubes.

Part two (52 pages) is divided from part one by a group of 23 plates which contains 45 micrographs and photographs and two line drawings. These are well chosen to illustrate the text and beautifully reproduced.

In part two ("Towards a Mechanism") Professor Canny outlines briefly various mechanisms which have been proposed to explain translocation, and discusses their drawbacks. He then describes his own model as he first based it, in 1962, on hypothetical membranes round transcellular strands proposed by Dr R. Thaine. But he goes on to say that "The prime objection to the model is that the strand structures it uses seem not in fact to be there, nor any such tonoplast-type membranes as were originally imagined as separating them from the sap". Filaments, perhaps gathered together in bundles are all that appear in the electron microscope to warrant his highly sophisticated calculations, which have been criticised[1,2]. He now seems to suggest that "accelerated diffusion" might be caused by flow within (and of?) 250Å diameter tubular filaments, or within "small aggregates of them", and driven by "mechanicochemical means". I find flow through such filaments hard to believe; their resistance would surely be enormous[3]. Far-fetched models have an irritant value which provides much of the spark for research into translocation. But they can become an entanglement.

There are numerous useful tables, graphs, and drawings in the text. These provide essential information from many different sources for those who wish to make their own calculations about translocation, or try their hand at model building. The book has author and subject indexes, and a bibliography with more than 330 references. There are four short appendices.

I found this a stimulating and thought-provoking book. It should be read by anyone who wishes to know more about translocation. It demonstrates vividly how enthusiastic speculation stimulates the search for hard facts.

RICHARD JOHNSON

1 MacRobbie, E. A. C., *Biol. Rev.*, **46**, 429–481 (1971).

2 Crafts, A. S., and Crisp, C. E., *Phloem Transport in Plants*, 277 (Freeman, San Francisco, 1971).

3 Weatherley, P., *Physiol. Vég.*, **10(4)**, 731–742 (1972).

Bacterial envelopes

Bacterial Membranes and Walls. Edited by Loretta Leive. Pp. xv+495. (Microbiology Series.) (Dekker: New York, December 1973.) $38.

IT is fashionable in some quarters to pretend that the prokaryotes are a spent force as research models in biology. This collection of essays, all written by distinguished experts, might be read with great profit by those who promulgate or accept such views. Chapters such as those by Mindich on membranes, by Kaback on transport, by Luria on the colicins, by Tomasz on the uptake of DNA, by Pardee, Wu and Zusman on bacterial division and, despite its specialised title, certainly that by Ghuysen and Shockman on the biosynthesis of peptidoglycan (mucopeptide, murein) all embody principles of general biological importance. In particular the necessary confluence of those interested in DNA replication and nuclear segregation with those interested in cell envelopes, to tackle the fundamental biological problem of cell division, is illuminating.

Although the subject of cell division is mentioned in the title of only one chapter, five out of the total of nine essays deal at lesser or greater length with it. Indeed, it might have been profitable to put together a separate section of the book on this subject, taking and expanding the relevant sections from some of the other chapters. For example, the part of Ghuysen and Shockman's article called "Relationship to Morphogenesis and Division" could well have stood on its own in such a section. This section would also have included the stimulating but maverick article by Henning and Schwarz which points its didactic finger in almost the opposite direction to several of the other chapters, as well as the thoughtful and cautious article of Pardee with its epilogue. This is not to criticise either the book or the editor who has persuaded her distinguished soloists to perform with such crystal clarity of tone, even though she did not integrate them into a consonant orchestra, had this been desirable. It is rather meant to emphasise unsatisfied intellectual hunger stimulated from the brief remarks often made almost as asides by some of the authors.

The generality of the title is a little misleading since some considerable lacunae exist. There are, for example, no serious considerations of energy metabolism, despite the siting of important parts of the electron transport chain in membranes, no general or detailed ultrastructural studies, no biophysical studies, nothing on the export of macromolecules other than DNA, and no serious study of the transport of ions. The latter might have allowed the supporters of the chemiosmotic hypothesis a fair chance to reply to Dr Kaback's strictures on them. A brief mention of the L-forms of Gram-positive bacteria such as *B. subtilis* or *Streptococcus haemolyticus* might have given pause for thought to Henning and Schwarz in their considerations of the determinants of cell shape for, to adopt their style—if membrane, not mucopeptide formation, is alone shape determining how is it that unstable L-forms of Gram-positive bacteria totally without regular shape or division and without mucopeptide

rapidly revert to normally shaped bacteria which divide regularly when penicillin, a quite specific inhibitor of one step in mucopeptide biosynthesis is removed from their growth medium? But this book is already nearly 500 pages long and refers to some 1,400 papers.

References in the main text extend to 1972 and a short appendix at the end of the book improves this to 1973 for some chapters; considering the publication date of 1973 this is very satisfactory. It is beautifully produced, well indexed and singularly free of typographical errors. Both the editor and the publishers are to be congratulated. I hope that Dr Leive may be persuaded to edit a second companion volume in this microbiology series that will deal with the subjects perforce omitted from the present one. The book is to be recommended to all those directly interested in cell surfaces. Individual chapters could be suggested as supplementary reading to postgraduate and final year undergraduate students in biochemistry or microbiology, but the perusal of some more general books on bacteria and bacterial walls and membranes would be a necessary preliminary.

HOWARD J. ROGERS

Taxonomy of ferns

The Phylogeny and Classification of the Ferns. Edited by A. C. Jermy, J. A. Crabbe and B. A. Thomas. Pp. xiv+284. (Supplement No. 1 to the Botanical Journal of the Linnean Society, Vol. 67.) (Academic: New York and London, December 1973.) £9; $25.

THIS is a collection of papers based on those presented at a joint symposium held in 1972 by the Linnean Society and the British Pteridological Society. The original programme has been expanded by the addition of three further papers, extensive reference lists and, judging by the concentrated factual content of some papers, additional detailed data. New approaches and techniques of the last 20 years have revealed errors and inadequacies of past taxonomic treatments without firmly establishing alternative classifications. In several of these papers the view is expressed that conclusions are tentative and await further critical investigations. It was the organisers' stated intention therefore that the symposium should provide an opportunity to take stock of the present situation and provide stimulus and direction for further work. In this they will have at least partially succeeded, by inviting leading researchers to give authoritative and up-to-date assessments in their own area of interest. Supported by detailed data and extensive bibliographies these add up



Fiddle-head of soft shield-fern *Polystichum setiferum.*

to a useful reference book for the specialist pteridologist.

But by basing the programme on the choice of speakers rather than on themes within the overall broad scope of the title, the programme lacks cohesion, and few of the papers are linked together in any way. As, furthermore, there is no record of any discussion which may have been stimulated by the papers, the reader is left with the impression that the unique feature of a symposium has been lost.

On the dust jacket, there are the further claims that this is "a reference book to the cytology, anatomy, palynology, chemisty and gametophyte studies of the Filicopsida" and, making a curious distinction, "a valuable purchase for many botanists and plant physiologists". These claims, perhaps those of the publisher rather than the editors, are misleading. The cytology is largely restricted to chromosome numbers, the anatomy and palynology occurs mostly as scattered references in papers on particular genera or families, and the gametophyte study takes no account of the effects of environmental conditions on the development of diagnostic characters, even though protonema and prothallus development is known to be particularly sensitive to such factors as temperature, light intensity and light quality. Several of the diagnostic characters discussed in Atkinson's paper on the gametophyte as an indicator of family relationships can be induced or suppressed at will in, for example, *Dryopteris* species merely by changing the cultural conditions. Botanists (and plant physiologists!) not already familiar with the ferns will find the several essentially traditional treatments of groups such as the dennstaedtoids, cheilanthoids, Oleandraceae, Thelypteraceae, Polypodiaceae and Aspidiaceae of limited value. Several areas of more general interest such as serological and numerical taxonomy, breeding systems and apomixis, developmental physiology and the ecological significance of phylogenetic changes are omitted and the biosystematic approach is restricted to one paper by Lovis on the Aspleniaceae. Professor Manton comments that the production of phyletic schemes for the Filicopsida involves every discipline in botany, but this is not fully reflected in this book.

Those with historical interests will enjoy tracing fern classification systems back to the time when it was commonly believed that fern spores collected on Midsummer's Eve and placed in the shoe rendered the wearer invisible (see Shakespeare, W., Henry IV Part 1, act 2, scene 1, line 96 (1576)). Also, botanists with any interest in taxonomy will find some of the more general papers worth reading, particularly those by Swain *et al.* on the possibilities for biochemical systematics in ferns, by Van Cotthem on the classification of stomatal types, by Lovis on the biosystematic approach in the Aspleniaceae and by Wagner on future challenges in fern systematics. Walker's comprehensive survey of fern chromosome numbers and Wood's and the Tryons' use of spore surface structure revealed by scanning electron microscopy may also be of some use to those working with other groups of plants.

The limited success of this volume in achieving its secondary aims only emphasises the need for a general text on the biology of ferns to interest students in these fascinating plants and to re-educate those botanists whose impressions of the Pteridophytes are based on their past experience of unimaginative teaching within a narrowly systematic context.

A. F. DYER

Petrol and polar bears

Man, Nature and Ecology. By Keith Reid, J. A. Lauwerys, Joyce Joffe and Anthony Tucker. Pp. 418. (Aldus, Jupiter: London, March 1974.) £3.95.

THIS large and lavishly illustrated book aims to give a background in ecology to the layman with an interest in doom. It is too diffuse to have a profound impact—the four authors cover ecology, anthropology, rare animals, and problems of population and resources. The layman who remembers his school biology and reads the newspapers will know much of it already.

The parts I found most interesting were those on the problems inherent in the use of pesticides, antibiotics and the products of the green revolution.

The book ends with a list of suggestions for an ecologically virtuous life. Ironically one of the recommendations is to save paper by using both sides when writing letters: the authors don't mention how many trees had to be felled to produce their book.

GILLIAN MOORE

Classify knowledge

The Fabric of Knowledge: A Study of the Relations Between Ideas. By J. L. Jolley. Pp. 130. Duckworth: London, October 1973.) £2.95.

THIS monograph will be of interest to anyone concerned with the problems of organising scientific or technical documents. It is more specialised than the title suggests, being an examination of the use of integrative level theory for the organisation of concepts in scientific and technical subjects. There are three parts. The first recounts the development of Mr Jolley's ideas, the second supports them by means of set theory and the third is a brief summary of previous ideas applied in the organisation of knowledge.

The first section is the most satisfactory and least controversial. It should be read in conjunction with the account of similar work by the Classification Research Group in *Classification for a General Index Language*, by D. J. Foskett (Library Association, 1970). Despite the common starting point there seem to be considerable differences of detail in the two sets of ideas. The levels identified are not exactly the same and their method of use varies: the CRG uses them for organising entity concepts only, whereas Mr Jolley includes all concepts. His semantic types used within the levels also differ from the categories used by the CRG. Unfortunately a full comparison is not possible since Mr Jolley has not written at sufficient length to do himself justice. A lot more examples would have helped to clarify the sometimes eccentric terminology and definitions that in themselves are inadequate for complete understanding.

There is no reason to doubt the usefulness of Mr Jolley's analysis, since it is based on many years of work in indexing consultancy. Even the set theory may be useful, but the exposition here is difficult to understand. Whether it is really necessary is another matter: the CRG have managed without it. It could be that they have merely failed to make explicit the real theory underlying their work, but the experience with categories should be a warning against Mr Jolley's claim to objectivity. There are no generally accepted categories of reality, and their use in classification can only be judged pragmatically. The only way to be objective in the classification of ideas is to examine the nature of knowledge rather than the nature of some supposed objective reality.

There seems little ground for Mr Jolley's optimism about the extension of his theory beyond science and technology. Integrative level theory relates to natural objects and there is no obvious reason why it should provide a structure for philosophy, history, religion or the arts. It is sufficient that it has a use in science and Mr Jolley's work is welcome independent support for that of the CRG. Both his suggested pattern of ideas and the use of set theory are worthy of further research.

D. W. LANGRIDGE

Analysis

Elementary Real and Complex Analysis. By Georgi E. Shilov. Revised English edition translated and edited by Richard A. Silverman, Pp. xi+516. (Mathematical Analysis: Vol. 1). (MIT: Cambridge, Mass. and London, 1973.) $14.95.

THIS translation, by R. A. Silverman is, as usual, of high standard. The appearance of the book is good, the printing and accuracy so meticulous, that an error on page 223, where $f(x_0)+h$ should read $f(x_0+h)$, comes as a shock.

The book covers much of the analytical knowledge demanded of a mathematics graduate—real and complex numbers, vector functions, metric spaces, limits, convergence, elementary functions, the theorems of the calculus, Reimann (–Stieltjes) integrals, analytical function theory (for piecewise smooth curves) and improper integrals. The chapters on limits (using directions) and improper integrals are probably the most refreshing. Functions of several variables, measure and Lebesgue integration receive only passing reference.

The author's declared aim is an easier 'Dieudonné'; nevertheless mathematical sophistication, and hard thought about abstract ideas, are demanded of the reader particularly in the earlier chapters. This may prove a deterrent to weaker students, scientists and engineers for whom the book is also intended. There is a good collection of examples, some normally accorded theorem status, some difficult even with the hints of the appendix.

This is a scholarly book; carefully used it could provide a logical, integrated and economic presentation of basic work.

MARGARET JACKSON

Domestic life in Africa

Man and Woman among the Azande. Edited by E. E. Evans-Pritchard. Pp. 197. 8 plates. (Faber: London, February 1974.) £4.50.

SIR EDWARD EVANS-PRITCHARD died towards the end of 1973, and this is the last book to come from his pen. As such it is appropriate that in it he returns to the study of the Azande people of Central Africa, the group about whom he first wrote more than forty years ago and about whose thought he published his first major work.

This book is a collection of Zande texts, some taken down in the 1920s, some written more recently. Evans-Pritchard links these with brief comments of his own, and in a few places discusses and clarifies obscure passages. He is always careful to allow his Azande informants to speak for themselves and his editorial presence never prevents his reader from having to deal for himself with the complexities, contradictions and individualities of Zande thought and belief.

Evans-Pritchard was a great and respected social anthropologist, and it is probably due to his eminence that he was able to publish this book, for it is hard to believe that a commercial publisher would risk issuing a series of native observations about the problems and joys of domestic life if these were not supported by the name of such a famous author. But we must be thankful for that eminence, for it has made available a series of fascinating documents about the relations of men and women in a single exotic culture. Material such as this has rarely been published in book form before.

One of the main qualities of this book is that it shows the almost-raw material from which anthropologists build up their analyses and abstractions. Women quarrel, men complain, an informant recalls a story heard about a neighbour, an old man criticises the conduct of the young. All this is faithfully and simply recorded here and it is left to the reader to make what he can of it. In some places he would clearly benefit from additional information or guidance from the editor, but this is not provided. To get the full worth of the book it is therefore necessary to read some of Evans-Pritchard's other writings on the Azande; once this is done the mass of data about male/female relations can be used more profitably.

In the final count anthropologists depend on the quality of their informants. Evans-Pritchard, like Malinowski and others before him, always stressed the need to record information *verbatim;* only in that way, he argued, could a true appreciation of native

ideas be developed. Although the texts published here are but a small proportion of those collected during his working life, they reveal his skill at eliciting information and also his tact and sensitivity in allowing people to say or write what they wanted and not what would have best suited him. Rejecting analysis in his final works, he concentrated on making known the views of those he studied; the present book is typical of this approach. Though one might wish for more of his own views and ideas, the work as a whole provides a mass of interesting and valuable data. It is also a tribute to Evans-Pritchard's lifelong fascination with the lives and ideas of other men and his deep respect for these in all their variety.

M. D. McLeod

Molecular sieves

Zeolite Molecular Sieves: Structure, Chemistry and Use. By Donald W. Breck. Pp. ix+771. (Wiley: New York and London, January 1974.) £18.

THERE have been several shorter monographs on particular aspects of zeolite chemistry and technology, mostly in Russian, and also several bibliographies. In 1968, 1971 and 1973 volumes have appeared embodying the papers read in three international symposia on molecular sieve zeolites. But Dr Breck's monograph is the first comprehensive text reviewing all aspacts of zeolites except their catalytic properties. A second monograph is being considered which will cover this important area. I hope this will be completed in due course in the same comprehensive pattern.

In the book under review the introductory chapter is followed by chapters on structure, mineral zeolites, synthetic zeolites, physical properties, chemical properties and reactions, ion exchange, adsorption and finally manufacture and properties of commercial sieve zeolites. A feature of the book is the amount of illustrative material presented as tables. In the field of molecular sieves progress in the basic science and in applications is rapid ar the incorporation of the most recent developments presents considerable problems. The author has nevertheless been successful in providing coverage up to about 1972. The discussions of zeolite structure, crystal chemistry, synthesis, chemical reactions and properties are particularly interesting.

One may ask whether Dr Breck's classification of zeolites will appeal to mineralogists since it produces some strange bedfellows, for example analcime, phillipsite, paulingite and yugawaralite are all given as members of the same group. Cages of linked tetrahedra of $(Si,Al)O_4$ are referred to as α–, β–, γ– . . . cages. In my opinion these cavities would be better designated as 14-hedra, 26-hedra, and so on since this conveys partial information about their nature. The chapters on adsorption and ion exchange reproduce many isotherms, although usually without the experimental points which allow one to assess the accuracy and reversibility achieved in the original researches. In the introductory chapter a brief review is given of several other materials having molecular sieve properties such as Saran charcoals and alkali metal graphites, but one of the more important types, the alkylammonium-exchanged clay minerals, receives virtually no attention.

There are various printing errors but they do not usually result in ambiguity and are small matters in a book of this compass. The author is to be congratulated on completing a monograph which will, despite its price, be indispensible to all those interested in molecular sieve science and technology, and which should become the first standard work on this subject. The format is attractive, clear and easy to read.

R. M. Barrer

Plant components

Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. By J. B. Harborne. Pp. x+278. (Chapman and Hall: London; Halsted Press, a division of Wiley: New York, 1973.) £4.80.

THE aim of the author is to provide undergraduate students and research workers with a series of proven methods of phytochemical analysis in a single volume at a reasonable cost. After a brief survey of general extraction, analytical and identification procedures, specific methods for each major class of organic constituent found in plants are considered. Much of the book (98 pages) is concerned with a comprehensive discourse of methods used for the analysis of phenolic compounds and terpenoids. The coverage of the organic acids, lipids and related compounds, nitrogen compounds and sugars, though not as extensive as the phenolics, is adequate. The macromolecules including the nucleic acids, proteins and polysaccharides are deliberately considered in less detail owing to space limitations and widespread coverage in other texts. It is unfortunate that more space could not have been allocated to this important area of research.

Each chapter contains sections on the chemistry, distribution and recommended qualitative and quantitative procedures for each component which have widespread application in research laboratories. Sufficient detail has been included to enable the inexperienced worker to attempt the methods and the practical experiments in each section should help the student gain experience with the procedures. The carefully selected references at the end of each section should aid the worker to quickly develop or modify the techniques to suit his own requirements. The author assimilates the rapidly expanding and difficult area of phytochemical methods into a well written and informative text which should be welcomed by both students and researchers.

G. Norton

Flies and humans

Flies and Disease. Vol 2: Biology and Disease Transmission. Bernard Greenberg. Pp. x+447. (Princeton University. Princeton, September 1973.) £4.50.

THIS second volume of *Flies and Disease* complements volume 1 which appeared in 1971.

The introductory chapter provides an historical survey of the association of flies with man throughout recorded history. This is one of the most fascinating chapters in the book, documenting both early myths and observations on the natural history of flies. A slip of paper in the mouth of a mummy "The maggots will not turn to flies within you" illustrates the Egyptians' appreciation of insect metamorphosis many centuries ago.

The second chapter deals with the life history and habits of ten common genera of synanthropic flies. There are several important omissions here (for instance, *Musca domestica*), justified on the grounds of treatment elsewhere in the literature, and the information given is occasionally rather disjointed because of the variety of interests of the original authors. The chapter ends with a discussion of the estimation of fly density (simple Lincoln index only) and dispersal: table 3 is a useful summary of the dispersal of more than 40 fly species.

Chapter 3 investigates flies as hosts of a variety of micro-organisms and describes the changes which take place in the gut fauna during metamorphosis. Many larval microbes are lost during pupation and emerging adults may even be sterile. The relevance of this to the vectorial capacity of saprophagous flies is examined.

The two final chapters discuss flies as disseminators of more than 50 human and animal diseases. In the absence of definitive parasite stages in the insect hosts, the importance of flies relative to other transmission pathways is often based on circumstancial evidence alone; for example a correlation of increased numbers of

disease cases with seasonal plagues of flies. Professor Greenberg deals with these examples in an open-minded way, admitting where the evidence is slight and emphasising where new research efforts should be directed.

The book has an extensive bibliography, including many early works. The style of the illustrations is very variable and occasionally disappointing, but the volume as a whole will be a useful source of information and reference for public health workers, epidemiologists and entomologists alike.

DAVID ROGERS

Image of a scientist

Science as a Career Choice: Theoretical and Empirical Studies. Edited by Bernice T. Eiduson and Linda Beckman. Pp. xv+735. (Russell Sage Foundation: New York, 1973.) £10.40.

WHY I feel able to embark upon the formidable task of reviewing this work, which is a vast book largely concerned with psychological theory and studies of the process of socialisation, is that the choice of career is a crucial factor in economic analysis of the way in which the labour market works. It is therefore necessary to review as much of the evidence as possible presented by a variety of disciplines in order to see what it is that drives people to make one choice rather than another.

This interesting study is a collection of papers concerned with the mechanisms of career choice and the way that people drift into the physical and other sciences, and what sort of people they are. Scientists apparently (page 670)

> "lead quite, unpretentious, even-tenored, rather steady lives. They come home regularly for dinner, and spend the time before and after dinner with their children and wives; pursue their hobbies systematically; and more often than not, spend the evenings reading journals, novels, magazines, or watching television. Most live on a fairly modest scale and do not spend much for theatres or entertainment... despite the fact that their home lives are interesting and varied, one gets the impression that these do not reflect their most exciting selves. For one thing, the interviews reveal that the scientists are not able to give the most significant parts of themselves to home and family."

To an outsider, this is an extraordinarily perceptive description of the home lives of many scientists, and also suggests the degree of secretiveness and obsession with work which has seemed so often a characteristic of scientists with whom one has been thrown into contact. Terman seems to have shown that scientists chose a subject early, and that their early socialisation tended to isolate them by making them feel different from other children. According to other studies, those people who are going to take up science develop an image of a scientist which tends to become self-confirming: the scientist is committed, studious, collaborative, concerned primarily with science; and that this perpetuation of a self-image which is assisted by highly developed social structures therefore tends to make the scientific community a fairly close one which is self-recruiting. This would also suggest that it was a social system which tended to push other people out, particularly people who developed an interest in science later in life, or people who had unusual social characteristics.

This description of the physical scientists contrasts markedly with those who call themselves social scientists. There seems to be some evidence that physical scientists come from individualistic and rather cold families (page 17) with high intellectual motivation. There is also, it seems, some evidence that the classic image of the pure scientific researcher clashes somewhat severely with most scientists' actual experience of their work. Interestingly enough, there are astonishingly few female or black scientists in the American studies which are cited here, and therefore the scientific community in the United States may be described as predominantly white and male, thus presumably reinforcing the tendencies towards a self-perpetuating community which have just been outlined.

The editors point out that the evidence, although very bulky, is in fact sketchy and derived from a great many partial studies of certain aspects of the problem, and therefore the very brief allusion which has been given here to some aspects of the work must be treated with great caution. Nevertheles, it does illustrate the probability that career choices between becoming a non-scientist or a scientist, and within the sciences, are largely affected by what economists would regard as irrational motivations, and also implies to the outsider that there must be a considerable number of people inside science who have been attracted into it by their own fantasies and who are now living in a world in which their fantasies clash rather sharply with reality. This may not be an untypical situation, but presumably it must be particularly disillusioning inside a closed profession like science, where so much of the original choice of career has depended upon high hopes and aspirations.

JOHN VAIZEY

Lymphocyte dogma

T and B Lymphocytes. By M. F. Greaves, J. J. T. Owen and M. C. Raff. Pp. vii+316. (Excerpta Medica: Amsterdam; American Elsevier: New York, 1973.) £14.08.

HERE is a book to fill with joy the hearts of cellular immunologists. So many pages devoted to T and B lymphocytes and only 4p per page.

T and B lymphocytes, by which is meant lymphocytes derived from the thymus and lymphocytes derived from the bursal equivalent (whatever that is) respectively, have after a shaky start in the 1960s become all the rage at the beginning of the 1970s. The present book represents a sort of consummation of this rage.

The history of the notion of two modalities of lymphocytes is given *pari passu* with a general account of the relationship between the immune response and the lymphoid system. The variously described properties of T and B lymphocytes are adequately documented. The authors go in detail into the mysteries of T and B lymphocyte participation in immunological responses and as a rousing finale consider the clinical applications of T and B lymphocyte theory. There is an appendix said to deal with the manipulation of T and B cell responses and an extensive and up-to-date reference list. The index could be more comprehensive but the diagrams and illustrations are good.

It seems on compelling evidence that the various chapters of the book have been written each by a different author but the blend overall is eminently readable and not markedly reiterative. Further, there are many and healthy instances in which ignorance in relation to particular points of fact is stressed. The book is heavily orientated towards work on mice but in this respect it reflects the activities of the bulk of contemporary cellular immunologists.

Greaves, Owen and Raff are to be congratulated on a timely and carefully prepared book which, despite its price, will be a boon to teachers of immunology. The only danger in it is that it will be seen as an orthodox statement of fact rather than what it is, the enunciation of a most interesting working hypothesis.

A. J. S. DAVIES

Erratum

IN the illustration "Butterfly Collection" (**248**, 289; 1974) the habitats of *Idea hypermnestra* and *Marpesia chiron* should have been given as Malaysia and South America respectively.

obituary

N. A. Mackintosh

NEIL ALISON MACKINTOSH, CBE, DSc, ARCS, the distinguished biological oceanographer and authority on the ecology and populations of whales, died on April 9, at the age of 73. He was educated at Westminster School and Imperial College where he very soon showed an aptitude for zoological research; in less than two years after graduation he had completed two papers on such widely different subjects as the chondrocranium of fish (*Proc. zool. Soc.*, 1923) and the crystalline style of gastropods (*Q. Jl. microsc. Sci.*, 1925).

He was in at the very beginning of that great enterprise, the Discovery Investigations of the Antarctic seas, when in 1924 he led the advance party to establish and take charge of the Marine Laboratory for the study of whales at South Georgia. Later he took part in three major expeditions of the *Discovery II*, being leader of two circumpolar voyages and bringing back a wealth of information on the plankton and hydrography of the Southern Ocean. In 1936 he had succeeded Dr Stanley Kemp as Director of Research until 1949 when the Investigations were merged with the new National Institute of Oceanography, of which he became deputy director, and since 1936 he had edited the long series of *'Discovery' Rep.* to which he made many outstanding contributions including one to be published in the thirty-sixth volume only four months before he died. In 1961 he established and directed the Institute's whale research unit at the British Museum (Natural History). He took a prominent part in the International Whaling Commission, being chairman



of its Scientific Committee. His contributions to knowledge lay in two main fields: the biology and conservation of whales, and the oceanic macroplankton.

It was at the laboratory adjacent to the whaling station at Grytviken, South Georgia, and on visits to stations on the African coast during the Antarctic winters (when the whales go north) that Mackintosh and Dr J. F. G. Wheeler, working together, amassed the enormous amount of information, based on the examination of nearly 1,700 whales, for their important monograph on the Southern Blue and Fin whales (*'Discovery' Rep.*, **1**, 1929). This and a number of later reports by Mackintosh laid the foundations of most of the current knowledge of the economically important species.

At Grytviken, Mackintosh and Wheeler discovered the remarkable rates of growth of the Blue and Fin whales. Mackintosh's book *The Stocks of Whales* (1965) is an important contribution to a study of their populations and conservation prospects based on a number of his earlier investigations, particularly his "Southern stocks of whalebone whales" in *'Discovery' Rep.*, **22**, 1942.

He made an extensive oceanographic survey of the macroplankton of the Atlantic sector of the Antarctic seas and was the discoverer of a remarkable seasonal vertical migration of many of the species whereby they are carried northwards in the surface waters during the summer months and then sink in winter to be returned towards the pole by a lower south-bound current ready to arise again the following spring. More recently, from the vast collections of earlier years, he renewed the investigation of the krill, producing two important studies: one relating the life cycle to ice and water conditions (*'Discovery' Rep.*, **36**, 1972) and another on the circumpolar distribution of the post-larval stages (*'Discovery' Rep.*, 1973).

Neil Mackintosh was one of the kindest and most modest of men, intensely disliking anything in the way of publicity. Indeed, his retiring nature was perhaps a fault, for because of it, so few in the outside world realised the greatness of the Discovery Investigations' achievements, including, of course, his own. He was awarded the Polar Medal in 1942 and the Patron's Medal of the Royal Geographical Society in 1954.

Announcements

Appointments

Henryk M. Wisniewski has been appointed Director of the Medical Research Council's Demyelinating Diseases Unit at Newcastle upon Tyne.

Gwynne James Morgan has been appointed Professor of Theoretical Physics at the University of Leeds.

C. D. Green, A. P. Halestrap, D. I. A. MacLeod, J. R. Rhodes, Barbara M. F. Pearse, Elizabeth C. Cartwright and **C. R. Hiley** have been elected to Beit Memorial Junior Fellowships for Medical Research.

David Anthony King has been appointed to the Brunner Chair of Computer Studies at the University of Liverpool.

Geoffrey Bernard Cook has been appointed to the Chair of Computer Studies at the University of Hull.

D. P. J. Browning, Violet R. Cane, K. M. Clayton and **T. W. Goodwin** have been appointed to the University Grants Committee.

A. S. V. Burgen, J. N. Walton, S. Cohen and **D. C. Phillips** have been appointed to the Medical Research Council.

Awards

Denys H. Wilkinson has been awarded the American Physical Society's 1974 **Tom W. Bonner Prize** in Nuclear Physics.

The **Linnean Gold Medal** of the Linnean Society of London has been awarded to **E. H. W. Hennig** and **J. Braun-Blanquet.** The **H. H. Bloomer Award** has been presented to **A. F. Millidge** and **George H. Locket.**

Corrigendum

IN the article "Heavy ion transfer reactions in nuclear astrophysical processes" by T. W. Conlon (*Nature*, **247**, 268; 1974) the third paragraph from the end should read as follows. "A study of the α-transfer reaction $^{12}C(^{16}O,^{20}Ne)^{8}Be$ at energies in the region of the Coulomb barrier has been made possible by the development of a particle identification system based on position-sensitive counters mounted in the focal plane of a Bueschner spectograph. Preliminary results indicate that significant amounts of ^{20}Ne are produced with respect to ^{24}Mg, which results from the principal compound nucleus channel (Table 2)."

In the same article, the following corrections should have been made; para. 1, line 12 should read "corresponds to the transfer of an α particle, . . ." para. 10, line 5, "available for later evolutionary . . ." and the last column in Table 1 should be "*Q* opitum (MeV) corresponding to (E min to E max)."

International meetings

July 8–10, **15th Annual Meeting of the Society for Economic Botany** (Mr Bruce Alderman, Kellogg Centre, MSU, S. Harrison Road, East Lansing, Michigan 48824).

July 23–24, **550th Meeting of the Biochemical Society** (The Meeting Officer, The Biochemical Society, 7 Warwick Court, Holborn, London WC1R 5DP).

August 5–9, **7th Semi-Annual Short Course on Laser Safety** (R. James Rockwell, Jr., Laser Safety Course, Conmed, 114 Medical College, Cincinnati, Ohio 45219).

August 26–28, **Pacific Slope Biochemical Conference** (Calvin S. McLaughlin, Secretary-Treasurer, Department of Molecular Biology and Biochemistry, University College Irvine, Irvine, California 92664).

Reports and Publications

Great Britain

Cancer Treatment Reviews, Vol. 1, No. 1, March 1974. Edited by K. Hellmann and S. K. Carter. Pp. 1–80. Subscription:— Vol. 1 (4 issues) March–December 1974. Inland £6.50 inclusive of postage; abroad £7.50 inclusive of postage. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1974.) [164

The Annual Report of the Science Policy Research Unit at the University of Sussex for the year 1973. Pp. 54. (Brighton: SPRU, University of Sussex, 1974.) [164

Medical Research Council. Memorandum No. 44: Haemophilia and its Related Conditions—a Brief Guide to Diagnosis and Treatment. By Rosemary Biggs. Pp. v + 42. (London: HMSO, 1974.) 34p net. [164

Flora of Tropical East Africa. Edited by R. M. Polhill. Gramineae (Part 2). By W. D. Clayton, S. M. Phillipd and S. A. Renvoize. Pp. 177–449. (London: Crown Agents for Oversea Governments and Administrations, 1974. Published on behalf of the East African Community.) £2.75. [164

Unilever. Report and Accounts for 1973. Pp. 48. (London: Unilever, 1974.) [164

Bulletin of the British Museum (Natural History). Zoology, Vol. 26, No. 3: Observations on *Trinema lineare* Penard (Testacea : Protozoa). By Ronald Henderson Hedley and Colin Gerald Ogden. Pp. 185–199 + 7 plates. (London: British Museum (Natural History), 1974.) £1.50. [184

Process Engineering of Pyrometallurgy. (Proceedings of a joint meeting of the Institution of Mining and Metallurgy and the Institution of Chemical Engineers.) Edited by M. J. Jones. Pp. vi + 105. (London: The Institution of Mining and Metallurgy, 1974.) £6.50. [194

The Way Ahead in Postgraduate Medical Education. Papers by Stanley Clayton, J. O. F. Davies, R. B. Hunter, Sir George Pickering, John Revans, Elizabeth Shore, George Smart, Sir Charles Stuart-Harris, Selwyn Taylor and G. I. Watson. Edited by Gordon McLachlan. Pp. 113. (London, New York and Toronto: Oxford University Press, 1974. Published for the Nuffield Provincial Hospitals Trust.) £1.25 net. [224

Agricultural Administration, Vol. 1, No. 1, January 1974. Edited by A. N. Duckham and John Pearce. Pp. 1–88. Subscriptions: Four quarterly issues per volume (approximately 320 pages)—£10 ($25, subject to exchange fluctuations.) London: Applied Science Publishers, Ltd., 1974. [224

Parliamentary and Scientific Committee. Annual Report for 1973. Pp. 15. (London: Parliamentary and Scientific Committee, 1974.) [234

Imperial Cancer Research Fund. Scientific Report to the Council by the Director of Research for the year 1973. Pp. 137. (London: Imperial Cancer Research Fund, 1974.) [234

The Fourth Television Network: a Question of Priorities. By Sir Charles Curran. Pp. 18. (London: BBC, 1974.) [244

Scientific Proceedings of the Royal Dublin Society. Series A. Vol. 5, No. 1: The Volcanic Rocks of the Carrigogunnel Area, Co. Limerick. By P. Strogen. Pp. 1–26 + plates 1–3. £1. Vol. 5, No. 2: The Structural Setting of the Leinster Granite, Ireland. By James C. Brindley. Pp. 27–36. 30p. Vol. 5, No. 3: Artificial Key for the Identification of the Fruits of Irish Grasses. By M. A. Farragher. Pp. 37–54 + plates 4–7. £1. Vol. 5, No. 4: Poaceae—Irish Members. Part IV: Classification. By M. A. Farragher. Pp. 55–76 + plate 8. £1. Vol 5, No. 5: The Petrography of some Red-Feldspar Pegmatites and Their Association with Late Hydrothermal Changes in the Leinster Granite. By P. S. Kennan. Pp. 77–84 + plates 9 and 10. 70p. Series. Vol. 3, No. 16: Some Recent Researches on the Growing of Fibre Flax. By M. Neenan and J. Devereix. Pp. 201–220 + plate 11. 50p. (Dublin: Royal Dublin Society, 1973 and 1974.) [244

The Zoological Society of London. Annual Report for 1973. Pp. 62. (London: The Zoological Society of London, 1974.) [254

Essential Oils. Pp. 32. (London: Bush Boake Allen, Ltd., Blackhorse Lane, E17, 1974.) [254

Bulletin of the British Museum (Natural History). Entomology. Vol. 30, No. 2: A Revision of Some Groups of *Liptena* Westwood (Lepidoptera : Lycaenidae). By H. Stempffer, N. H. Bennett and S. J. May. Pp. 107–181 + 6 plates. £4.75. Zoology. Supplement 6: The Cichlid Fishes of Lake Victoria, East Africa—The Biology and Evolution of a Species Flock. By P. H. Greenwood. Pp. 1–134 + 1 plate. £3.75. (London: British Museum (Natural History), 1974.) [264

Fisons Limited. Report and Accounts for the year ended 31 December 1973. Pp. 31. (London: Fisons Limited, 1974.) [294

British Museum (Natural History). Mineralogy Leaflet No. 5: Natural Crystals. Pp. 12. 9p. Simple Crystal Models, Sheets 1 and 2. (London: British Museum (Natural History), 1974.) [304

Journal of Archaeological Science, Vol. 1, No. 1, March 1974. Edited by G. W. Dimbleby, D. R. Brothwell and H. Barker. Pp. 1–116. Subscription: 1974, Vol. 1, 4 issues, Inland £8 including postage; abroad £9.10 including postage. (London and New York: Academic Press, 1974.) [15

Other Countries

Physics Reports of the Kumamoto University, Vol. 1, No. 1, 1973. Pp. 1–72. (Kumamoto: Department of Physics, Kumamoto University, 1973.) [164

Marine Aquariums in the Research Laboratory. By John M. King and Stephen Spottle. Pp. 40. (Eastlake, Ohio: Aquarium Systems, Inc., 33208 Lakeland Blvd., 1974.) $2. [164

Alberta Research—Annual Report for 1973. Pp. 65. (Edmonton, Alberta: Research Council of Alberta, 1974.) [164

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Paper 72–20: Stratigraphy of the Southern Part of the Devonian Ancient Wall Carbonate Complex, Jasper National Park, Alberta. By E. W. Mountjoy and W. S. Mackenzie. Pp. vii ; 121 (8 plates). $3. Paper 73–9: Reconnaissance Studies of Proterzoic and Cambrian Stratigraphy, Lower Mackenzie River Area (Operation Norman), District of Mackenzie. By J. D. Aitken, R. W. Macqueen and J. L. Usher. Pp. vii + 178 (22 plates). $4.50. Paper 73–33: Deltaic Sedimentation in the Northeastern Bowser Basin, British Columbia. By G. H. Eisbacher. Pp. 13. $2. (Ottawa: Information Canada, 1973 and 1974.) [174

Smithsonian Contributions to Zoology, No. 127: A Revision of the Family Lichomolgidae Kossmann, 1877, Cyclopoid Copepods Mainly Associated with Marine Invertebrates. By Arthur G. Humes and Jan H. Stock. Pp. v + 368. (Washington, DC: Smithsonian Institution Press, 1973. For sale by US Government Printing Office.) $4.50. [174

Volume One of the Report and Recommendations of the National Advisory Commission on Multiple Sclerosis—an Overview. Pp. i + 67. (Bethesda, Md.: US Department of Health, Education and Welfare, Public Health Service, National Institute of Health, 1974.) [184

Republic of South Africa: Department of Industries. Sea Fisheries Branch Investigational Reports. No. 106: Growth of Juvenile Rock Lobster *Jasus lalandii*. By D. E. Pollock. Pp. 16. No. 108: Oceanic Circulation Deduced from Plastic Drift Cards. By L. V. Shannon, H. G. Stander and J. A. Campbell. Pp 31. (Sea Point, Cape Town: Sea Fisheries Branch, 1973.) [184

Science Council of Canada. Background Study No. 30: A Technology Assessment System—a Case Study of East Coast Offshore Petroleum Exploration. By M. Gibbons and R. Voyer. Pp. 114. (Ottawa: Information Canada, 1974.) $2. [184

Energy, Mines and Resources Canada—Annual Report 1972/1973. Pp. 33. (Ottawa: Information Canada, 1974.) [184

New Zealand: Board of Health. Report Series No. 18: Drug Dependency and Drug Abuse in New Zealand—Second Report. Pp. 247. (Wellington: Government Printer, 1973.) [194

New Zealand + National Radiation Laboratory. Environmental Radioactivity: Fallout from Nuclear Weapons Tests Conducted by France in the South Pacific During July and August 1973, and Comparisons with Previous Test Series. Pp. 29. (Christchurch, NZ: National Radiation Laboratory, 1973.) [194

Smithsonian Contributions to Zoology. No. 155: The Familial Phylogeny of the Tetraodontiformes (Acanthopterygii: Pisces) as Evidenced by Their Comparative Myology. By Richard Wonterbottom. Pp. iv + 199. $2.80. No. 157: A Bibliography of the Catalogs, Lists, Faunal and Other Papers on the Butterflies of North America, North of Mexico. Arranged by State and Province (Lepidoptera: Rhopalocera). By William D. Field, Cyril Dos Passos and John H. Masters. Pp. 104. $1.70. No. 160: Systematics of the Subterranean Amphipod Genus *Stygobromus* (Gammaridae), Part I: Species of the Western United States. By John R. Holsinger. Pp. iii + 63. $1.45. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) [224

Institut Royal Météorologique de Belgique. Publications. Série A, No. 83: Repré senations Analytiques des Composantes du Rayonnement Lumineux Solaire: Conditions de Ciel Serien. Par R. Doniaux. Pp. 24. Série A, No. 85: L'Evapotranspiration Potentielle des Bassins Hydrographiques en Belgique. Par Franz Bultot et G. L. Dupriez. Pp. 61. Série B, No. 75: Some Remarks About the Temperature Effect on Meson Telescope Data. By Dr. A. Van Gysegem. Pp. 11. (Bruxelles: Institut Royal Météorologique de Belgique, 1974.) [234

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Paper 73–2: Age Determinations and Geological Studies, K–Ar Isotopic Ages, Report 11. By R. K. Wanless, R. D. Stevens, G. R. Lachance and R. N. Delabio. Pp. 139. $3. Paper 73–7: Geological Survey of Canada—Radio carbon Dates XIII. By J. A. Lowdon and W. Blake, Jr. Pp. vii +61. $2. Paper 74–18: Sulphur in Archean Volcanic Rocks of the Canadian Shield. By E. M. Cameron. Pp. 9. $2. Paper 74–19: Analysis of Rocks and Minerals Using an Atomic Absorption Spectrophotometer. Part 5: An Imptoves Lithium-Fluoborate Scheme for Fouteen Elements. By S. Abbey, N. J. Lee and J. L. Bouvier. Pp. iv + 26. $2. (Ottawa: Information Canada, 1973 and 1974.) [254

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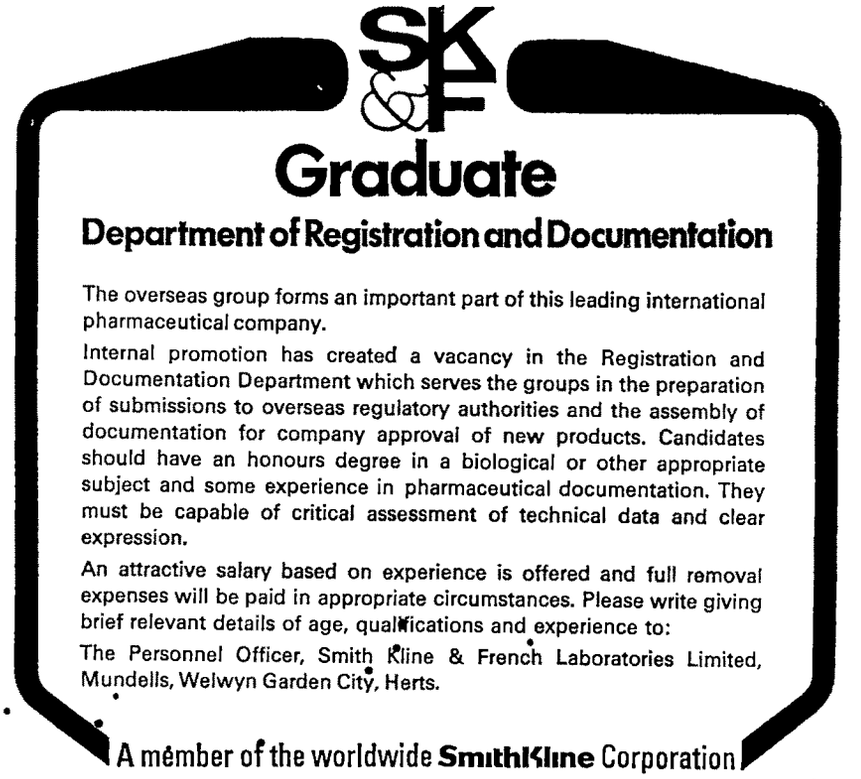
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Please apply stating qualifications, age and experience to The Secretary, Lister Institute of Preventive Medicine, Elstree, Hertfordshire, from whom further particulars can be obtained. (2374)

CITY OF LONDON POLYTECHNIC

SIR JOHN CASS SCHOOL OF SCIENCE AND TECHNOLOGY

READER IN CELL PHYSIOLOGY

Applications are sought from highly qualified persons for the post of Reader in the Department of Biological Sciences.

In addition to providing a wide range of undergraduate and postgraduate teaching and research the School is a nationally recognised centre for biomedical studies. The successful candidate will be expected to initiate and develop lines of research in cell physiology preferably with some relationship to health or environment—and will also be expected to play a significant rôle in the development of new course work in the biological sciences particularly in relation to undergraduate programmes within the B.A./B.Sc. (C.N.A.A.) Modular Degree Scheme, the Higher National Certificate in Medical Laboratory Science and the Diploma in Medical Technology.

The post is tenable from September 1, 1974 or as soon as possible thereafter.

Salary scale—4 consecutive points within the scale £4,380 to £4,824 (London Weighting Allowance of £118).

Full details and an application form, may be obtained from the Assistant Secretary, City of London Polytechnic, 117/119 Houndsditch, London, E.C.3. (2515)

ANIMAL BREEDER
UNIVERSITY OF GUELPH

Ph.D. in Animal Breeding with background in statistical genetics. Experienced in data analyses and interested in working with animal industry breeding developments. Responsible for developing a research programme and teaching undergraduate and graduate students. Appointment at Assistant Professor level. Applications accepted to August 31, 1974.

Apply to: W. D. Morrison, Professor and Chairman, Department of Animal & Poultry Science, Ontario Agricultural College, University of Guelph, Guelph, Ontario, Canada N1G 2W1. (2492)

UNIVERSITY OF DURHAM
DEPARTMENT OF BOTANY

Applications are invited for studentships tenable from October 1, 1974, from chemists, biochemists or botanists, to work on the biochemistry of grain legume improvement. Successful candidates may register for a higher degree.

Applications to Professor D. Boulter, Department of Botany, Science Laboratories, South Road, Durham DH1 3LE. (2516)

CONSERVATION OFFICER

required by Dorset Naturalists' Trust. Degree in Biology/Ecology, plus conservation training or experience. Ability work with amateurs. Minimum starting salary £1,600 p.a. Contributory pension scheme. Full details from Hon. Sec., Dorset Naturalists' Trust, 58 Pearce Avenue, Poole, Dorset. (2510)

MARINE PHYSIOLOGIST

The Rosenstiel School of Marine and Atmospheric Science has an opening for a whole animal physiologist with at least a secondary interest in environmental physiology, beginning as early as January, 1975. Responsibilities will include teaching a graduate level course on the physiology of marine animals. Appointment will be at a rank and salary commensurate with experience and qualifications. Applicants must have completed the Ph.D. degree. Send curriculum vitae and names of five referees to Dr Barrie F. Taylor, Chairman of Search Committee, University of Miami, R.S.M.A.S., 4600 Rickenbacker Causeway, Miami, Florida 33149. Applications close on August 31, 1974. (2509)

DIRECTOR

The Jackson Laboratory invites applications for the position of Director of the Laboratory, which will become open on October 1, 1975. Qualified applicants are invited to submit a curriculum vitae and summary of experience to one of the following:

Dr James D. Ebert, Chairman, Search Committee, Marine Biological Laboratory, Woods Hole, Massachusetts 02543;

Dr James F. Crow, Member, Search Committee, Department of Medical Genetics, University of Wisconsin, Madison, Wisconsin 53706;

Dr Douglas L. Coleman, Secretary, Search Committee, The Jackson Laboratory, Bar Harbor, Maine 04609.

AN EQUAL OPPORTUNITY EMPLOYER. (2508)

SUB-EDITORS

BUTTERWORTH'S have two vacancies in their Scientific Publishing department. Previous experience of sub-editing manuscripts and all aspects of book publishing is essential. A degree in chemistry or the life sciences is necessary.

The offices are situated in pleasant rural surroundings at Borough Green, near Sevenoaks, and company transport is available from certain surrounding districts. The minimum salary for a qualified sub-editor is £2,405 p.a. and new rates are at present under negotiation.

Please write, giving details of age, experience and qualifications to:

Mrs D. Lulham,
Butterworth & Co. (Publishers) Ltd.,
88 Kingsway,
London WC2B 6AB. (2511)

The University of Birmingham



Wellcome

THE WELLCOME FOUNDATION LIMITED

Structure, Physiology and Biochemistry of Insect Neuromuscular Junctions

Applications are invited for a CASE postgraduate studentship in the above field of work.

The student will work primarily in the Department of Zoology and Comparative Physiology at Birmingham University with some time spent in the Unit of Arthropod Physiology at the Wellcome Research Laboratories, Berkhamsted Hill, where biochemical aspects of work in this field are currently in progress.

The Studentship commences on 1st October, 1974 and is tenable for 3 years. The current value of the Studentship is £695 per annum.

Further details may be obtained from the Academic Supervisor:

Dr. M. P. Osborne,
Dept. of Zoology & Comparative Physiology,
University of Birmingham,
Edgbaston, BIRMINGHAM.

(2362)

SK&F

Technical Information in a Sales Environment

This post provides the opportunity for someone with a background in technical information work to move closer to the marketing sphere.

As a member of a team dealing with our full range of products, the prime responsibility will be for the provision of a full technical input to the sales function for our range of biological products. This will involve maintenance of product information dossiers, preparation of product licence applications and assistance with preparation of promotional material and technical training. There will also be a responsibility to represent the company's interest at conferences and seminars, and for the co-ordination of activities relating to the introduction of new products.

The Company, SmithKline Animal Health, is concerned with the development, manufacture and sale of veterinary medical products and feed additives on a worldwide basis.

Applicants should be in their mid to late twenties and will have already gained experience of technical support or information work in the health-care field. A biological science degree and knowledge of a European language is desirable. Salary will depend on the immediate contribution which the candidate is capable of making and an excellent range of benefits apply.

Please write giving brief career details to Mr. A. Maltby, SmithKline and French Laboratories Limited, Mundells, Welwyn Garden City, Herts.

(2567)

A member of the worldwide **SmithKline** Corporation

Tropical Products Institute, London

Food Scientist

■ Develop new food uses of tropical produce ■ Biochemical/chemical research relevant to food processing ■ Opportunity to work independently overseas.
☐ 1st/2nd hons degree in Biochemistry, Chemistry ☐ Some relevant experience in food industry desirable ☐ Age under 27 ☐ Appointment as Scientific Officer (£1663–£2557, under review, plus threshold payment) ☐ Ref: SA/24/JD.
☐ Application forms (for return by 16 July 1974) from Miss C. A. Hall, Tropical Products Institute, 127 Clerkenwell Road, London EC1R 5DB

Tropical Products Institute, Slough, Bucks

Entomologist

■ Research on biological problems relating to insect infestation of stored produce ■ Investigation of integrated control methods ■ Assist with training technologists from developing countries ■ Opportunity to work overseas.
☐ Degree in Zoology or Biology ☐ Age under 30 ☐ Appointment as Higher Scientific Officer (£2221–£2854) or Scientific Officer (£1435–£2329) ☐ Additional threshold payments ☐ Salaries under review ☐ Ref: SA/23/JD.
☐ Application forms (for return by 16 July 1974) from Miss C. A. Hall, Tropical Products Institute, 127 Clerkenwell Road, London EC1R 5DB.

Department of Health and Social Security London

Information Scientist

■ In Medical Division ■ To compile information on chemical contamination of food including additives, colouring, insecticides and pesticides, and on environmental health problems ■ Help develop data storage and retrieval system.
☐ Degree or equivalent in chemical or biological subject ☐ Age under 27 ☐ Appointment as Scientific Officer (£1663–£2557, under review, plus threshold payment) ☐ Ref: SB/2/K.
☐ Application forms (for return by 15 July 1974) from Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, telephone Basingstoke 29222 ext. 500 or 01-839 1992 (24 hour answering service).

Ministry of Agriculture, Fisheries and Food Royal Botanic Gardens, Kew

Botanist

■ In Jodrell Laboratory ■ Supervise assistants carrying out anatomical research ■ Undertake, after initial training, the identification of plant fragments and organs ■ Develop new techniques ■ Use optical and scanning electron microscopes ■ Give technical advice to visiting research workers.
☐ Degree in Botany ☐ Interest in literature on natural history and geography ☐ Age under 30 ☐ Appointment as Higher Scientific Officer (£2331–£2964) or Scientific Officer (£1545–£2439) ☐ Additional threshold payments ☐ Salaries under review ☐ Ref: SB/10/AH.
☐ Application forms (for return by 15 July 1974) from Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, telephone Basingstoke 29222 ext. 500 or London 01-839 1992 (24 hour answering service).

Ministry of Agriculture, Fisheries and Food Plant Pathology Laboratory, Harpenden

Plant Scientist

■ Responsible for technical and scientific aspects of plant health in Crop Certification Schemes to produce improved propagation material ■ Liaise with UK official bodies and growers' organisations ■ Travel involved ■ Advise on administration of plant disease legislation ■ Investigate mycological problems.
☐ 1st/2nd hons degree or equivalent in Plant or Agricultural Science, or related subject ☐ Knowledge of plant pathology and ability to organise essential ☐ Knowledge of Agriculture desirable ☐ At least 2 years post-graduate experience ☐ Age normally under 32 ☐ Appointment as Senior Scientific Officer (£2798–£3895) or Higher Scientific Officer (£2221–£2854) ☐ Additional threshold payments ☐ Salaries under review ☐ Ref: SB/3/AG.
☐ Application forms (to be returned by 12 July 1974) from Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke 29222, ext. 500 or London 01-839 1992 (24 hour answering service).

Science group
CIVIL SERVICE

(2523)

TOXICOLOGISTS

Veterinary Graduates, preferably with experience, required for Short and Long Term Toxicological Studies of compounds of potential therapeutic importance. Excellent opportunities for advancement in modern, well-equipped laboratories and Animal House. Pension and Assurance Scheme. Application forms from the Secretary, Biorex Laboratories Ltd., Biorex House, Canonbury Villas, London N1 2HB.

(2490)

BIRKBECK COLLEGE
(UNIVERSITY OF LONDON)
DEPARTMENT OF ZOOLOGY
M.Sc. DEGREES FOR PART-TIME STUDENTS
IN
ENTOMOLOGY
AND IN
COMPARATIVE PHYSIOLOGY

Each Course extends over a period of two calendar years and involves attendance on three evenings each week during term. Examination is by written papers and course assessment.

Full details may be obtained from The Secretary, Department of Zoology, Birkbeck College, Malet Street, London WC1E 7HX, to whom application should be addressed. (2507)

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL
(UNIVERSITY OF LONDON)
COURTAULD INSTITUTE OF BIOCHEMISTRY
RESEARCH ASSISTANT

Applications are invited from Honours Graduates in Biochemistry for the above post for work on lipid metabolism in the nervous system in relation to Multiple Sclerosis. Registration for a higher degree would be possible for a suitable graduate. Starting salary £1,371 per annum (under review). Applications in writing including a curriculum vitae and the names and addresses of two referees, to the Director, Professor R. H. S. Thompson, Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London W1P 5PR, by July 15, 1974, quoting ref. M.S.

(2496)

HISTOLOGY TECHNICIAN

required for Pharmacological and Toxicological Laboratory. Experience essential in the preparation and processing of animal tissues. Good working conditions. Pensions and Assurance Scheme. Application Forms from: Secretary, Biorex Laboratories Ltd., Biorex House, Canonbury Villas, London NI 2HB. (2489)

UNIVERSITY OF GLASGOW
DEPARTMENT OF CHEMISTRY

Applications are invited for an S.R.C. Postdoctoral Research Assistantship for work on the synthesis of enzyme models.

The appointment will be for one year in the first instance and, according to experience, within the salary range £2,118 to £2,412 per annum of the new Lecturers' salary scale which will be effective from October 1, 1974.

Applications giving a brief curriculum vitae and the names of two referees should be sent to Dr A. G. Cairns Smith, Department of Chemistry, University of Glasgow, Glasgow G12 8QQ, from whom further information can be obtained. In reply please quote Ref. No. 3478M. (2498)

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF HISTOLOGY
TECHNICIAN

required to work on a research project concerned with subcellular aspects of secretion in the anterior pituitary. An interest in radioimmunoassay, tissue culture or electron microscopy would be an advantage. Candidates should possess a degree, H.N.C. or at least O.N.C. in an appropriate subject. The appointment will be tenable for two years in the first instance. Salary range up to £1,920 per annum. Application forms may be obtained from the Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote ref. RV/N/276077. (2494)

ICI Pharmaceuticals Division

Graduate Biochemist

We have a vacancy for a Graduate Biochemist with a good honours degree in Biochemistry to work on the metabolism of parasites (helminths). Previous research experience would be an advantage, but knowledge of parasite biochemistry is not required.

The successful candidate will be expected to undertake original and applied research into helminth biochemistry, the mode of action of drugs on helminths, and the design of novel drugs, under the direction of a Research Scientist.

Pharmaceuticals Division is attractively situated in rural North Cheshire, within easy reach of main road and rail routes. Assistance given to married men in moving to this area is designed to attract and retain staff of high calibre.

Applications in writing, requesting an application form should be sent to:

M F Losse
Personnel Officer
ICI Pharmaceuticals Division
Mereside, Alderley Park
Macclesfield, Cheshire.

(2551)

DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH
NEW ZEALAND

Scientist

The Physics and Engineering Laboratory of the Department of Scientific and Industrial Research has a vacancy for a scientist at the Geophysical Observatory, Christchurch.

The Upper Atmosphere Division of the Physics and Engineering Laboratory, of which the Geophysical Observatory is part, is undertaking research into the physics of the ionosphere and magnetosphere, using a variety of experimental research and observatory ionosondes and magnetics) programmes from the Pacific Islands to Antarctica. Satellite telemetry facilities are also available.

Qualifications: 1st class honours degree in physics plus relevant experience.

Salary: Up to NZ$10,232 p.a.

Further information may be obtained from the High Commissioner for New Zealand, New Zealand House, Haymarket, London SW1Y 4TQ. Applicants should quote Ref. Imm. 2/284 and forward applications to the High Commissioner for New Zealand by August 1 1974.

(2487)

DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH
NEW ZEALAND

Scientist

The Industrial Chemistry Section of the Chemistry Division, DSIR, Lower Hutt, has a vacancy for a Chemical Engineer or Chemist with experience in the treatment of industrial waste. Duties will be to develop treatment processes for industrial effluents, and to carry out both laboratory and pilot scale studies.

Qualifications: Good Honours degree or Ph.D. required.

Salary: Up to NZ$10,232 p.a. depending on qualifications and experience.

Further details, passage assistance etc., may be obtained from the High Commissioner for New Zealand, New Zealand House, Haymarket, London SW1Y 4TQ. Applicants should quote Ref. Imm. 2/285 and forward applications to the New Zealand High Commissioner by July 31 1974.

(2486)

THE ANIMAL VIRUS RESEARCH INSTITUTE

PIRBRIGHT, WOKING, SURREY

Applications are invited from Honours graduates interested in chemical and biochemical studies of the structure and multiplication of animal viruses, for a post in the Biochemistry Department. Facilities are available for the successful applicant to work for a higher degree.

The appointment will be in the S.O. (£1,435 to £2,329) or H.S.O. (£2,221 to £2,854, grade, starting salary depending on qualifications and experience. FSSU Superannuation Allowance of 4½%. House available for married man. Application forms and further particulars from Secretary. (2513)

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF BOTANY
LECTURER IN APPLIED ECOLOGY

Applications are invited for the post of Lecturer in applied ecology commencing on October 1, 1974. Applicants should possess a good honours degree and postgraduate experience in plant ecology, soil science or agronomy. An interest or experience in applying ecological principles to solving practical problems of the environment such as land reclamation or pollution would be an advantage.

There will be excellent opportunities for collaborating with a very active research group in environmental rehabilitation which has strong links with industry and local government.

Initial salary within the range £2,118 to £2,412 on a scale rising to £4,896 per annum.

Applications together with the names of three referees should be received not later than July 19, 1974 by the Registrar, The University P.O. Box 147, Liverpool L69 3BX from whom further particulars may be obtained. Quote ref. RV/N/276073. (2495)

UNIVERSITY OF MANCHESTER
DEPARTMENT OF MEDICAL BIOCHEMISTRY

Applications are invited for a Postdoctoral RESEARCH ASSOCIATESHIP financed by the British Heart Foundation. The project involves the isolation of endothelial cells from major blood vessels, their establishment in culture and studies of the synthesis of the collagen and glycoprotein components of the endothelial basement membrane. Experience in cell culture and/or protein synthesis would be advantageous. The appointment from October 1, 1974 is tenable for two years. Salary range p.a.: £2,118 to £2,412 plus F.S.S.U. Applications giving the names of two referees should reach the Secretary, Department of Medical Biochemistry, University of Manchester Medical School, Manchester M13 9PT, as soon as possible. (2481)

Bass Charrington

Physical Chemist
BURTON-ON-TRENT

A Graduate with a good honours degree specialising in Physical Chemistry is required by a major Brewery Company for a period of three years to work on projects related to the Physico-Chemical characteristics of foam formation and collapse in beer.

The appointment and planned projects would be most applicable to a recent graduate wishing to proceed to an external Ph.D. qualification whilst working in an industrial research environment and earning a competitive salary with attractive fringe benefits.

Detailed applications should be sent to:—

The Staff Personnel Manager,
Bass Production Limited,
High Street,
Burton-on-Trent DE14 1JZ.

(2564)

TRENT POLYTECHNIC NOTTINGHAM
DEPARTMENT OF LIFE SCIENCES
SENIOR LECTURER IN BIOCHEMICAL PHARMACOLOGY
LECTURER GRADE II IN BIOCHEMISTRY

To commence September 1974 or as soon as possible thereafter. Salary scales (subject to ratification): Senior Lecturer—£3,525 to £3,915(bar) to £4,212; Lecturer Grade II—£2,700 to £3,474.

For further particulars and application forms please send self-addressed foolscap envelope to The Chief Administrative Officer, Trent Polytechnic, Burton Street, Nottingham NG1 4BU. Closing date July 5. (2474)

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL
(UNIVERSITY OF LONDON)
COURTAULD INSTITUTE OF BIOCHEMISTRY
RESEARCH ASSISTANT

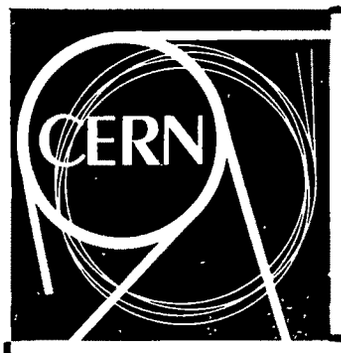
Applications are invited from Honours Graduates in Biochemistry for the above post to work with Dr B. Ketterer on studies of cellular proteins which interact with carcinogens. Registration for a Ph.D. degree would be expected. Starting salary £1,371 per annum (under review). Applications in writing including a curriculum vitae and the names and addresses of two referees, to the Director, Professor R. H. S. Thompson, Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London W1P 5PR by July 15, 1974, quoting ref C.R.C. (2506)

Electron Microscopy Technician (grade 3)

Required to assist generally in a fully equipped unit serving research and teaching in Biological Ultrastructure. The post offers opportunities in the development of techniques and instrumentation for applicants of appropriate interests and experience. Salary in the range £1,825 to £2,095 per annum.

Applications in writing to Mr. R. Adams, Departmental Superintendent, Department of Botany, Imperial College, London SW7 3BB.

(2473)



European Organization for Nuclear Research

Organisation Européenne pour la Recherche Nucléaire

CERN is a modern research laboratory, situated near Geneva, which offers first-class social and financial conditions of employment, and the opportunity to work in an international atmosphere.

A junior staff position in the Theoretical Study Division is open to scientists working on theoretical aspects of elementary particle physics or intermediate energy nuclear physics. Applicants should have a few years of post-doctoral experience and have accomplished interesting original research.

This position is for a period of three years, with the possibility of renewal for a further three years, and implies a basic salary of between 44,000 Sw.Frs., and 55,000 Sw.Frs., per annum, tax free, according to the qualifications of the selected person.

Candidates are invited to apply irrespective of nationality. However, among candidates with similar qualifications, preference will be given to nationals of CERN Member States.

Applications, including curriculum vitae and list of publications, are invited. They should be addressed to:

Professor D. Amati, Leader of the Theoretical Study Division, CERN, 1211 Geneva 23, Switzerland, and received before 15 August, 1974.

(2543)

UNIVERSITY OF OXFORD
PROFESSORSHIP OF CLINICAL BIOCHEMISTRY

The electors intend to proceed to an election to this professorship, which has been established with effect from August 1, 1974. The stipend is £7,559 a year. Further particulars may be obtained from the Registrar, University Registry, Broad Street, Oxford, by whom applications (twelve copies), naming three referees but without testimonials, should be received not later than July 13, 1974.

(2491)

UNIVERSITY OF NEWCASTLE UPON TYNE
DEPARTMENT OF ZOOLOGY

The University invites applications for the post of DEMONSTRATOR in the Department of Zoology. The appointment will be for a period of three years, subject to annual renewal, and will be tenable from September 1, 1974. Preference will be given to candidates experienced in animal behaviour (including relevant aspects of neurophysiology). Applicants should possess a higher degree or equivalent postgraduate research experience.

Salary will be at an appropriate point on the scale £1,929 to £2,223 (from October 1974 £2,118 to £2,412) according to age, qualifications and experience. Membership of F.S.S.U. required.

Further particulars may be obtained from the Registrar, The University, Newcastle upon Tyne NE1 7RU with whom applications (3 copies), together with the names and address of three referees, should be lodged not later than July 12, 1974. Please quote reference N. (2483)

UNIVERSITY OF ABERDEEN
DEPARTMENT OF MEDICAL PHYSICS
PHYSICIST

required for duties in hospital isotope service, which includes thyroid, haematological and scanning procedures. The department has an extensive research and teaching programme. Some experience of radiotherapy physics would be an advantage.

Salary on Lectureship Scale £1,929 to £4,548 (after October 1, 1974 £2,118 to £4,896) with placing according to qualifications and experience.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies), giving two referees, should be lodged by July 8, 1974. (2500)

THE UNIVERSITY OF LEEDS
DEPARTMENT OF COLOUR CHEMISTRY

Applications are invited for a RESEARCH STUDENTSHIP to examine novel triarylmethane dyes of potential biological interest.

The project is financed by Allen & Hanbury's Research Ltd., and the grant has the same value as a SRC Studentship. The person appointed will be able to read for a higher degree.

Applicants should have a good Honours degree (or equivalent) in Chemistry and should write by July 7 to Dr G. Hallas, Department of Colour Chemistry, The University, Leeds LS2 9JT, from whom further particulars may be obtained.

(2499)

EDITORIAL ASSISTANT

required for Genetics Abstracts. Degree in Biological Sciences preferably in Genttcs essential, foreign language an advantage. Apply in writing to Dr. E. S. Krudy, Information Retrieval Limited, 1 Falconberg Court, London W1V 5FG. (2504)

UNIVERSITY OF NOTTINGHAM
ELECTRONICS ENGINEER
SENIOR SCIENTIFIC OFFICER

Applications are invited for a new technical post in the Department of Zoology from qualified electronics engineers with experience of servicing and designing electronic equipmept. The appointee will occupy a senior position in a new Electrosphysiology Research/Teaching Unit in the Zoology Department and will be expected to play a major rôle in advising graduate students and academic staff on the technical aspects of their research and teaching programmes. Previous experience of biomedical electronics would be an advantage. Salary within the range £2,382 to £2,751 (bar) £2,862 to £3,555 per annum.

Application forms from Staff Appointments Officer, University of Nottingham, University Park, Nottingham quoting ref. 375. Closing date: June 30, 1974. (2484)

RESEARCH POST AT THE OXFORD HAEMOPHILIA CENTRE

Applications are invited from medically qualified graduate scientists with some experience of research in some aspect of haemostasis for appointment as a member of a small team of Medical Research Council staff working at the Oxford Haemophilia Centre, under the direction of Dr Rosemary Biggs The successful candidate will be expected to cooperate with other members of staff on projects already progressing at the Centre and to initiate a research project or projects in the general field of interest of the work at the Centre. The work of the M.R.C. staff is integrated with that of the N.H.S. Clinical Unit, many projects being undertaken jointly, and an Honorary Clinical contract with the United Oxford Hospitals may be sought at a level appropriate for the successful candidate The M.R.C. and N.H.S. staff also work in close co-operation with the staff of the Lister Institute Plasma Fractionation Laboratory and joint research projects may be planned. The appointment will be to the M.R.C. staff on the appropriate scientific grade and will initially be for a period of five years with the possibility of renewal at the end of this period. Superannuation provision.

Applications should be addressed to Dr Rosemary Biggs, Director, Oxford Haemophilia Centre, Churchill Hospital, Headington, Oxford OX3 7LJ (2477)

INFORMATION SCIENTIST

Senior management in the Malaysian natural rubber industry needs to be kept informed of techno-economic and statistical trends in rubber consuming countries. An abstracts-based information bulletin has been started as the core of a new information service for management, and a computerized retrieval system is now being developed.

A qualified information scientist is required to develop and operate this service at the Malaysian Rubber Producers' Research Association, which is shortly moving to new premises on an attractive site near Hertford.

Application forms and further details from the:—

Director,
Malaysian Rubber Producers' Research Association,
56 Tewin Road,
Welwyn Garden City,
Herts, AL7 1AD.

(2533)

POSITION AVAILABLE

ENTOMOLOGIST, ASSISTANT OR ASSOCIATE PROFESSOR RANK

Qualifications: Recent Ph.D., preferably with teaching, research or extension experience in applied entomology.

Duties: Teaching—To offer a course in economic entomology to the Diploma in Agriculture students; to assist in entomology courses for degree students; to supervise graduate students.

Research—To conduct research in pests of vegetables, ornamentals or greenhouse crops.

Extension—To provide extension information in his area of research; to identify insects sent in by home owners, and others and provide information concerning them.

Salary: Open.

Apply: Professor F. L. McEwen, Chairman, Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1. (2493)

KINGSTON POLYTECHNIC
RESEARCH ASSISTANTSHIP
SCHOOL OF GEOLOGY

The research assistant will be required to work upon A SEDIMENTARY AND FAUNAL ANALYSIS OF SOUDLEYAN/LONGVILLIAN (CARADOC) ROCKS IN SNOWDONIA, NORTH WALES.

This research will be conducted under the supervision of Dr. J. S. W. Penn. The selected candidate will be expected to register for a higher degree. Commencing salary £1,427.

Application forms and further details from Appointments Officer, Kingston Polytechnic, Penrhyn Road, Kingston upon Thames KT1 2EE. 01-549 1366. (2502)

THE MIDLAND CENTRE FOR NEUROSURGERY & NEUROLOGY

PATHOLOGY DEPARTMENT

Senior Technician—Electron Microscopy

Applications are invited for the post of Senior Technician in Electron Microscopy. The department is engaged on studies of the ultrastructural pathology of material from the central and peripheral nervous systems and muscle. Two Siemens microscopes are in use and an unusually comprehensive range of equipment is available. The successful candidate will be required to assist in instrument maintenance and in developing electron microscopy. Previous experience in election microscopy necessary.

The Department may be visited by appointment (021-558 2311).

Applications with names of two referees to
Area Administrator,
P.O. Box 50,
Shaftesbury House,
High Street,
West Bromwich,
B70 9LE.
(2526)

Faculty Position

ELECTRICAL ENGINEERING

Applications are invited for a position as Associate or Full Professor in the field of Electronic or Communication Engineering. Ph.D. and industrial experience required. Duties will include undergraduate and postgraduate teaching and laboratory development. The applicant should be able to initiate and lead research in electronics engineering; particular interest in communications or biomedical engineering would be preferable. Salary according to qualifications. Send applications to: O. K. Gashus, Head, Electrical Engineering Department, Nova Scotia Technical College, Halifax, Nova Scotia, Canada. (2527)

Ph.D or M.D. TO LEAD IMMUNOLOGICAL RESEARCH DEPARTMENT

A Ph.D. or M.D. is required by a French Pharmaceutical Research Laboratory of international size to direct a new Department: Immunology applied to therapeutics.

He (she) must be able to formulate research programmes and develop them.

Preference is given to a Ph.D. of (and) M.D. with about ten years postgraduate experience in industrial research.

No matter the nationality.

Send your resume in complete confidence to Box No. 2531, Nature, T. G. Scott & Son Ltd., Clement's Inn, London WC2A 2ED. (2531)

University College of North Wales
CHAIR OF FORESTRY

Applications are invited for the post of Professor and Head of the Department of Forestry and Wood Science.

The appointment will be from a date to be arranged and the salary will be not less than £5,973.

Further particulars can be obtained from the Secretary and Registrar, University College of North Wales, Bangor, LL57 2DG. TEN copies of the application (ONE from overseas candidates), together with the names of three referees, should be sent to reach the Secretary and Registrar by July 8, 1974. (2532)

STRANGEWAYS RESEARCH LABORATORY CAMBRIDGE

Cell biologist required to join a group in the Strangeways Research Laboratory concerned with cell motility in relation to cancer invasion. The work will mainly be in electron microscopy and tissue culture. The post which is financed by the M.R.C., is for a maximum of 4 years at a commencing salary of not less than £1,830 + F.S.S.U. Applications, together with the name of two referees should be sent to the Administrative Secretary (Ref. M.A.) Strangeways Research Laboratory, Wort's Causeway, Cambridge. (2534)

UNIVERSITY OF WESTERN AUSTRALIA
Perth

CHAIR IN MATHEMATICS

Applications for appointment to a Chair in the Department of Mathematics during 1975, preferably before September, are invited from persons who have made significant contributions in the field of applied mathematics and who, preferably, have had experience of teaching at university level. The present research strength of the Department in applied mathematics is primarily in classical areas, but applications will be welcomed from persons with interests in other areas.

The appointee will replace Professor J. J. Mahony who has resigned from the end of the year and will occupy one of four Chairs in the Department. Professor A. L. Blakers (Mathematics Education) and Professor J. P. O. Silberstein (Pure Mathematics) occupy two of the other Chairs. The third, which is earmarked for Mathematical Statistics, is vacant. The headship of the Department is filled periodically from among the professors by decision of the University Senate. The present Head (Chairman) of the Department is Professor Blakers. The appointee will share in the determination of policy and in the administration of the Department.

The present salary for a Professor is SA19,614 p.a. Benefits include superannuation similar to FSSU, fares to Perth for appointee and dependent family, removal allowance, study leave, long service leave and housing loan scheme. Further information incuding conditions of appointment and details of present staff and research in the applied area may be obtained from the Staffing Officer, or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications in duplicate stating full personal particulars, qualifications, experience and the names and addresses of three referees should reach the Staffing Officer, University of Western Australia, Western Australia 6009, by 20 July 1974. (2519)

RESEARCH ON TRANSFERABLE DRUG RESISTANCE ENTERIC REFERENCE LABORATORY

Applications are invited for two posts in the Enteric Reference Laboratory, Public Health Laboratory Service, Colindale, directed by Professor E. S. Anderson. Successful candidates will join a team investigating transferable drug resistance.

(1) A microbial geneticist to study epidemiological and ecological aspects of transferable drug resistance in enterobacteria.

(2) A microbial or molecular geneticist for research on the genetic and molecular characterisation of plasmid transfer systems.

Candidates with a 1st or 2nd class honours degree or a Ph.D. will be considered. Starting salary in accordance with age and experience.

Application with full curriculum vitae and names of 2 referees to Personnel Officer, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT. (2517)

Imperial College
Department of Biochemistry

BIO-ORGANIC CHEMIST

Applications are invited for an S.R.C. Post-Doctoral Assistantship for work on the Biosynthesis of Fungal Sesquiterpenes in collaboration with Dr G. Mellows.

Candidates should have an interest in and preferably experience of biological chemistry. The appointment is tenable for one year, commencing October 1, 1974. Salary range up to £2,247 p.a. with FSSU, plus £162 London allowance.

Applications, including curriculum vitae, research experience and the names of two referees should be sent as soon as possible to Dr G. Mellows, Department of Biochemistry, Imperial College, London SW7 2AY. (2525)

THE ROYAL DENTAL HOSPITAL OF LONDON SCHOOL OF DENTAL SURGERY
(University of London)

A Graduate Research Assistant is required by the Department of Physical Sciences to study ion-exchange and adsorption phenomena at the surface of hydroxyapatite, the mineral constituent of bones and teeth. Applicants are expected to have or to obtain a good degree in Physics or Chemistry. The appointment, which is for three years in the first instance, will be on a salary scale from £1,417 to £1,713 (under review) plus a London Allowance and F.S.S.U. benefits. Alternatively a part-time appointment would be considered.

Further particulars may be obtained from Dr N. E. Waters and applications should be sent to the School Secretary. (N) (2520)

RESEARCH MICROBIOLOGIST

Following a promotion a vacancy has arisen at our Research Laboratories in Edgbaston, Birmingham, for a chemical microbiologist to work on the production of chemicals and enzymes by fermentation processes.

Applicants, aged about 25, should have at least an upper second class degree preferably in chemistry with two or three years post graduate microbiological experience either academic or industrial. The Company operates staff bonus and pension schemes and assistance with removal expenses will be given where appropriate. Write, giving full details of education and career to date to:

Mr. P. E. Milsom,
JOHN & E. STURGE LTD.,
1 Wheeleys Road,
Birmingham, B15 2LE.
(2547)

WANTED
CYTOGENETICIST

To join team investigating genetic and immunological aspects of birth defects and leukaemia. The main responsibility of the successful applicant will be to carry out, with technical assistance, chromosome studies on amniotic cells and lymphocytes as part of the diagnostic and the research activities of the department. Previous experience desirable. This post is annually renewable initially and will be appointed on the appropriate Whitley Council scale.

Apply to:—

Dr. R. Harris,
Department of Medical Genetics,
St. Mary's Hospital,
Manchester 13,

stating experience and giving the names of two individuals to whom reference may be made. (2536)

UNIVERSITY OF NEW SOUTH WALES

PROFESSOR OF BIOCHEMISTRY

Applications are invited for appointment to the third chair to be established in the School of Biochemistry. A Chair of Biochemistry is held by Professor E.O.P. Thompson, the present Head of School, and Dr W. J. O'Sullivan has recently accepted the Chair of Medical Biochemistry. Applicants should have appropriate quafifications and research experience in a major field of biochemistry, with an interest in university teaching and a capability for academic leadership.

The School has extensive undergraduate programmes for students in science, applied science and medicine as well as postgraduate research activities for higher degrees. The new professor will supervise and participate in teaching and examining undergraduate and postgraduate students in the School, with particular reference to the lecture courses and practical training for students in science and applied science, and will engage in and promote research and advanced study in the field of his chair.

Salary $A19,614 per annum. Subject to the consent of the University Council, professors may undertake a limited amount of higher consultative work. The University reserves the right to fill any chair by invitation.

Details of appointment, including superannuation, study leave and housing scheme, may be obtained from the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close in Australia and London on **August 30, 1974.** (2522)

WHITTINGTON HOSPITAL LONDON N.19.

DEPARTMENT OF CHEMICAL PATHOLOGY

BASIC GRADE BIOCHEMIST

Required to work in this well-equipped Department.

The Post would suit a recent graduate and could provide a comprehensive training in Clinical Chemistry.

For further details please contact Miss S. McFarlane, Senior Biochemist, Whittington Hospital, Highgate Hill, London N.19. (Tel: 01-272 3070 Ext. 233).

Application forms available from Hospital Personnel Officer (Address as above). (2518)

CHARING CROSS HOSPITAL MEDICAL SCHOOL

DEPARTMENT OF BIOCHEMISTRY

TECHNICIANS

Applications are invited for the following posts:

1. Senior research technician to join research group headed by Professor A. Neuberger, F.R.S., working on glycoproteins, supported by the Medical Research Council. Applicants should hold HNC, AIST or equivalent qualifications and have suitable laboratory experience for this senior position. This appointment is from 1st July 1974 until 31st March 1977. The salary is on Whitley scale commencing at £2,400 plus London weighting.
2. Research technician to join Professor A. Neuberger's group working on the biosynthesis of porphyrins, supported by the Wellcome Trust. Applicants should hold recognisable qualifications for this post which is from 1st July 1974 until 31st March 1977, salary £1845 plus London Weighting. Applications, in writing, to the Secretary, Charing Cross Hospital Medical School, Brandenburgh House, Fulham, W.6. (2542)

THE NORTH OF SCOTLAND COLLEGE OF AGRICULTURE

Applications are invited for the post of SPECIALIST ADVISORY OFFICER in Animal Husbandry. In addition to Specialist Advisory Work duties include participation in Research and Development. Posting, for some years at least, will be to Inverness to serve the six counties of the Highland Region.

Salary Scale: Grade IV £1,584-£2,148

Grade III £2,328-£4,061

Cost of living safeguard allowance payable in addition. Superannuation Scheme.

Forms of application and further particulars of the post (Reference Number 46/74) may be obtained from the Secretary, The North of Scotland College of Agriculture, School of Agriculture Building, 581 King Street, Aberdeen AB9 1UD with whom application should be lodged **not later than 10th July, 1974.**

(2545)

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF CANCER STUDIES

Research Technician, Grade III, is required to work on a project involving a study of the mechanism of carcinogenesis by chemical compounds. Biochemical work, tissue culture work and some animal experimentation will be involved. Preference will be given to candidates with experience in either biochemistry or tissue culture techniques. Applicants must have an ONC in an appropriate subject and several years laboratory experience. Salary will be on the scale £1,650-£1,920. Applications should be sent with a curriculum vitae and the names of two referees to the Personnel Department, University of Birmingham, Birmingham, B15 2TT. Ref. 450/B/264. (2546)

UNIVERSITY OF READING

DEPARTMENT OF PHYSIOLOGY AND BIOCHEMISTRY

RESEARCH DEMONSTRATOR

required for work on the regulation of luteinising hormone secretion in the chicken, involving the use of neurophysiological and radioimmunoassay techniques. Candidates should have a degree in Physiology or Zoology or a related discipline and may register for a higher degree. The post will be for three years from October 1, 1974 with possible extension and involves about 12 hours demonstrating per week during term. Salary in the scale £1,047 by £51 to £1,149 p.a. (under review). Apply with names of two referees, quoting Ref. TN 48, to Assistant Bursar (Personnel), University of Reading, Whiteknights, Reading RG6 2AH. (2560)

UNIVERSITY OF BRISTOL

Postdoctoral Protein Crystallographer

Applications are invited for an S.R.C. post to work on the crystal structure of pyruvate kirase or phospho glucose isomerase. The research will involve the development of computer programs to handle data collected by an oscillation camera. The appointment is for a period of two years with an initial salary of up to £2,058 per annum depending on experience. Previous research experience in crystallography is essential.

Applications, including a brief curriculum vitae and names and addresses of two referees, should be sent to Dr. H. Kinase, Department of Biochemistry, University Walk, Bristol BS8 1TD. (2558)

JOHN INNES INSTITUTE

DEPARTMENT OF ULTRASTRUCTURAL STUDIES

The Department of Ultrastructural Studies is accommodated in a new laboratory with extensive facilities for studying plant cell systems with the aid of electron microscopy and light microscopy.

A vacancy exists in the Department for an assistant to work on the ultrastructure of plant cells and protoplasts. An interest in the visualisation of cell structure is essential. Some experience in microscopy will be an advantage but the successful applicant will be given training in electron microscopy and tissue culture techniques.

The appointment will be as a SCIENTIFIC OFFICER (£1,435 to £2,329) or ASSISTANT SCIENTIFIC OFFICER (£961 to £1,729). Grade and starting salary will be determined by qualifications and experience. H.N.C. or pass degree qualifications are required for appointment to the Scientific Officer grade. Superannuation benefits are provided under the Institute's Scheme for Clerical and Technical Staff with 5¼% allowance in addition to salary to offset contributions.

Applications with full curriculum vitae and the names of two referees should be sent to the Secretary, John Innes Institute, Colney Lane, Norwich NOR 7OF, by July 5, 1974. (2540)

TECHNICIAN (Grade 3)

required in Department of Microbiology, University of Reading, to assist in research on bacterial classification and to prepare materials for classes. Interest in photomicrography an advantage. Salary in scale £1,650 to £1,920 p.a. Apply stating qualifications and experience and names of two referees, quoting Ref. TN46, to Assistant Bursar (Personnel), University of Reading, Whiteknights, Reading RG6 2AH. (2559)

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF PHYSIOLOGY AND ENVIRONMENTAL STUDIES

Applications are invited for a Lectureship in the above Department in the School of Agriculture. Areas of interest should preferably include the regulation of enzyme activities, the control of photosynthetic processes, and cellular aspects of growth and development, although other areas will not necessarily be excluded. The salary will be on the Lecturers scale: £2,118 to £4,896 although the appointment will initially be made within the first three points of the scale. The appointment is tenable from October 1, 1974.

Application forms and conditions of appointment available from Staff Appointments Officer, University of Nottingham, University Park, Nottingham NG7 2RD. Please quote ref. 377. Closing date June 30, 1974. (2553)

INSTITUTE FOR MARINE ENVIRONMENTAL RESEARCH, EDINBURGH

ECOLOGISTS

The research programmes of the Edinburgh Oceanographic Laboratory of I.M.E.R. are designed to describe and analyse variability and to formulate models of pelagic ecosystems, based largely on the Continuous Plankton Recorder survey of the North Atlantic Ocean and North Sea for which 27 years' data and computing facilities are available.

Applications are invited for two posts in the existing team of biologists and physicists at Edinburgh which is expected to move to Plymouth within the next three years:

ZOOPLANKTON ECOLOGIST (Post 38): Research on spatial and temporal variability, and population dynamics, of zooplankton in the C.P.R. survey. First or good second-class honours in zoology, preferably with experience of marine ecology and knowledge of computing.

SYSTEMS ECOLOGIST (Post 42): Research on structure and dynamics of plankton communities in relation to environmental variability, leading to formulation and validation of models of pelagic ecosystems. First or good second-class honours in a relevant biological or mathematical science, with experience of ecosystem analysis and modelling.

Grades and starting pay dependent on age, qualifications and experience. Candidates will be considered for appointment at Higher Scientific Officer/Senior Scientific Officer level (salary range £2,221 to £3,895) and exceptionally well qualified candidates will be considered for Principal Scientific Officer (£3,715 to £4,895) (Scales under review).

Non-contributory Pension Scheme.

For application forms or further particulars write (specifying post 38 or 42) to the Director, Institute for Marine Environmental Research, 67/69 Citadel Road, Plymouth PL1 3DH, Devon.

CLOSING DATE: JULY 10, 1974. (2541)

UNIVERSITY OF SURREY LECTURER/INDUSTRIAL TUTOR

DEPARTMENT OF BIOCHEMISTRY

Applications are invited from suitably qualified graduates with experience in the pharmaceutical or food industry for this new post in the Department of Biochemistry. The successful candidate will be required to organise the year of industrial experience of students reading for the 4-year B.Sc. Courses in Biochemistry and Nutrition, and to participate in the teaching, administration and research of the Department.

The teaching of the Department includes courses in biochemistry, clinical biochemistry, food science, nutrition, pharmacology and toxicology. Preference will be given to candidates with teaching/research interests in food science, pharmacological, or clinical biochemistry.

Salary, within the scale £2,118 to £4,896 per annum, with F.S.S.U., from October 1, 1974.

Further particulars of the post may be obtained from the Academic Registrar (L.F.G.), University of Surrey, Guildford GU2 5XH. Telephone: Guildford 71281, extension 452, and to whom applications should be sent, in the form of a curriculum vitae, including present salary and the names and addresses of two referees, by Friday, July 12, 1974. (2552)

THE ROYAL FREE HOSPITAL

DEPARTMENT OF NEUROLOGY

RESEARCH ASSOCIATE

Postdoctoral fellow required for work in muscle spindle morphology. Preference will be given to those with experience in electron microscopy.

The post is available from September 1, 1974 and the appointment would be for 3 years. Salary to a maximum of £2,232 first year depending on qualifications and experience.

Applications, naming two referees, to the Secretary, Department of Neurology, The Royal Free Hospital, Grays Inn Road, London WC1X 8LF. (2538)

UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF SPACE RESEARCH

Applications are invited for two posts, supported by S.R.C. grants:

X-RAY ASTRONOMY

Research Associate to carry out theoretical and laboratory studies of grazing incidence optical systems for use in X-ray telescopes. Previous experience of X-ray techniques useful but not essential. The posts is available for two years in the first instance.

ION-MOLECULE COLLISIONS

Research Associate within a group carrying out measurements of ion-ion and ion-molecule reaction rates using an advanced flowing afterglow apparatus. Experience with these techniques or with spectroscopic techniques would be advantageous but is not essential. The post is available for three years.

Salary scale (under review): Research Associate £1,569 to £2,223: Exceptionally: Senior Research Associate £2,223 to £2,718 plus F.S.S.U.

Applications (3 copies) naming 3 referees to the Assistant Registrar (S), University of Birmingham, Birmingham B15 2TT by July 22, 1974. Quote Ref. NL7. (2562)

MEDICAL RESEARCH COUNCIL
NATIONAL INSTITUTE FOR MEDICAL RESEARCH
MOLECULAR GENETICIST

A Scientist with postdoctoral experience is required to join a small group working on the molecular biology and genetics of yeast. Primary interests of the group are in the replication and function of nuclear and mitochondrial DNA and of the molecular and genetic interrelationships between the nuclear and mitochondrial genomes.

Incremental salary according to age and qualifications within the range £2,223 to £3,378 p.a. plus £162 p.a. London Allowance and F.S.S.U. provision.

Applications, giving the names of two professional referees, should be sent to the Director, National Institute for Medical Research, Mill Hill, London NW7 1AA. (2565)

SEALE-HAYNE COLLEGE
LECTURER IN THE DEPARTMENT OF BIOLOGICAL SCIENCES

Applications are invited from suitably qualified AGRICULTURALISTS or BIOLOGISTS. Candidates should have interests in one or more of the following:

CROP PHYSIOLOGY
ANIMAL PHYSIOLOGY
ECOLOGY

The main courses taught at the College include five Higher National Diplomas, two Ordinary National Diplomas as well as post-graduate/post-diploma certificate courses.

The appointment will be at either the Lecturer Grade I or Lecturer Grade II, Scales of Salaries for Teachers in Further Education, according to age and experience. Further particulars may be obtained from The Principal, Seale-Hayne College, Newton Abbot, Devon, to whom applications should be sent by July 12, 1974. (2566)

PHARMACOLOGISTS/ TOXICOLOGISTS

Honours Graduates/Postdoctoral Fellows, with some experience preferred, required for research and toxicological studies of compounds of potential therapeutic importance. Excellent opportunities for advancement in modern, well-equipped laboratory. Pension and Assurance Scheme. Application forms from the Secretary, Biorex Laboratories Ltd., Biorex House, Canonbury Villas, London N1 2HB. (2563)

UNIVERSITY COLLEGE DUBLIN
APPOINTMENT IN PHARMACOLOGY

Applications are invited for a teaching post in the Department of Pharmacology. Candidates must have a Ph.D. or an equivalent qualification *either*

(i) in pharmacology or physiology

or

(ii) in a cognate subject, provided that the candidate has had a significant undergraduate broad training in physiology.

The current salary scales are:
College Lecturer £3,514 by £162 to £4,648.
Assistant Lecturer £2,420 by £129 to £3,323.

Entry point on the relevant scale will be in accordance with qualifications and experience.

A non-contributory pension scheme and family allowances are additional to salary. An alternative contributory F.S.S.U. type scheme is also available.

Further information and details of application procedure may be obtained from:
Mr J. P. MacHale,
Secretary and Bursar,
University College,
Belfield,
Dublin 4.

Latest date for receipt of completed applications is Friday, July 5, 1974. (2537)

INSTITUTE OF CANCER RESEARCH
SUTTON, SURREY BRANCH
RADIATION CHEMIST/BIOCHEMIST Ph.D.

A vacancy exists on the full time research staff for a suitably qualified scientist to work within an M.R.C. sponsored research program. The successful applicant will be applying pulse radiolysis and other radiation techniques to radiobiology and radiotherapy problems. Salary on the lectureship scale (M.R.C. Grade II) with F.S.S.U. Candidates from overseas who may be attending the Seattle Congress of Radiation Research July 14-20 could be interviewed there by contacting Professor Boag.

Application in duplicate with names of two referees should be sent to the Secretary, 34 Sumner Place, London SW7 3NU, quoting Ref. 300/9/75(a). (2535)

THE NEW SOUTH WALES INSTITUTE OF TECHNOLOGY
SYDNEY, AUSTRALIA
PRINCIPAL LECTURER AND HEAD OF DEPARTMENT OF CELLULAR PATHOLOGY
in the
SCHOOL OF LIFE SCIENCES
SALARY $A15,744 to $A16,389 p.a.

The School occupies a new six-storey building adjacent to a large teaching hospital (Royal North Shore). There are well equipped laboratories for general biology, biochemistry, microbiology, environmental biology, physical biology and biomedical subjects.

The appointee will be responsible to the head of the School for the teaching and the development of degree level programmes in cellular pathology.

This will include developing courses in haematology, blood storage, histopathology and histochemistry. The appointee will also be required to study current trends in the fields mentioned, and to make recommendations on new undergraduate and postgraduate courses.

It is anticipated that the appointee will be medically qualified, possess appropriate postgraduate qualifications and have substantial professional experience. Depending on his interests and experience he may be offered an association with the Royal North Shore Hospital, in which case a right of limited private practice may be approved under similar terms and conditions to other such appointments to the hospital.

The position is offered with tenure. Superannuation, long service leave and an appropriate housing loan scheme are available. Also fares will be paid for the successful appointee and a contribution will be made towards initial accommodation expenses.

Enquiries regarding the Institute may be made with the Agent-General, New South Wales Government Offices, 66 Strand, London WC2 5LZ.

Applications close with the Agent-General on July 26, 1974. (2549)

THE UNIVERSITY COLLEGE OF WALES
ABERYSTWYTH
DEPARTMENT OF PHYSICS

Applications are invited for postgraduate work in the Department of Physics for sesssion 1974-75. The Science Research Council is prepared to offer to suitable candidates a limited number of Research Studentships for ionosphere and space research and studies of atomic collisions in plasmas, shock and detonation waves, and the flow of non-Newtonian fluids.

The Science Research Council has also accepted the postgraduate course on Physics of the Atmosphere as suitable for tenure of its Advanced Course Studentships.

Applications to Professor W. J. G. Beynon, F.R.S., Department of Physics, University College of Wales, Aberystwyth. (2478)

UNIVERSITY OF SOUTHAMPTON
DEPARTMENT OF CHEMISTRY

Postdoctoral Research Fellowship available from October 1974 to work on time-resolved fluorescence spectroscopy of aromatic molecules using mode-locked laser as excitation source. Starting salary up to £2,058 p.a. (under review) according to age and experience.

For further details please write to Dr D. Phillips, Department of Chemistry, University of Southampton, Southampton SO9 5NH. (2479)

THE SCHOOL OF PHARMACY
UNIVERSITY OF LONDON
RESEARCH STUDENTSHIP IN NEUROPHARMACOLOGY

An M.R.C. Research Studentship is available in the Department of Pharmacology for 3 years from October 1974 for neuropharmacological studies on sympathetic ganglia using electrophysiological and/or radio-active tracer techniques. Applicants should hold, or expect to attain, a good honours degree in a relevant subject and would be encouraged to register for a higher degree in the University of London.

Enquiries and applications should be made to Dr D. A. Brown, Department of Pharmacology, The School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX. (2497)

THE UNIVERSITY OF MANCHESTER
DEPARTMENT OF MEDICAL BIOCHEMISTRY
S.R.C. RESEARCH STUDENTSHIP

Applications are invited for an S.R.C. Research Studentship tenable from October 1, 1974, to study aspects of the biosynthesis of connective tissue macromolecules. Applicants are expected to have, or to obtain, a first or upper second class honours degree in biochemistry or an allied subject. Further information is available from Professor D. S. Jackson, Dept. of Medical Biochemistry, University of Manchester Medical School, Oxford Road, Manchester M13 9PT, to whom applications including a brief curriculum vitae and the names of two referees should be forwarded as soon as possible. (2480)

UNIVERSITY OF BIRMINGHAM
FACULTY OF MEDICINE AND DENTISTRY
DEPARTMENT OF PHYSIOLOGY

Applications are invited for a POSTDOCTORAL RESEARCH FELLOWSHIP to collaborate in a study of the physiological and pharmacological regulation of renin release under the supervision of Dr B. Singer.

Starting salary up to £2,553 p.a. plus F.S.S.U., depending on age and experience.

Applications, with curriculum vitae, naming two referees, should be sent to Professor S. M. Hilton, Head of the Department of Physiology, The Medical School, Birmingham L15 2TJ. (2476)

UNIVERSITY OF LONDON
REACTOR CENTRE
(IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY)
S.R.C. RESEARCH STUDENTSHIPS

The Science Research Council is prepared this year to offer to suitable candidates a limited number of research studentships tenable at the University of London Reactor Centre. The value of these awards will be £695 p.a. (under review).

The areas of research for which studentships may be available are the following:

(i) Heat transfer studies on the U.L.R.C. reactor;

(ii) Fast reactor physics;

(iii) Neutron activation analysis.

Candidates who possess, or hope shortly to obtain, a good honours degree in a relevant subject are invited to write for further information to: Dr T. D. MacMahon, University of London Reactor Centre, Silwood Park, Sunninghill, Ascot, Berkshire SL5 7PY. (2503)

FELLOWSHIPS AND STUDENTSHIPS

KINGSTON POLYTECHNIC
RESEARCH STUDENTSHIP
SCHOOL OF CHEMICAL AND PHYSICAL SCIENCES

Applications are invited from good honours graduates in chemistry for a postgraduate research studentship. The successful candidate will be registered for a higher degree and will be involved in the study of the rôle of Boric Acid in the Synthesis of Anthraquinone Dyestuffs, in collaboration with Borax Consolidated Ltd. and I.C.I. (Organic Div.).

Enquiries to Dr P. B. Brindley, School of Chemical and Physical Sciences, Kingston Polytechnic, Penrhyn Road, Kingston upon Thames KT1 2EE. (2501)

UNIVERSITY OF VIENNA
DEPARTMENT OF PHYSICS
POSTDOCTORAL RESEARCH FELLOWSHIP

Applications of Physicists and Metallurgists are invited for Basic Research on the deformation of metals. The group guided by Prof G. Schoeck works on plasticity of b.c.c. single crystals. Electron-microscopy, low temperature and hydraulic deformation facilities are available. Knowledge of German not essential. Salary approx. £2,000 p.a., after tax. Address applications and enquiries to H. Kirchner, II. Phys. Inst., A-1090 Strudlhofgasse 4. (2505)

LANCHESTER POLYTECHNIC
S.R.C. RESEARCH
STUDENTSHIPS

The Science Research Council is prepared to offer to suitable candidates two CASE Research Studentships at Lanchester Polytechnic to undertake research for a PhD Degree in the fields of:

SOLID STATE PHYSICS
CORROSION FATIGUE

Applicants should possess, or expect to obtain this summer, a first or upper second class Honours Degree or the equivalent in an appropriate subject.

Application forms and further particulars available from the Assistant Secretary (Personnel), Lanchester Polytechnic, Priory Street, Coventry CV1 5FB, returnable by July 8, 1974. (2475)

PH.D STUDENTSHIPS
INSTITUTE OF CANCER RESEARCH
(UNIVERSITY OF LONDON)

Two postgraduate Studentships will be available from October 1974 for studies on:

1. The chemical mechanisms involved in radiobiology and enzymology. The work will involve the application of rapid mixing techniques combined with pulse radiolysis, and rapid freezing, to investigate the radiation chemistry of radiosensitisers and of the mechanistics of fast enzymatic processes.
2. Development of tissue culture methods for the study of Sertoli cells from animal and human tissues with special reference to their structure and biochemical function.

Applicants should have or expect to have at least an upper second class honours degree in biochemistry, chemistry or possibly physics (No. 1) or in biochemistry, zoology or a related subject (No. 2).

Applications to The Dean, Institute of Cancer Research, 26 Neville Street, London SW7 3AS. (2482)

University of Kent at Canterbury

RESEARCH FELLOWSHIP
IN BIOCHEMISTRY

Applications are invited for a post-doctoral research fellowship involving work on nucleic acid and protein metabolism of malaria parasites and the effects of drugs. The appointment will be initially for one year, with the possibility of extension. Salary in the range £2,118-£2,412 with F.S.S.U. benefits. Application forms and further particulars may be obtained from the Assistant Registrar, Faculty of Natural Sciences, Chemical Laboratory, The University, Canterbury, Kent CT2 7NH to whom completed applications should be returned by July 19, 1974. Please quote ref. A60/74. (2530)

UNIVERSITY OF BIRMINGHAM
NEUROCOMMUNICATIONS
RESEARCH UNIT

A vacancy occurs in the above Unit for a Research Fellow, preferably with relevant research experience, to study the electrophysiology of the mammalian cortex in relation to complex sound stimuli. Appointment for a period of one year from August 1, 1974, or as soon thereafter as possible.

Salary scale (under review): £1,929 to £2,223 p.a. or, exceptionally, to £2,388 p.a. plus F.S.S.U.

Further particulars from Assistant Registrar (RS), University of Birmingham, P.O. Box 363, Birmingham B15 2TT, to whom applications (five copies) naming two referees should be sent by July 10, 1974. (2561)

THE EDINBURGH SCHOOL OF AGRICULTURE

RESEARCH STUDENTSHIP

Applications are invited for a three-year research studentship financed by the British Egg Marketing Board for work on biochemical aspects of food intake regulation in poultry. The successful candidate will be required to possess a good honours degree in biochemistry, physiology or agricultural science. The value of the award is £750 per annum plus post-graduate fees.

Further information and application form from the Secretary, The Edinburgh School of Agriculture, West Mains Road, Edinburgh EH9 3JG. (2524)

NEW ZEALAND

Senior and Post-doctoral Research Fellowships

The New Zealand National Research Advisory Council invites applications for 10 Senior and Postdoctoral Fellowships to be taken up in New Zealand in 1975. The awards will be for scientific research in Government Departments. A good working knowledge of English is required.

The emoluments will be: Senior, within the range NZ$10,288 to NZ$15,265, Postdoctural NZ$8,637. All emoluments are taxable.

Senior Fellowships will normally be for a period of from nine months to year and Postdoctoral Fellowships for a period of from two to three years.

Application forms and further particulars may be obtained from: The Senior Administration Officer, National Research Advisory Council, Box 8004, Wellington, New Zealand; The Senior Scientific Adviser, New Zealand High Commission, New Zealand House, Haymarket, London SW1Y 4TQ England; from the New Zealand Embassy, 19 Observatory Circle, N.W., Washington D.C. 20008, U.S.A.; from the New Zealand High Commission, Suite 804, Commonwealth Building, 77 Metcalfe Street, Ottawa, Canada K1P 5L6; from the New Zealand High Commission, Commonwealth Ave., Canberra 2600, A.C.T., Australia; from the New Zealand Embassy, 20-40 Kamiyama-cho Shibuya-ku, Tokyo 150, Japan; from the New Zealand Embassy in Bangkok, Bonn, Brussels, The Hague, Jakarta, Lima, Paris, Rome, Saigon, Santiago, Seoul; from the New Zealand Consulate-General in Athens, Geneva, Los Angeles, New York, San Francisco, Sydney; from the New Zealand Commission in Hong Kong; and from the New Zealand High Commission in Kuala Lumpur, and Singapore.

Applications must reach the Council's office by September 30 1974.

(2488)

University of Aberdeen

DEPARTMENT OF ZOOLOGY

An S.R.C. Research Studentship is available in the Department of Zoology, University of Aberdeen, for any one of the following projects—

1. Characterisation of information transfer between macrophages and lymphocytes.
2. The effect of polyanions on immunosuppressed mice.
3. Tracheal gas composition and oxygen uptake in aquatic insects.
4. Ligand induced redistribution of cell surface receptors.
5. Electron microscope studies of uterine cancers.

Further information and applications forms may be had from Dr. D. T. Gauld, Department of Zoology, Tillydrone Avenue, Aberdeen AB9 2TN. (2528)

UNIVERSITY OF HULL
PSYCHOLOGY DEPARTMENT
RESEARCH STUDENTSHIP
SOCIAL PSYCHOLOGY OF PET-KEEPING

Applications are invited for this studentship which is sponsored by Pedigree Petfoods Ltd. Tenable for three years, the studentship commences in September 1974 and is valued at £1,200 tax free, per annum, with an annual allowance for research expenses.

Candidates should hold a first or second class honours degree in psychology. Some experience in market research or of field-work in a related area would be an advantage. Further details can be obtained from Mr J. G. M'Comisky, Department of Psychology, The University, Hull HU6 7RX.

Applications (three copies) giving the names of two referees, should be sent to the abovenamed by July 1, 1974. (2485)

THE UNIVERSITY OF LEEDS
PROCTER DEPARTMENT OF FOOD AND LEATHER SCIENCE

OPPORTUNITIES FOR POSTGRADUATE RESEARCH

Several postgraduate studentships will be available in the department from October 1974. Areas of work for which grants may be available are:

Application of food technology to the catering industry;
Basic studies of leather processing;
Biochemical aspects of utilisation of fish proteins, citrus waste, beet waste;
Carbohydrate-protein interactions;
Cereal foods of developing countries;
Electron-microscopy of foodstuffs;
Electron microscope studies in leather manufacture;
Fat-globule membrane in ice-cream;
Flavour studies using combined G.L.C.-mass spectrometry;
Fluidisation of particulate foods;
Lipid-protein interactions in relation to meats;
Mechanical behaviour and structure of skin and leather;
Microstructure of cakes with special reference to the rôle of emulsifiers;
Pectolytic enzymes;
Physical and chemical properties of flour doughs;
Rapid methods of food analysis;
Structure of proteins in foods; and
Studies of ancient leathers and parchments.

Depending on the area of interest, a good degree in chemistry, biochemistry, physics, chemical engineering, or a biological subject would be appropriate. All awards permit registration for a higher degree.

Enquiries and requests for application forms to Professor A. G. Ward, Procter Department of Food and Leather Science, The University, Leeds LS2 9JT. (2555)

POST DOCTORAL RESEARCH FELLOW

required to work in the Division of Experimental Pathology. The vacancy is for three years, and will involve some work being carried out in the Department of Experimental Pathology, University of Birmingham. The project is M.R.C. supported, and will be concerned with studies of the kinetics of lymphoid cells in the mouse. Background and experience in immunology, lymphoid physiology and/or autoradiographic techniques will be an advantage.

Applications with curriculum vitae and names of two referees to The General Secretary, Kennedy Institute of Rheumatology, Bute Gardens, London W6 7DW. (2548)

RESEARCH STUDENTSHIPS

Applications are invited for two three-year Research Studentships, tenable from October 1, 1974, at the M.R.C. Dunn Nutritional Laboratory, Cambridge, in the following areas:

(1) The relationship between protein metabolism and hormonal status in animals subjected to protein-energy malnutrition.

(2) The biochemical effects of riboflavin deficiency in an experimental animal, to relate the known parameters of subclinical riboflavin deficiency with biochemical lesions which may have pathological consequences. These studies will form part of a new programme, the aim of which is to examine various aspects of human nutritional status in relation to the water-soluble vntamins.

Applicants should have, or be expecting this summer, a first or upper second class honours degree, preferably in Biochemistry or Nutritional Science, and should send their curriculum vitae and names of two referees for (1) to Dr P. G. Lunn and (2) to Dr C. J. Bates, at the Dunn Nutritional Laboratory, Milton Road, Cambridge CB4 1XJ, not later than June 30, 1974. (2556)

UNIVERSITY COLLEGE CARDIFF

DEPARTMENT OF APPLIED MATHEMATICS AND ASTRONOMY

Applications are invited from those possessing or expecting to have a First or Upper Second Class Honours Degree in Mathematics or Physics for two S.R.C. Studentships. The first is in the areas of astrophysics and general relativity and the second is in atomic scattering theory, plasma physics or solid state (with special reference to disordered systems or theory of small particles). Applicants will be expected to register for a Ph.D. Applications to Professor N. C. Wickramasinghe, Department of Applied Mathematics and Astronomy, University College, P.O. Box 78, Cardiff CF1 1XL. Please quote ref. 0594. (2557)

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Please write for further information and application form to the Secretary, Thames Polytechnic, Wellington Street, London SE18 6PF. (2550)

GRANTS

THE NATIONAL KIDNEY RESEARCH FUND

invites applications for grants for renal research at units within the United Kingdom. Application forms are available from The Secretary, 184B Station Road, Harrow, Middlesex HA1 2RH (01-863 4460). Completed applications will be required by July 31, 1974. (2454)

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RESEARCH SCHOLARSHIP

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Further particulars and application form from Secretary, NVRS, Wellesbourne, Warwick CV35 9EF. (2529)

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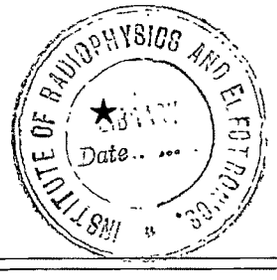
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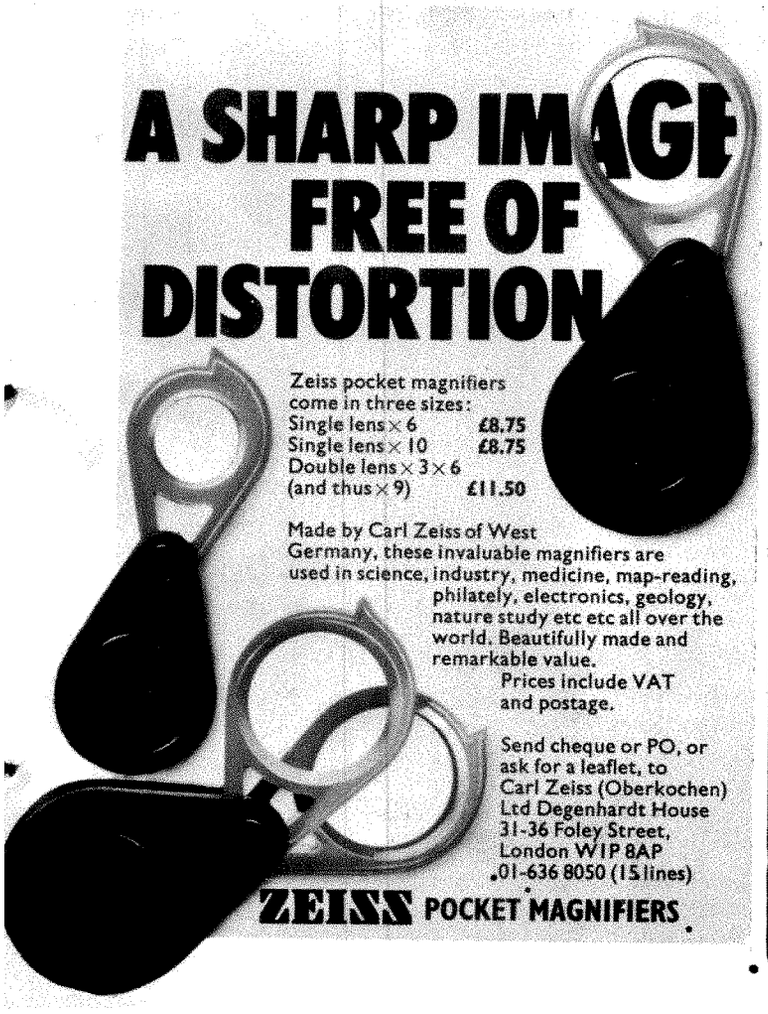
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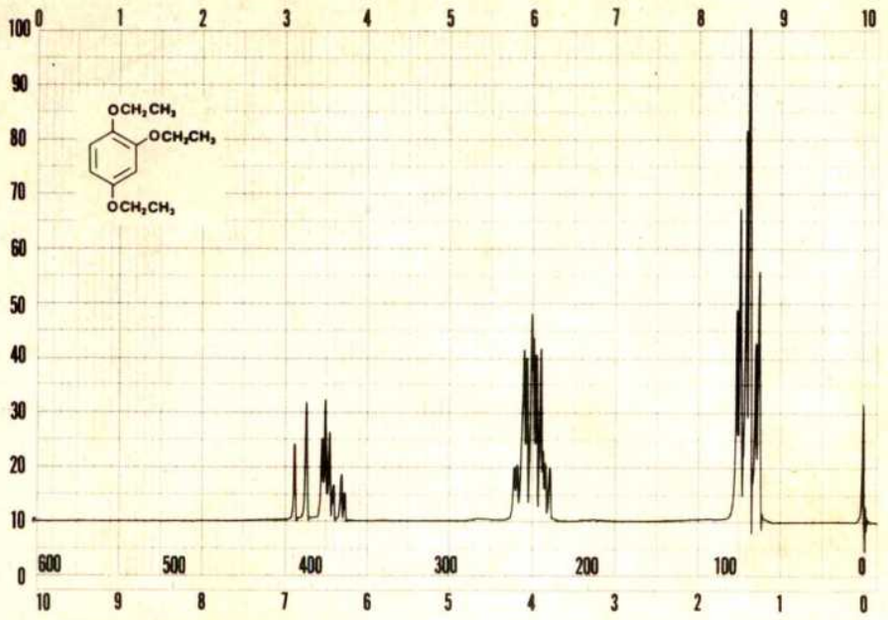
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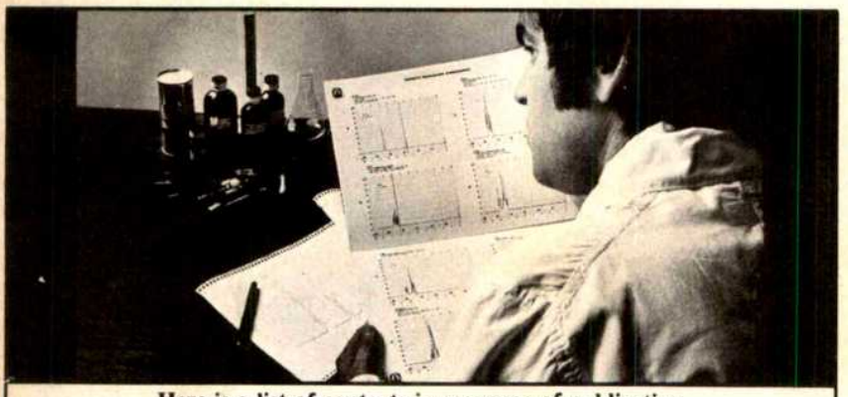
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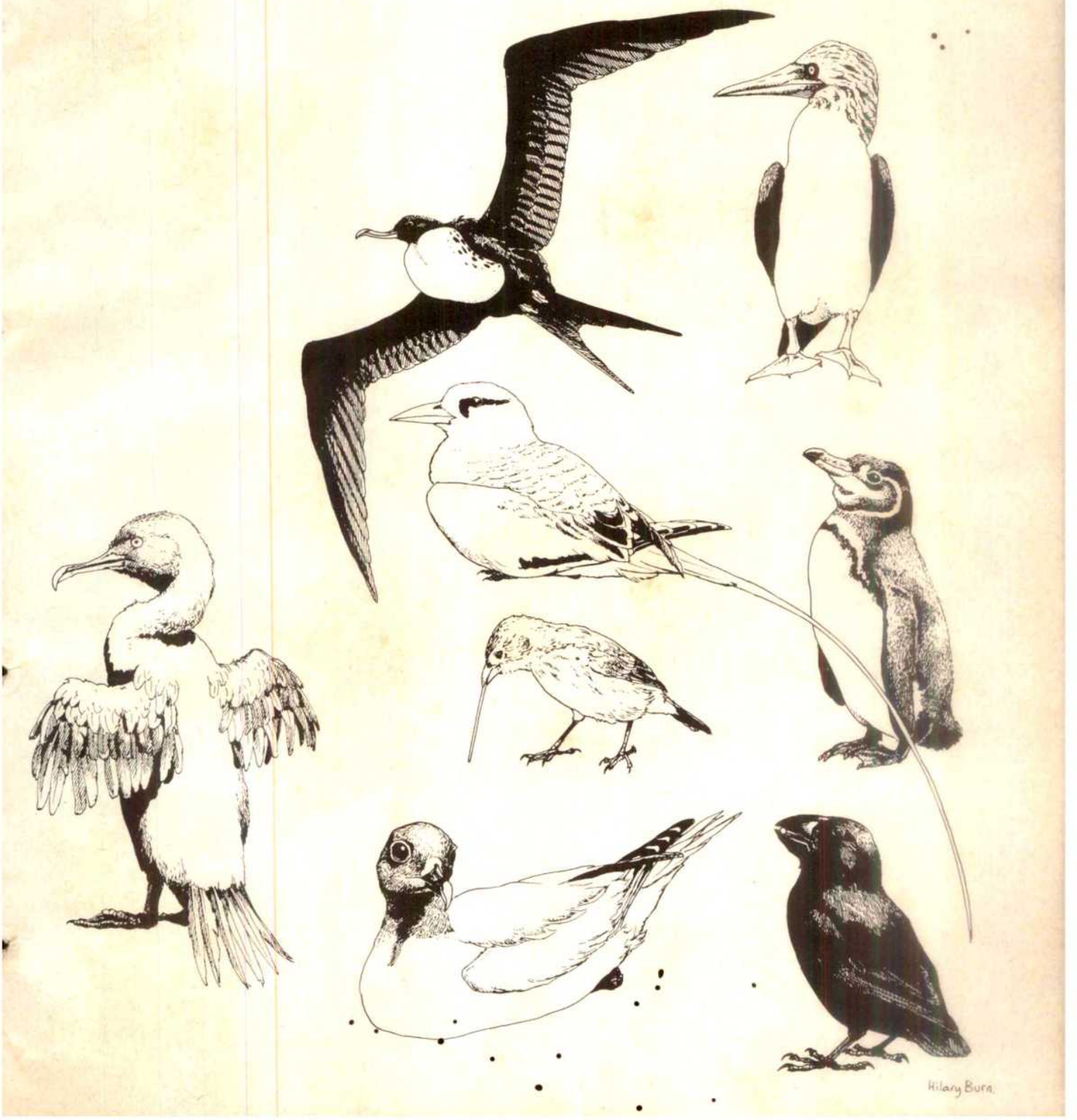
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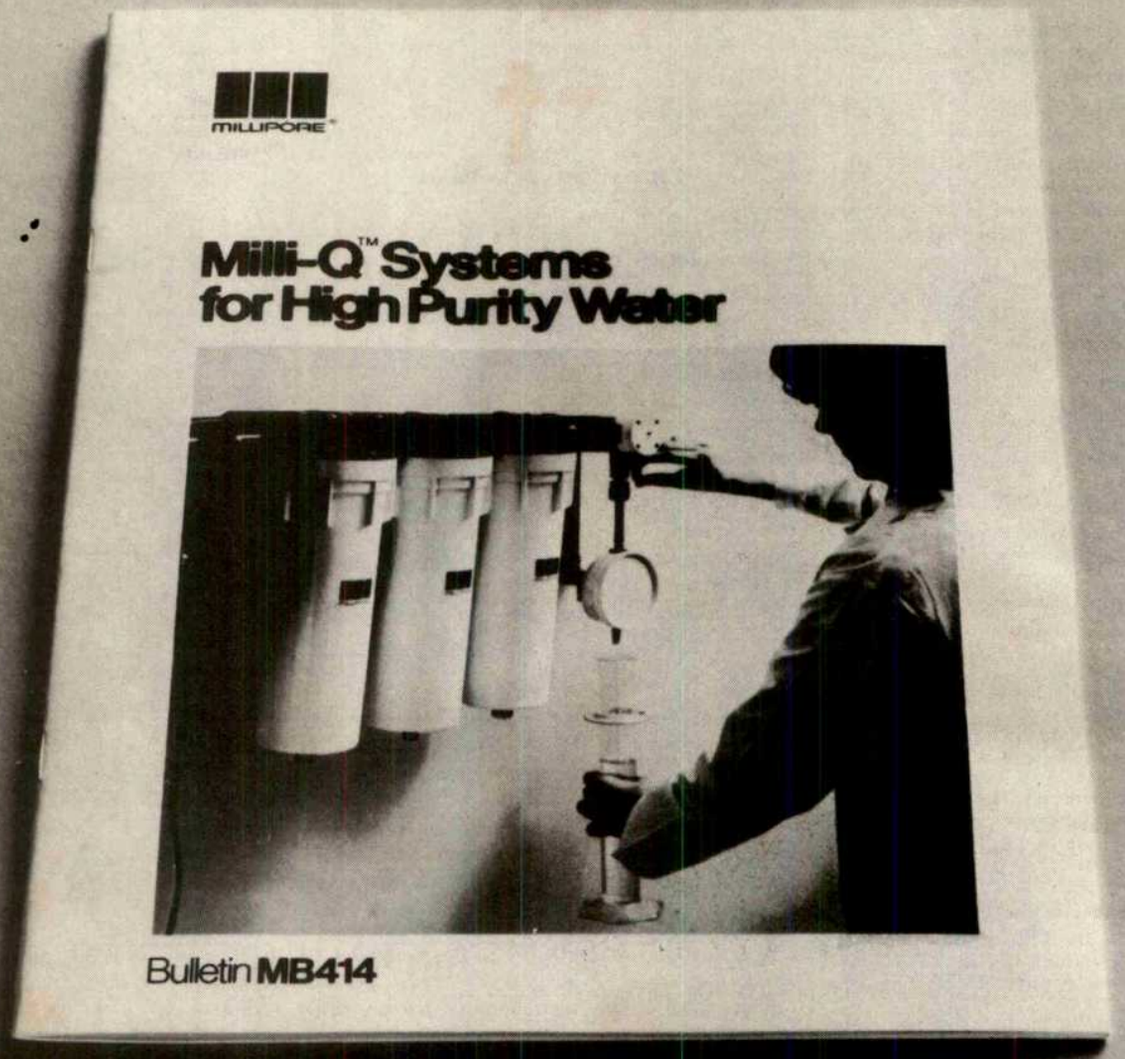
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nature

Volume 249 June 28, 1974

and now . . . nuclear hypocrisy in Britain

IT has been a quite remarkable two months in which the issue of nuclear weapons testing has emerged from the shadows in which it has been lurking for ten years. The United States and Soviet Union have made noises about a further treaty; India has detonated her first device; China has fired another one (her sixteenth); France has ventured on a new series. It was only Britain of the nuclear powers who had not contributed to the drama, and now she too has obliged in characteristic fashion—by being found out.

The revelation by Mr Chapman Pincher in the *Daily Express* that there was shortly to be a British test in Nevada was greeted officially by refusals either to confirm or deny. Now Mr Wilson has announced that the test has already taken place. He must have strong feelings of *déjà vu*, as he found himself with a similar legacy from the Conservative government in 1965 when he last stopped Britain's testing activities.

Since this test surfaced as the result of a leak—and not a government orchestrated one at that—one is bound to ask whether this really is the first for nine years or whether previous testing in Nevada has been kept more effectively behind a security screen. There is really no way that anyone can find out, as testing in Nevada goes on so regularly that there would be nothing untoward to the outside world about the occasional additional test. Obviously whilst Mr Wilson was in power in the sixties there would be ideological objections, but Mr Heath was Prime Minister for nearly four years and would have no commitment to self-restraint. It seems inconceivable that it would take four years from the brakes being released until as vigorous an organisation as Aldermaston could find time to test.

The tragedy of the recent revelations is that this British obsession for secrecy in all matters of defence has rebounded so vigorously on prospects for British diplomacy. A few weeks ago *Nature*, along with almost everyone else in the West, was rebuking India for becoming a nuclear power, and the main line that most, including ourselves, pursued was that it was simply hypocritical to talk of a nuclear explosive as capable of being labelled peaceful. Britain, of course, could be particularly smug since she had renounced the provocative gesture of firing nuclear devices years ago. Our restraint was an example to the world. Now we find ourselves with much the same sort of hypocrisy on our doorsteps—a failure to be at least elementally honest about nuclear affairs; worse, a willingness to have our own test labelled, by default, as American. It is difficult to see why secrecy should extend this far. Obviously the Labour Government were not going to reveal the hot potato they were passed, but surely the Conservative government would have been in no danger in giving prior notice. The announcement of a test would barely have stirred a ripple; the discovery is bound to make waves.

The real significance of recent events, however, is that they show how inextricably intertwined are British and American nuclear interests. It is just not possible for one country to detonate explosions at another country's facility without there being such a high degree of liaison that one is bound to conclude that the two countries act as one in nuclear matters. Of course, the possession of Polaris submarines already ensures this to a large extent; the nuclear test simply brings it once more to our attention. This liaison reduces Britain's independence in the discussion of nuclear issues on a world stage to an ineffectual level. This is very unfortunate at a time when the two superpowers appear to be on the verge of agreeing between themselves on some form of nuclear test treaty.

The form most widely discussed is of a threshold agreement, restricting tests not to exceed a certain seismic magnitude. There have been strident objections to this throughout the scientific community, based on a feeling that such a treaty is easy to find loopholes in, leaves room for ambiguity and has not been given a fair debate in wide enough a community. It is possible to sense qualms in British circles too, but what hope is there of an independent and questioning line under these circumstances?

The entanglement, however, does not simply constrain Britain in nuclear matters. It is impossible to see how Atlantic and European relationships on all diplomatic matters can fail to be influenced to a degree by the nuclear commitment. It is high time that the nuclear relationship and its influence on foreign policy was looked at with great care.

100 years ago



MR. JAMES LICK, of San Francisco, California, having in the course of his life accumulated a large fortune, has recently concluded a deed by which he conveys all his property to seven persons upon trust to be applied to various worthy objects. Among these, 700,000 dols. are to be applied to the construction of a more powerful telescope than any yet made, to be erected at an observatory in California, and 300,000 dols. to found, in California, a school of the mechanical arts.

From *Nature*, **10**, 171, July 2, 1874.

Kill a Pinta goat a day . . .

Science and conservation in the Galapagos Archipelago must swim against a fast running tide of tourism and goats. David Davies looks at the way in which the Ecuadorian Government and the Charles Darwin Foundation are trying to maintain the islands.

"I FOUND long ago that I cannot live happily without the Galapagos Islands; even if I am not there I need to know that they exist undestroyed by goats or men . . ." So wrote Peter Kramer, retiring director of the Charles Darwin Station on Santa Cruz in the Galapagos Islands in December 1973.

The Galapagos Archipelago is on the equator about 1,400 kilometres off the Ecuadorian coast. Darwin was there for 35 days in 1835, and was deeply influenced by what he saw. His field notes were dominantly geological; he was struck by the youthful volcanic nature of the rocks. It was however, the biological scene which was ultimately to have most impact on him—giant tortoises, finches (Darwin's finches) whose bills were "modified for different ends", marine iguanas, flightless cormorants and many others. By the time Darwin was writing *Origin of Species* he had absorbed much else and the effect of his stay in the Galapagos is less obvious, but in his early evolutionary writings the significance of islands is crystal clear; in 1842 he wrote on the back of a notepad "islands are the nursery of new species".

He was not the only visitor to the Galapagos. In the nineteenth century whalers would put in on their long journey from the Pacific to the American East Coast round Cape Horn, and would collect a giant tortoise to keep in the hold of the ship for later consumption. Many other ships also put in to the Galapagos, caring nothing about what species they brought in. One such import about seventy years ago was the Norwegian black rat which had such a grip on Pinzon, for example, that by 1965 no tortoises younger than thirty could be found; all tortoise hatchlings were being killed by rats. Other feral animals such as the pig have also wrought havoc on the tortoise population of various islands by eating the eggs. But the most dramatic and visible introduction has been the goat.

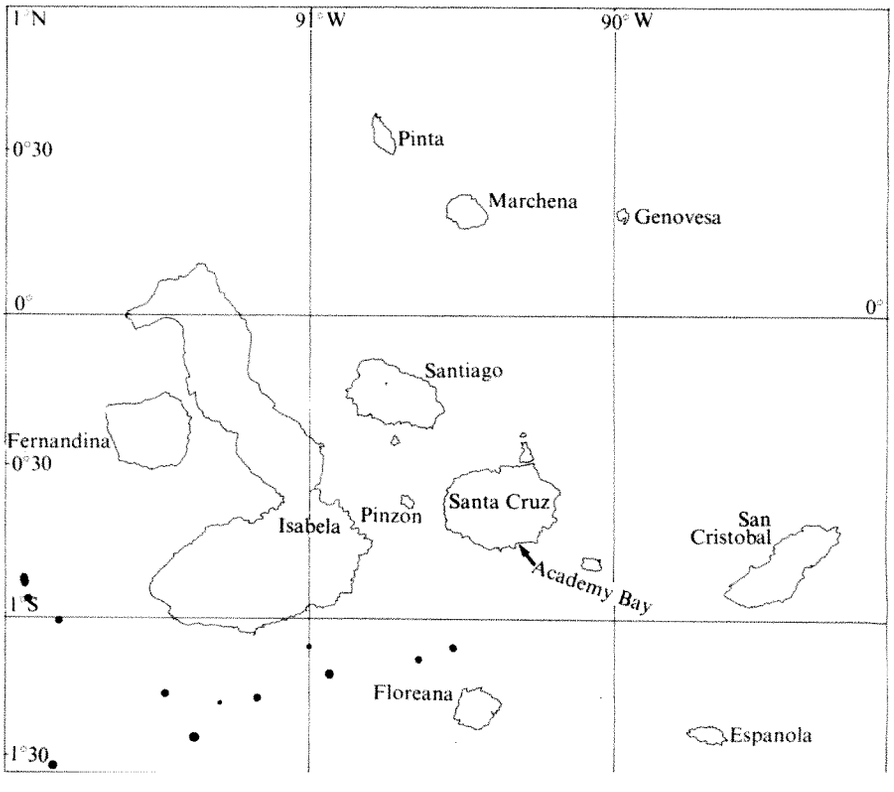
The local population has been in the habit of depositing goats on the more remote islands to ensure a food supply for fishing expeditions. Thus in 1958, a male and two females were left on Pinta. By 1968 the number had reached 4,000 to 5,000 and in 1971 the estimate was "not below 20,000". Hardly surprisingly, species of plants unique to the archipelago are threatened by this population on Pinta and elsewhere.

Not all the impact on the Galapagan environment has come from man's works. There are many historically active volcanoes in the west of the archipelago, and in 1968 the caldera collapse of Fernandina was the world's largest collapse in seventy years. In addition latitudinal oscillations of the Oceanic Equatorial Counter Current (the 'El Niño' phenomenon) lead to long term fluctuations in rainfall and have striking effects on the life of the islands. The most recent threat, however, has been man himself, as resident and tourist.

The population of the islands was less than 1,000 in 1960; now it is more than 4,000. Santa Cruz and San Cristobal have the most inhabitants whilst some islands, particularly the active volcanoes, are prudently uninhabited. Many of the inhabitants are farmers but an increasing number are needed to service a tourist industry which now provides for several thousand annually compared with 200 in 1969. Much of this tourism has been stimulated by the natural environment of the Galapagos and has been appropriately careful, but a growing number of private yachts visit the island on Pacific cruises and they are causing serious problems. As one scientist put it, "man has now replaced goats as the greatest threat".

The conservation issue is not simple. Although biologists would agree that there is an obvious case for the strictest of measures to preserve the islands intact, they are also the home of many Ecuadorians. In addition they occupy a strategic naval position. In two World Wars it was "he who holds the Galapagos holds the Panama Canal". There is thus a strong naval presence and in the past has even been some suspicion by the military that scientific operations have had more sinister overtones. There is thus a delicate diplomatic balance necessary in order that residents, military, tourists and nature coexist.

Ecuador has had a formal interest in Galapagan conservation since 1934 when legislation was enacted to ban the export of protected animals and the import of domestic ones. Nature reserves were also established, but



safeguards or international inspection rights (it is in fact the only reactor apart from the Canadian-Indian plant which is unsafeguarded). According to representative Melvin Price, Chairman of the Congressional Joint Committee on Atomic Energy (JCAE), it is capable of producing 8 kg of plutonium a year, which is almost enough for a crude bomb. The country is not known to have constructed a plant for separating out the plutonium from the spent fuel rods, but according to one US official, it could do so relatively easily. Thus, whatever safeguards are applied to the proposed power reactor, Israel would still be capable of joining the nuclear club if she chooses to do so. It should also be noted that neither Israel nor Egypt has ratified the Non-proliferation Treaty, although Egypt has signed it.

Be that as it may, the nuclear deals are likely to be developed as follows. Israeli and Egyptian officials have been in Washington this week to negotiate contracts for the supply of fuel to the reactors. These negotiations, ironically, have to be completed before agreement is reached on the reactor sales because the Atomic Energy Commission has set a June 30 deadline for new contracts involving the delivery of enriched uranium to foreign countries in the early 1980s. Then, a few weeks later, negotiations will begin on the sale of the reactors themselves. If agreement is eventually reached the JCAE will be informed and Congress will have 30 days in which to react. The agreement could be prevented from going into effect only if Congress passes a bill disapproving the terms under which the sale will take place. But the Bill would be subject to a possible presidential veto.

Representative Price has promised that the JCAE will give the matter a full and open review, and he has said that he "will keep (Congress) appropriately informed." of developments, during the negotiations.

But several members of Congress are dissatisfied with that arrrangement, and are attempting to give Congress veto power over any international agreement involving the export of nuclear technology. Representative Lester Wolff has already introduced a Bill into the House which will prevent the Middle East agreements from going into effect until Congress gives its express approval of the deal, and Senator William Proxmire has announced his intention to propose an amendment to the Export Administration Act when it comes before the Senate in the next few weeks, which will require that "no nuclear technology, nuclear materials, or associated equipment could be sent to any country until both houses of Congress give approval in a vote". According to a staff member of the JCAE, however, such a requirement would place a huge burden on Congress because in the past six months alone the United States has entered into some 50 separate agreements involving such transactions.

In contrast to the nuclear agreements, the scientific exchange proposals announced by President Nixon have scarcely raised a flicker of interest in the United States. As far as Egypt is concerned, Nixon and President Sadat announced in a joint communique that their two governments will establish a series of working groups to meet in the near future to prepare concrete projects and proposals for bilateral research in the fields of agriculture, "technology, research and development in scientific fields including space, with special emphasis on the exchange of scientists," and medical research.

Such activities will supplement the present limited scientific cooperation between the two countries, which is funded to the tune of about $1.5 million a year through excess US currency in Egypt (so-called PL480 funds). At this stage, the proposal is simply an agreement to cooperate and no concrete projects have been decided. Similar arrangements are proposed with Jordan and Saudi Arabia.

As for Israel, the agreement is less specific, referring only to the need for "further encouragement of the fruitful links already existing between the two countries in the scientific and technical field, including space research.

New King Coal

by Eleanor Lawrence

A "NEW ERA" for coal is forecast by the Secretary of State for Energy, Mr Eric Varley, in his introduction to the interim report of the examination into the coal industry (published by the Department of Energy) carried out jointly by the government, the National Coal Board and the mining unions.

After the depression in the British coal industry in the 1960s the report paints a much more hopeful picture of the future for the industry into the 1980s and beyond. Future demand for coal will be at least equal to if not greater than the present 130 million tons a year, predicts the report. But to satisfy this demand the industry will have to "run very fast to keep still" to the tune of £600 million in capital investment by 1985. New capacity of 42 million tons a year will be needed by 1985 to replace the coal lost by exhaustion of existing mines.

Twenty million tons of this is expected to come from new fields. The big Selby coalfield in Yorkshire will contribute up to 10 million tons a year by 1985 (it is hoped) and Mr Varley, in reply to a question in Parliament, said that the possibility of developing the rich seam of coal under Oxfordshire is being actively explored.

Nine million tons a year would come from the life extension of pits which would otherwise be quickly exhausted, and the remaining 13 million tons from new schemes at existing pits. With opencast mining a total production of 150 million tons a year is to be the ultimate target.

The upswing in the fortunes of coal follows the dramatic rise in the oil prices during the past year, which has again made coal a fuel competitive with oil. Economic conditions for a thriving industry are right for the first time in twenty years, says Mr Varley. Now the coal industry can be both competitive and commercially self-sufficient.

The electricity boards are the coal industry's main customers, taking 58% of National Coal Board (NCB) production in 1973, and the future of coal depends to a large extent on their long term policies. The Central Electricity Generating Board (CEGB) has told the committee that, providing coal remains competitive with oil, the boards will continue to take as much NCB coal as they can get. The CEGB reckons that in 1985 it will be able to burn about 90 million tons (compared with the 76 million tons it took in 1973). But the industry fears that this potential demand will not be realised unless new coal-fired generating capacity is built.

In the event the NCB has shown itself amply justified in its whole-hearted endorsement of the future of coal even when things looked blackest. The industry contracted from an output of 210 million tons and a workforce of 770,000 men in 1959 to 130 million tons and 270,000 men in 1973. The government is obviously determined not to return to the situation of the 1960s, when the increasing dependence on cheap oil led to the running down of the industry, and promise in the report that the industry shall not be at the mercy of short term fluctuations in the price of competing fuels.

Mr Varley said in Parliament last week that it would need "superhuman effort" from the industry to meet the ultimate target of 150 million tons a year. Production is down this year and two major strikes in the past three years have cast doubts on the essential condition of security of supply.

General Atomic's new fusion venture

from David Fishlock

WHILE discussions continue on the design—and location—of JET, the Joint European Tokamak, plans are ready for a start in La Jolla next month on the first of the new generation of thermonuclear fusion machines. These are the machines plasma physicists believe will demonstrate later this decade the feasibility of fusion, by reaching 'break-even'—the point at which the experiment's energy output equals the input.

Dr Tihiro Ohkawa, head of fusion research at General Atomic, the joint nuclear venture between Gulf Oil and Royal Dutch - Shell, has received approval from the United States Atomic Energy Commission for his plans for Doublet III. If all goes well by about the end of 1977 Doublet III will be ready to demonstrate plasma confinement times as long as one second.

Doublet III is the successor to a very successful experiment in magnetic containment called Doublet II, carried out by General Atomic between 1971 and 1973. Dr Ohkawa demonstrated how the plasma itself could be used as an electrical conductor to generate intense magnetic fields that remain unperturbed by the plasma. Doublet III will be a large scale version of this experiment and is expected to cost about $26 million.

A site has been chosen in the valley below General Atomic's hilltop research campus, so that the power lines supplying some 5 million amperes can conveniently be kept out of sight. The dumpling-shaped structure, some 13 feet tall and 18 feet in diameter, is expected to take 3–5 years to complete. Meanwhile, to assist the designers Doublet II has been stripped down and is now being rebuilt as Doublet IIA, with magnetic facilities for fine tuning the plasma configuration.

In the late 1960s, when the physics of fusion was still in chaos, Dr Ohkawa's fusion research group suffered far more severely than the British Culham Laboratory. After Texan utilities withdrew their support his group numbered no more than seven. But, encouraged by Dr C. L. Rickard, vice-president in charge of advanced systems development, Ohkawa rebuilt confidence among sponsors. Today he can boast the largest fusion team in the private sector anywhere in the world. It numbers 80 and this year will spend about $7 million. Next year it will number 120.

It draws these funds from three sources—the company, a group of electrical utility sponsors and the United States Atomic Energy Commission. Of this year's research budget, about $2 million is being spent on plasma physics, $4 million on large fusions systems (mostly Doublet IIA) and the remaining $1 million on technology for the fusion reactor. But this last sector is expected to grow quickly, so that by 1980 it will be at least the size of the other two parts of the programme.

Already a conceptual design is taking shape of a candidate for the prototype fusion reactor which the Atomic Energy Commission hopes to start building in 1976. General Atomic is thinking in terms of a reactor based on Doublet III that will 'piggyback' on the company's experience with helium-cooled fission reactors. Instead of using a liquid-metal-cooled system, as is usually proposed for extracting heat from a fusion reaction, it plans to use helium as the coolant and to drive gas turbines directly with the hot but inactive gas. For the present, therefore, Dr Ohkawa is confining his forays into fusion reactor technology to work on a ceramic torus—of silicon carbide and carbon—in which the plasma will be confined.

Death-blow dealt to Moscow seminar

from Vera Rich



Slepak : leading activist

INTENSIFIED pressure on the part of the Soviet authorities would seem to have dealt a death-blow to the Moscow 'underground' seminar on "Collective Phenomena and the Applications of Physics to Other Fields of Science" scheduled for July 1-5, 1974. A wave of arrests in the Soviet Union of Jewish dissidents, supposedly in connection with security arrangements for the Nixon visit, coupled with the blocking of visa applications from intending foreign participants, almost certainly mean that the seminar can no longer take place as originally envisaged.

The campaign began, apparently, on Wednesday, June 19, with an attempt to arrest an electronics engineer, Vladimir Slepak, who, although not a member of the Seminar Programme Committee, is a leading Jewish activist in the Soviet Union. Slepak apparently resisted arrest by barricading himself into his apartment; on Friday, however, the police broke in with crowbars. It is reported that when his fifteen-year-old son Leonid attempted to contact western journalists by telephone, the KGB intervened.

On the same day, Committee Member Viktor Brailovskii, in hiding to avoid forcible conscription, failed to keep a prearranged telephone contact with the international organisers of the seminar, while a telephone call to Tanya Levich (the wife of committee member Venyamin Levich) was established and cut off three times so that no message could be conveyed. It now seems that Lerner, Brailovskii and also committee members Mark Azbel, Aleksandr Lunts and Aleksandr Voronel were arrested on Friday or Saturday. Voronel, whose Moscow apartment was to have been the venue of the seminar, was arrested at the home of a friend. He is reported to have "given himself up" to the police—in view of the details of Slepak's arrest, however, this probably means no more than that he opened the door to them. Since all contacts between the dissidents and the outside world have now been broken, it is quite possible that this list of arrests is by no means complete.

Simultaneously with the news of the arrests, the foreign "participants" learned that they themselves would not be able to travel to Moscow. Some 40 scientists from the United States (including seven Nobel Prize winners) have been refused visas. Similar refusals are reported from Israel and France. The British contingent, for whom more details are available, reveal an interesting picture of blocking tactics. Those intending to travel alone were told, on submitting their applications, that they must provide an official invitation from some recognised scientific organisation in the Soviet Union (which, of course, they were unable to do). Since then they have heard no more of their applications. The others, who planned to travel as a group, have not met a definite refusal; but since the last date by which the travel agents needed visa clearance in order to complete the necessary arrangements has now passed, this amounts to *de facto* refusal.

Asked to comment on the situation Dr Norman Chigier of the University of Sheffield, one of the three International Secretaries of the Seminar, observed: "It appears that the Soviet authorities are determined to destroy the seminar".

Egg collector collected



Wood Sandpiper: collected

CITIZENS of the United States are entitled under their constitution to bear arms, and they believe in using them. In addition to shooting public figures and wild game, they are also accustomed to collect any rarities they encounter "for scientific purposes". There is a conspiracy of silence over this in North America, where for example the senior ornithological journal has refused to publish any comments on reports that specimens of the first Little Gulls *Larus minutus* and Wood Sandpipers *Tringa glareola* found nesting in North America were promptly collected despite the fact that they are easily recognised and that there was already ample material available. Even the supposedly militant Audubon Societies have said nothing about the situation. There has, however, been a growing chorus of complaint where the same policies have been followed abroad. Many of the atrocities have been perpetrated by young and inexperienced people, brought up in an unhealthy tradition by their seniors who tend to escape the blame, so it is worthy of note that one Daniel has at least been brought to judgement in his own courts.

For several months now there has been an excited twittering in international ornithological circles as the news went round that Professor Charles Sibley, formerly of Cornell University and now of Yale University, who was awarded the American Ornothologists' Union's Brewster Memorial Award for the electrophoretic analysis of egg-white proteins in 1971 and subsequently elected a Vice-President, has been found participating in the international egg trade. It seems that by a skilful piece of deduction Richard Porter of the British Royal Society for the Protection of Birds, concluded that a local egg collector must be dealing with foreign parts, located allegedly incriminating correspondence with Professor Sibley and referred it to the American authorities. Professor Sibley's files at Yale were then inspected, with the result that on May 20 he was fined $3,000 under the Lacey Act for six cases of "illegally importing bird parts taken abroad in violation of foreign wild life laws", five of them in Britain.

The situation is made worse by the fact that for at least fifteen years the British Home Office Advisory Committees had already been stretching the law to the limit to enable Professor Sibley to obtain material legally, and many British ornithologists had been assisting him with this. He justified his decision to exceed his allocation by telling the British *Sunday Times* (June 16) that it was an insult to his scientific standing imposed by emotional bird conservation groups with little knowledge of bird population dynamics, which he felt entitled to ignore because "the idea that taking a few eggs could endanger a species is the most ridiculous thing you could imagine . . . I say 'fine, we'll pay the penalty, but you are going to have to listen to the real experts, because you are not the real experts' ".

The British Home Office Advisory Committees consist of people of a most unemotional sort who are internationally recognised to have made more important contributions to the study of population dyanmics than Professor Sibley. They must have been watching the amount of material that he had already received through legal means, which is known to have been large, rather carefully, and it would be interesting to know where Professor Sibley expects to find greater "experts". It is surprising to find a person in such a responsible position at such a distinguished institution first behaving in such a way and then indulging in such an outburst when he was found out. If senior people behave like this, it is hardly surprising that the conduct of some junior American ornithologists is not all it should be. It is good to see the American authorities take the first steps towards putting their house in order. But there is still bigger game in the woods.—From W. R. P. Bourne.

Smallpox report

THE British government has accepted in principle the main recommendations contained in the report of a committee which enquired into causes of the London smallpox outbreak of 1973, when two people died and widespread disruption was caused as vaccination was demanded for four to five millions.

The circumstances leading to the appointment of a committee of enquiry were that a graduate laboratory technician employed at the London School of Hygiene and Tropical Medicine was admitted to hospital on March 16th suffering from suspected meningitis or glandular fever. In fact she had contracted smallpox, and after being placed in an open ward at St. Mary's Hospital, Harrow Road, she came into contact not only with other patients but with two visitors who subsequently contracted the disease and died.

There was serious disquiet that a laboratory worker could have become infected while working in the laboratory; that having become infected, she could spend a week in the general ward while the disease remained undiagnosed; and further, that even after a provisional diagnosis of smallpox, another twelve days elapsed before the visitors were traced, by which time it was too late to save their lives.

The committee found, among other things, that inadequate safety measures were in force at the London School of Hygiene and Tropical Medicine, where the technician was infected while watching the harvesting of pox virus. The pox virus laboratory was said to be old fashioned, poorly decorated, overcrowded and dirty — conditions which, said the report, led to a lowering of safety standards.

Lethal smallpox virus samples were stored in a corridor in an unlocked deep-freeze, workers from other laboratories passed to and fro, using equipment housed in the pox virus lab, and there was an inadequate immunisation policy in the School's Department of Microbiology. Each unit within the department was left to make its own arrangements in relation to its own hazards.

The report recommended the establishment of a permanent committee to draw up and enforce a code of practice for work on pathogens constituting a major threat to public health; the appointment of a safety officer at the School (already implemented); and, since many of the shortcomings resulted from cash shortages in the first place, financial aid to implement the safety proposals.

Failure to trace contacts in the case were caused by a catalogue of administrative errors, coupled with the fact that no one realised the urgency of the situation, says the report, recommending the appointment of regional specialists to supervise control of outbreaks, consideration of compulsory isolation of contracts, and better diagnostic guides for doctors.

correspondence

Unreal Science

SIR,—As a qualified and practising scientist I wish to dissociate myself from your editorial of April 12. You allude to Geller and Velikovsky with terms like "nonsenses", "beliefs beyond science", and "pseudo-scientific ideas", and contrast these with "real science" and "science based on conventional ideas about the way a scientific investigation should proceed", and you declare that "scientists want to fight this distressing drift from the scientific way of thinking".

History is littered with ideas shown to be false by people bold enough to question their contemporary conventional science, often in the face of personal ridicule and even persecution.

Presumably you class Einstein's relativity theory with 'real' science, yet to back his theory Einstein makes a couple of postulates, not yet proven, whilst the unreal Velikovsky quotes source after source and fact after fact to back his. Where Velikovsky leaps eagerly into the jig-saw puzzle of the past and fits piece after piece together (rather unscientifically perhaps, but incorrectly . . . ?) Einstein treads warily (and of course scientifically) through his mathematical maze, and deigns to emerge into the real world only at the end to explain Mercury's perihelion drift and to predict the deflection of light by the Sun. But wasn't there someone recently who found that the Sun was a little flatter than was previously thought which could account for half that drift (doesn't that make Einstein at least half wrong?), and isn't there someone else suggesting there's a planet, Vulcan, in there somewhere, or even maybe a whole asteroid belt? And just for one careless moment suppose that Velikovsky were right . . . no, of course —much too unscientific!

I am not saying Einstein is wrong and Velikovsky right, but I believe both men's theories worthy of serious consideration. You say "*Nature* has a responsibility (that) guides us in the choice of papers for publication". You have a right and a duty to edit, but that statement smacks of censorship. Velikovsky encountered censorship. More recently Dingle has met similar problems for daring to question Einstein.

I am not young nor am I by any means a pseudo-scientist, nor are a number of colleagues who share my opinion. I want no part in any science which operates with a closed mind, and I will encourage the young, and old, to drift from such a way of thinking, though I hardly need to. Your own editorial will do that well enough by itself.

P. WARLOW

Southminster, Essex, UK

Cancer research

SIR,—Your correspondent Brian Ford comments (*Nature*, May 24) on the abundance of data and paucity of concepts in cancer research. A cursory survey of the literature strongly supports this point.

For example, 10 randomly chosen issues of *Excerpta Medica—Cancer* were found to cite about four thousand papers, of which a total of nine were classified as Theories of Carcinogenesis. It is as though 99.8% of the time taken to solve a jig-saw puzzle went on gathering heaps of pieces and 0.2% went on fitting the pieces together. This imbalance conflicts with the widespread belief that cancer research needs "more facts and fewer theories". The opposite appears to be the case.

Yours faithfully,

T. E. WHELDON

Department of Clinical Physics and Bioengineering,
Glasgow, UK

Geology ⊃ geophysics?

SIR,—The President of the Geological Society of London, the Director of the Institute of Geological Sciences and Professor P. Allen have informed us[1] "that geophysics is a necessary and important part of geology" and in case the reader misses the point they say it again " . . . of earth science, that is of geology." That a present and past—and one hopes future—president of the oldest Geological Society in the world should so write is doubtless a Freudian slip, as earlier this century the society refused to take geophysics under its wing, this important task being faithfully discharged since then by the Royal Astronomical Society to the mystery of our friends abroad. In the Cambridge Tripos Part II twenty years ago the question was set: "Geophysics dots the i's and crosses the t's of geology. Comment." I seem to recall that Professor Allen was an examiner. Perhaps those who simply answered "But, of course" qualified for firsts!

I have always worked, as have your correspondents, for more understanding and dialogue between geophysicists and geologists, but also between geophysicists and astronomers and physicists: problems of the Earth's rotation, Earth and planetary magnetic and gravitational fields, solid state physics of interiors, lunar and planetary formation are of more interest to the latter groups than to geologists. Geologists on both sides of the Atlantic who suppose—perhaps understandably as the highways of earth science are presently congested by careering bandwagons—that continental drift, seafloor spreading, plate tectonics and plumes is the whole of geophysics might do well to read again Sir Harold Jeffreys's *The Earth* or study Professor A. H. Cook's *Physics of the Earth and Planets.*

As Sir Peter Kent is also Chairman of the Natural Environment Research Council (NERC), it seems desirable to emphasise that geophysics is a discipline in its own right separate from —and one might add older than— geology. The NERC awards of research studentships for the Earth Sciences seem to me to be seriously overweighted[2] at present in favour of geology as against geophysics by about 7:1. This disparity does not reflect the relative need for highly trained people in these two disciplines nor the present intellectual challenges in the two subjects: it reflects the fact that there are some 45 departments of geology in British Universities.

Thus, as Chairman of the Royal Society committee which launched the European Geophysical Society, I welcome the 1975 "Europe from crust to core" meeting as a further step in European scientific cooperation. I hope that the formation of a 'Pan-European Society of Earth Scientists' (as distinct from a European geological society) is not seriously mooted if only on logistic grounds. Geophysicists interested in geological problems will surely come, as I hope to myself, to Reading and geologists concerned especially with tectonics will continue to join us at the European Geophysical Society's annual meetings.

Yours faithfully,

S. K. RUNCORN

University of Newcastle upon Tyne

[1] *Nature*, June 14, **249**, 608 (1974).
[2] *N.E.R.C. Research Studentships 1974.*

news and views

Testing for carcinogens and mutagens

It has been known since the work of Pott in the eighteenth century that certain forms of cancer are associated with certain occupations and it is now generally accepted that the disease can be and is caused by chemicals in the environment. The number of chemicals that have been shown experimentally to induce cancer in laboratory animals is very large and constantly increasing but the numbers known with reasonable certainty to be active in human beings is very much smaller. Recognised human carcinogens were listed by Hueper in 1971 (*Health Phys.*, **21**, 689–707; 1971) and included asbestos and some metals, several aromatic amines, various tar and pitch products and mustard gas. Since then adenocarcinomas of the vagina in young women and girls has been linked with exposure to diethylstilboestrol *in utero* following therapeutic administration of the compound to their mothers during pregnancy (Herbst *et al.*, *New Engl. J. Med.*, **284**, 878–881; 1971), the occurrence of benign tumours of the liver has been reported in women taking oral steroidal contraceptives (Baum *et al.*, *Lancet*, ii, 926–929; 1973) and, more recently of all, angiosarcoma of the liver has been found in men exposed to vinyl chloride during the manufacture of polyvinylchloride (PVC) one of the most extensively used plastic materials (*Br. Med J.*, **1**, 590–591; 1973).

It is highly significant that these human tumours are all exceedingly rare and therefore the occurrence of even quite small numbers of cases led to the suspicion of an environmental factor in their causation. It is very unlikely indeed that an environmentally-caused increase in a common human tumour would be so readily detected, for example cancer of the lung or colon in the United Kingdom. The realisation that many chemicals are mutagenic has been relatively recent and the problems of detection of altered mutation rates in human populations are formidable. It is therefore of the utmost importance to develop reliable tests for carcinogenicity and mutagenicity of environmental chemicals.

There is substantial agreement on methods for conventional long-term carcinogenicity tests in rodents and these provide the best available means for assessment of possible carcinogenic hazard to man. They are, however, very expensive and require at least 3 years for completion. Methods for mutagenicity testing in mammals are less well established. The specific locus test in the mouse can only be applied to a limited number of chemicals because it requires very large numbers of animals and it is extremely expensive. The dominant-lethal test and cytogenetic analysis *in vivo* require far fewer animals but they are still expensive and time consuming. There is thus a very real need for simpler and less costly tests for carcinogens and mutagens in the environment and much effort is currently being directed towards the development of *in vitro* test systems using cultured cells and microorganisms. Although such tests are unlikely to give conclusive results they may provide valuable information for the assignment of priorities for more definitive test procedures.

The molecular mechanisms of chemical carcinogenesis are not understood but it is now clear that most of the chemicals that cause cancer (precarcinogens) do so as a result of metabolic conversion into more carcinogenic substances (proximate carcinogens) and finally to chemically reactive electrophilic products (ultimate carcinogens) which react with nucleic acids, proteins and other cellular components and which are also mutagenic (Miller, *Cancer Res.*, **39**, 559–576; 1970). Without assuming the same mechanism for chemical carcinogenesis and mutagenesis it may be concluded therefore that demonstration of mutagenic activity in a chemical should give warning of its possible carcinogenicity.

Apart from *Drosophila*, the organisms commonly used in research on chemical mutagenesis including *Escherichia coli*, *Salmonella typhimurium*, *Neurospora* and *Saccharomyces*, do not contain the mixed function oxidases necessary for activation of precarcinogens and premutagens and do not therefore undergo mutation after exposure to these chemicals. This difficulty has been overcome in the host-mediated assay (Gabridge and Legator, *Proc. Soc. exp. Biol. Med.*, **130**, 831–834; 1969) in which test microorganisms are injected into the peritoneal cavity or elsewhere in mammals, usually rodents, and the putative mutagen is given by another route. The organisms are recovered after an interval and scored for mutants. Several chemicals that are inactive by direct application to the organisms become active in this assay, which makes use of the microsomal or other enzymes of the host. A logical extension of this procedure was to use an *in vitro* system in which the test compound is incubated with mammalian liver microsomes in the presence of the necessary co-factors and suitable indicator microorganisms. For example, dimethylnitrosamine was actively mutagenic when incubated under such conditions with mouse liver microsomes (Malling, *Mutation Res.*, **13**, 425–429; 1971) and human liver preparations have recently been shown to be similarly active (Czygan *et al.*, *J. natn. Cancer Inst.*, **51**, 1761–1764; 1973). Although these *in vitro* methods are relatively easy and quick they still require appreciable time and experimental skill and the process has been simplified further by Ames and his colleagues (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 2281–2285; 1973) who have incorporated a post-mitochondrial liver homogenate into the agar plate used for culture of the test organisms. Using this procedure several chemical carcinogens, mainly planar aromatic compounds, were shown to be frame-shift mutagens. Further work with more and different types of carcinogens is needed before the value of the Ames procedure for large-scale screening of environmental chemicals can be adequately assessed. If it proves sufficiently sensitive and reliable it should, by virtue of its simplicity and cheapness, provide a valuable addition to currently available tests.

More recently Durston and Ames (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 737–741; 1974) have used the combined agar plate-microsome method for the detection of mutagens in urine and the communication by Commoner, Vithayathil, and Henry on page 850 of this issue of *Nature*, reports similar experiments. Both groups of workers investigated the urine of rats given the potent carcinogen 2-acetylaminofluorene (AAF) and Commoner *et al.* also studied *p*-dimethylaminoazobenzene (DAB). Both groups found mutagenically active metabolites in the urine of rats treated with AAF and the activity was increased after addition of

β-glucuronidase. Commoner *et al.* also found that the urine from DAB-treated animals contained active mutagenic components but only after treatment with β-glucuronidase and take-diastase; this is of interest because Ames *et al.* had previously reported that DAB itself was not mutagenic after exposure to liver homogenates *in vitro*. Clearly many more carcinogens and other compounds must be tested in this system before it can be properly evaluated. Nevertheless, as the authors point out, the test system for urinary mutagens could readily be included in toxicity studies on laboratory animals and it could also be used to screen the urine of human populations for the presence of conjugated and unconjugated mutagens. In this way, the prior ingestion of carcinogens and mutagens in the diet and from other environmental sources might be revealed.

P. N. MAGEE

Destruction of atmospheric ozone?

THE Earth's atmosphere is extremely effective in filtering out that part of the Sun's ultraviolet radiation which is harmful to life, that is, radiation with wavelengths below about 300 nm. Ultraviolet radiation with shorter wavelengths, below about 200 nm, is absorbed by molecular oxygen, O_2. The rarefied, but nevertheless strongly absorbing, ozone molecule, O_3, traps the remaining longer wavelength ultraviolet radiation up to about 290 nm, a region in which O_2 is virtually transparent. The main ozone layer extends very roughly between altitudes of 15–50 km above the Earth's surface.

As severe radiation damage to the surface cells of plant and animals is causd by intense ultraviolet light at wavelengths of less than 300 nm, changes in the very low atmospheric ozone concentrations, which filter solar radiation, are of vital concern. Normally, atmospheric ozone concentrations are held in rough balance between the rates of production and destruction of O_3. Ozone is produced by the photochemical dissociation of O_2, followed by recombination of the oxygen atoms with O_2 in the presence of a third gas molecule M,

$$O_2 + h\nu \rightarrow O + O$$
$$O + O_2 + M \rightarrow O_3 + M$$

The destruction of O_3 can occur through its reaction with O atoms, or alternatively, by reaction with nitric oxide, NO, which is known to be a trace constituent even of unpolluted atmospheres.

Ozone is a highly reactive molecule, and it has been suggested recently that artificial injection of certain chemical species into the stratosphere may diminish the ambient ozone concentrations by increasing its rate of destruction, thus upsetting the natural ozone balance. The consequently increased flux of far ultraviolet light incident on the Earth's surface would obviously have far reaching, deleterious consequences.

Particular attention has been focused on the possible role of nitric oxide (or equivalent nitrogen oxides, often referred to generally as NO_x) in affecting stratospheric ozone. Nitric oxide, which is a minor product of combustion processes, is emitted into the stratosphere by high flying aircraft and by other aerospace vehicles. Johnston, at Berkeley, has proposed the following two-step catalytic cycle for ozone destruction by added nitric oxide:

$$NO + O_3 \rightarrow NO_2 + O_2 \quad (1)$$
$$O + NO_2 \rightarrow NO + O_2 \quad (2)$$

Thus, reaction (1) and (2) together consume O_3 and O atoms, and provide a mechanism for recycling NO. The rates of reactions (1) and (2) are now well known, and so there is no doubt that sufficient quantities of nitric oxide, if well mixed with the stratospheric atmosphere, could consume ozone in this way. The exact concentrations of the relevant atmospheric species are not well enough known, however, nor are the effects of the local injection of NO_x—as opposed to the addition of 'well mixed NO_x'—clear. There is no general agreement about whether stratospheric ozone levels will be affected by NO_x injected into the atmosphere by known plans for fleets of supersonic aircraft. Nevertheless, an appreciation of the possibility of a perturbation of the ozone cycle by for example, nitric oxide, is clearly most important. Indeed, concern has been expressed in the British press, following Pittock's report (*Nature*, **249**, 641; 1974) of a significant downward trend (since June, 1965) in atmospheric ozone concentrations at altitudes up to 25 km above the Earth's surface. It remains to be seen whether or not Pittock's conclusion, based on carefully executed, but difficult, experiments, is confirmed by other workers. At any rate, there is growing awareness of the importance of the possibility that the natural ozone cycle may be upset by atmospheric pollutants.

That awareness led recently to the discussion of a possible chlorine oxide, ClO_x, cycle, based on two steps involving chlorine atoms, Cl, and the chlorine monoxide free radical, ClO:

$$Cl + O_3 \rightarrow ClO + O_2 \quad (3)$$
$$ClO + O \rightarrow Cl + O_2 \quad (4)$$

Stolarski and Cicerone, elsewhere, and Molina and Rowland, on page 810 of this issue of *Nature*, consider this cycle, and suggest that Cl atoms are formed in the stratosphere. The suggested methods of formation include the photochemical dissociation of HCl from rocket exhaust gases, and of freons which diffuse upwards from the Earth's surface. Freons, compounds which contain only carbon, chlorine and fluorine, are manufactured extensively as aerosol propellants and refrigerants. Molina and Rowland, working at the University of California at Irvine, consider that the freons CF_2Cl_2 and $CFCl_3$ have a long residence time in the lower atmosphere. This would allow their concentrations to build up, without any obvious sinks for their removal. Rough calculations suggest that virtually all of the freons ever manufactured now remain in the atmosphere. The upward diffusion of freons, followed by photochemical dissociation, could result in chlorine atoms at stratospheric altitudes.

Watson and I at Queen Mary College, University of London, have determined recently the rates of several of the relevant chemical reactions involving Cl atoms, and O_3 and ClO radicals. Molina and Rowland, using these and other results, point out that, at stratospheric temperatures, the attack of ClO on O (reaction (4)) is six times as rapid as that of NO_2 on O (reaction (2)). Consequently, under some conditions, the Cl/ClO cycle can be considerably more efficient than the NO/NO_2 cycle in the catalytic conversion of $O_3 + O$ to $O_2 + O_2$. At some altitudes, a mixed NO_x — ClO_x cycle may also be important:

$$Cl + O_3 \rightarrow ClO + O_2$$
$$NO + ClO \rightarrow Cl + NO_2$$
$$O + NO_2 \rightarrow NO + O_2$$

Molina and Rowland conclude that the Earth's atmosphere has only a finite capacity for absorbing chlorine atoms without important consequences. According to their view, the present understanding of atmospheric chlorine chemistry suggests that this absorptive capacity may be insufficient even to cope with the number of Cl atoms equivalent to the quantities of freons at present introduced into the atmosphere on a world-wide scale. More accurate estimates of the absorption capacity are desirable, and no account can be taken of possible heterogeneous reaction between Cl atoms and particulate matter in the stratosphere. If the conclusions of Molina and Rowland are correct, then the effects of the stratospheric photodissociation of CF_2Cl_2 and $CFCl_3$ could not be removed immediately even if no more freons were introduced at ground level. That is because of the postulated long residence time of these species in the lower atmosphere.

The inevitably approximate nature of this type of calculation, and the far reaching assumptions required, should be borne in mind. Discussions such as that of Molina and Rowland, however, serve a most useful purpose in initiating discussion of a new aspect of atmospheric ozone. It is reassuring that interest in these and related problems is being taken by the US Department of Transportation, in its support of the Climatic Impact Assessment Program, under the auspices of which several valuable discussion documents are being issued.

It is conceivable that sulphur dioxide, emitted in relatively large quantities during the combustion of many natural hydrocarbon oils, such as aviation fuel, might also affect ozone concentrations by the photochemical dissociation of SO_2 to SO + O, followed by the reaction of SO with O_3, which has a rate comparable to that of NO with O_3.

This possibility, which requires further consideration, is raised in order to highlight the fragility of the ozone layer and the consequent need for thorough knowledge of the mechanisms and rates of chemical reactions involving ozone.

M. A. A. CLYNE

Phosphorus and the eutrophication problem

SCHINDLER of the Freshwater Institute, Winnipeg, has described in *Science* (**184**, 897–898; 1974) the effects on the growth of planktonic algae of fertilising small, unproductive Canadian lakes with compounds of phosphorus, nitrogen and carbon. If nitrogen or carbon were added there was no marked change in the phytoplankton. If phosphorus was added as well or by itself, there were large increases in phytoplankton. When the additions of phosphorus were discontinued the phytoplankton soon returned to the pre-fertilisation levels. Hence Schindler concludes that a large decrease in the phosphorus content of sewage effluents is the essential remedy for the over-enrichment of waters by man or, as it is called, cultural eutrophication. Polyphosphates in detergents should be banned since they form some 50% of sewage phosphorus (somewhat less in Britain). He agrees with those people who advocate the chemical treatment of sewage to remove a substantial amount of all waste phosphorus. But rapid action is needed and the removal of polyphosphates from detergents will meet this need.

Nearly all limnologists who have investigated the problem will agree with Schindler that phosphorus is a major cause of eutrophication. He refers to several lakes which have benefited from the removal of sewage effluents but it is less clear whether the removal of detergent phosphorus alone would have prevented undesirable eutrophication. Some action has been taken. Canada has prohibited the sale of detergents containing more than 2.2% of phosphorus and some American States have passed similar legislation (see Schindler's article); others will remove phosphorus by sewage treatment.

If polyphosphates are to be removed from detergents, in which they play a vital part, what is to replace them? At present there is no generally agreed answer. In Canada nitrilotriacetic acid (NTA) is a permitted alternative. A governmental warning against its use, has, however, been issued in the United States (see statements by Environmental Protection Agency Administrator and Surgeon General (December 18, 1970) and by Chairman, Committee on Public Works, US Senate (December 19, 1970)). As a result substances such as washing soda and sodium silicate have been used and the detergents containing them are said to be of poor quality, as may be expected. Experience of the world-wide use of several organic compounds suggests that extensive and intensive tests are necessary before they are accepted as safe. Phosphates, though bad for eutrophication are otherwise harmless. One must also realise that any increased cost of a replacement will be passed on to the consumer. It seems therefore that there are arguments for and against Schindler's viewpoint.

European experience supports Schindler's view that phosphates are the commonest major cause of cultural eutrophication and that nitrogenous substances are the next most important cause (Vollenweider, OECD Report DAS/CSI/68.27; 1968). Observations for more than 30 years and experiments with large enclosures in the English Lake District (Lund, *Proc. R. Soc.*, **B180**, 371–382; 1972; *Verh. int. Verein. theor. angew. Limnol.*, **18**, 71–77; 1972 and unpublished data) have also shown that phosphorus is an important factor in eutrophication, though it is an over-simplification to blame everything on one element. The same is likely to be true in other British waters.

The UK Standing Committee on Synthetic Detergents (12th Progress Report, HMSO; 1971) is against the present use of NTA and does not consider that phosphorus removal is as yet justified. Nevertheless its reduction from sewage before it enters the already eutrophic Lough Neagh, Northern Ireland has been advocated (Wood and Gibson, *Water Res.*, **7**, 173–187; 1973). Schindler says that the removal of phosphorus from sewage "while fine in principle . . . will take several years to implement to any effective degree, considering the time lags and uncertainty inevitable in financing, planning and constructing such facilities". Sweden and Switzerland, however, have gone ahead vigorously with just such a scheme; other countries are doing so or preparing to do so to various extents. In Sweden, in particular, it seems that an added gain is a general improvement in the final effluent.

The eutrophication problem is not the same everywhere. It is doubtful whether the best remedy for one lake or lake region is the best for all. Phosphate enrichment may produce unwanted changes in one lake but will not do so in other lakes. Moreover, opinions differ about which substances are the most harmful. It seems reasonable that each lake or lake area should be considered separately, bearing in mind the general, though still imperfect, knowledge of eutrophication. Then it can be decided whether remedial measures are necessary, what they might be, whether they are acceptable on economic or other grounds and whether legislation is necessary to implement them.

J. W. G. LUND

Survival of the Teesdale rarities

from Peter D. Moore
Plant Ecology Correspondent

RARITY is an attribute which makes an organism peculiarly attractive to mankind, even to biologists. When many rare organisms are gathered together in one place, it is inevitable that biological attention should be focused on that spot. In the British Isles there is no better example of this than the rare plants of Teesdale, especially since they have been threatened and partially obliterated by the development of the Cow Green Reservoir. One good result of this incident is the burst of research activity which it has stimulated, partly as a result of the finance generated by the setting up of the ICI Teesdale Trust Fund, a kind of sad consolation for depleted biological resources.

The major biological problem presented by Teesdale is a phytogeographical one, namely the explanation of the surprising distribution patterns of some of the rarities, such as *Gentiana verna*, *Minuartia stricta*, *Tofieldia pusilla* and *Kobresia simpliciuscula*. In 1956, Pigott (*J. Ecol.*, **44**, 545) presented a detailed survey of the plant communities in Teesdale and suggested possible explanations for the unusual assemblages of species found there. Following the arguments of Godwin (*J. Ecol.*, **37**, 140; 1949), he considered that these communities were relicts of late-glacial vegetation which had survived because of the poor growth of forest in the area during the postglacial climatic optimum. Factors such as severity of climate and instability of soil may have assisted such survival.

Direct evidence of postglacial survival has been found in the local peat deposits, where plant fragments and pollen grains are preserved (Squires, *Nature*, **229**, 43; 1971 and more recently Turner *et al.*, *Phil. Trans. R. Soc.*, **B265**, 327; 1973). Peat deposits, some dating back to the late-Devensian (last glacial) period, show that light demanding plant species have been present in some quantity throughout the Flandrian (postglacial) period. Woodland cover can never have been complete, therefore, and the refugium hypothesis of Pigott gains support. Furthermore, some of the rare and uncommon plants (such as *Gentiana verna*, *Dryas octopetala*, *Polemonium caeruleum* and *Polygonum viviparum*) have a fairly continuous pollen record, indicative of postglacial survival. Many of the other rare plants, however, cannot be identified to the level of species on the basis of their pollen so that evidence for them is still lacking.

An alternative approach to the problem is the study of the ecophysiology of the rare plants. Arnold (*New Phytol.*, **73**, 333; 1974) has examined the growth of seedlings of two Teesdale plants, *Kobresia simpliciuscula* and *Plantago maritima*. Seeds of these species were germinated at 18° C and the seedlings were grown at 10°, 14° and 18° C at a light intensity of 60 W m^{-2}. Records were kept of leaf length, width and number and net assimilation rate. Both species grew faster in all respects at higher temperatures but, in the case of *Kobresia*, there was a marked increase in growth at 18° C when compared with that at 14° C. Mean summer temperatures in Teesdale are between 10° C and 14° C; temperatures of 18° C were exceeded on only about 3% of summer days. Arnold remarks that *Kobresia* would be favoured by a warmer environment and she finds difficulty in explaining its occurrence at Teesdale in terms of its temperature response. The answer seems to be that temperature response is not an important parameter in this situation. The experiments demonstrate that these species are living suboptimally in Teesdale as far as temperature is concerned, but this is not surprising. The survival of such plants is due to their ability to maintain a positive growth rate where many other more robust species (including trees) fail to do so. The relevance of these temperature response data, if indeed they are relevant, will only be appreciated when further figures are available from the potential competitors of the Teesdale rarities.



Aerial view of Upper Teesdale (looking north-west) showing the Cow Green area before it was flooded for the reservoir built by the Tees Valley and Cleveland Water Board (Imperial Chemical Industries photograph).

Genes for N_2 fixation on the move again

from a Correspondent

Two years ago Dixon and Postgate reported (*Nature*, **237**, 102; 1972) their success in transferring nitrogen-fixing ability from a N_2-fixing strain of *Klebsiella pneumonia* to the common colon bacteria *Escherichia coli*. Greater complications in using plant nodule bacteria from the genus *Rhizobium* as donors of nitrogen-fixing ability were anticipated since the presence of N_2-fixing activity in *Rhizobium* cannot be shown in laboratory cultures, and the genus *Rhizobium* lacks an effective genetic system for mobilising genes. This difficulty now seems to have been overcome by Dunican and Tierney of University College, Galway (*Biochem. biophys. Res. Commun.*, **57**, 62; 1974) who have been able to transform a derepressed antibiotic resistance transfer factor into *R. trifolii*. This work greatly increases the possibility of mobilising *Rhizobium* genes since this same factor is known to mobilise with an appreciable frequency genes in other bacterial systems. With this R factor present the *Rhizobium* acted as if it had had a sex factor and transferred the ability to fix nitrogen when mated with a non-fixing strain, *Klebsiella aerogenes*. Hybrids capable of fixing nitrogen, measured by the acetylene reduction technique, oc-

curred with a frequency of 10^{-7} or higher. Some of these colonies retained the ability to fix nitrogen when subcultured and in these cases maintenance of the R factor was not essential for retention of N_2-fixing ability. Not unexpectedly, the N_2-fixing *Klebsiella* hybrids acted like most naturally-occurring free-living N_2 fixers in fixing nitrogen only under anaerobic conditions and in the absence of combined nitrogen.

The ability of *Rhizobium* strains to fix nitrogen only when within nodules on the roots of legumes has puzzled scientists for more than half a century. These bacteria grow in culture media but have never been shown to fix nitrogen *in vitro*, a fact which has in the past often prompted the suggestion that the legume plant contributes at least part of the genes concerned with fixation. Dunican and Tierney suggest that the N_2-fixing ability is present though not phenotypically expressed in *R. trifolii* and this ability is expressed when transferred to a new bacterial host. The alternative explanation that genetic material transferred from the *Rhizobium* activates certain latent N_2-fixing genes in *K. aerogenes* cannot be entirely ruled out.

It is anticipated that this genetic system may be useful in studies of the physiology of legume root nodules and may also provide a means of improving strains of *Rhizobium* used in agriculture as seed inoculants. Legumes such as soybeans fix only about one quarter of their nitrogen requirements and a significant improvement in field fixation rates may be possible.

Radius of neutron distribution in lead

from Peter E. Hodgson
Nuclear Theory Correspondent

DURING the past few years there has been great interest in determining the sizes of atomic nuclei, and in particular the relative spatial extents of the constituent neutron and proton distributions. The proton distributions can be determined accurately from analyses of electron elastic scattering and muonic X rays, but the neutron distributions can only be determined indirectly, and the results are less accurate due to the lack of knowledge of the nucleon–nucleon interaction. Many different methods have been used, and the results are in substantial agreement.

On the whole it is found that the proton and neutron distributions are quite similar, with perhaps a slight tendency for the neutron distribution to extend further from the centre of the nucleus than the proton distribution, particularly for heavier nuclei. There is also the possibility of a 'neutron halo', a very diffuse neutron cloud extending far beyond the nuclear surface, where protons cannot reach due to the Coulomb field.

A quantity of particular interest is the ratio of the root mean square neutron and proton radii, as this is a convenient way of specifying the relative extents of the neutron and proton distributions. Recent work has tended to give a value of about 1.05 for this ratio though some studies have given values below unity.

A recent measurement of this quantity has used the (p,n) reaction to the isobaric analogue state of the target nucleus. This reaction concerns principally the outer neutrons and protons, and so is particularly sensitive to their densities in the surface of the nucleus. The final state has the same structure as the initial state and differs from it only by the substitution of a proton for a neutron in the highest quantum state. Another way of describing this is to say that the final state is formed from the initial state just by an isospin flip that changes a neutron into a proton. The interaction is thus brought about only by the isospin component of the nucleon–nucleon interaction, which determines the isospin term in the nucleon optical potential. Also affecting the isospin potential is the difference between the neutron and proton density distributions, so the reaction is sensitive to just the physical quantities of interest. All the interactions responsible for the (p,n) reaction are quite well known, and so is the proton distribution, so comparison between the experimental data and a series of neutron density distributions enables the latter to be determined.

The dependence of the cross section of these quantities is particularly simple if the distorted wave Born approximation is used. Then the cross section is proportional to the square of the transition amplitude

$$T(\mathrm{p,n}) = [2(N-Z)^{\frac{1}{2}} / A] \int \chi_n^-(r)\, U_i(r)\, \chi_p^+(r)\, d\mathbf{r}$$

where $\chi_n^-(r)$ and $\chi_p^+(r)$ are the waves describing the outgoing neutron and incoming proton respectively and the isospin potential

$$U_i(r) = [A/(N-Z)] \int u_t(|\mathbf{r}-\mathbf{r}'|) \{\rho_n(r') - \rho_p(r')\}\, d\mathbf{r}'$$

where $u_t(|\mathbf{r}-\mathbf{r}'|)$ is the isospin component of the nucleon–nucleon interaction and $\rho_n(r')$ and $\rho_p(r')$ are the neutron and proton density distributions respectively.

Schery, Lind and Zafiratos (*Phys. Rev.*, **C9**, 416; 1974) have measured the cross section for the (p,n) reaction to the isobaric analogue state of ^{208}Pb at 25.8 MeV. They calculated the cross section from the above formulae using standard values of $u_t(|\mathbf{r}-\mathbf{r}'|)$ and the optical potentials that fix the distorted waves $\chi_n^-(r)$ and $\chi_p^+(r)$. They assumed that the neutron density distribution has the form

$$\rho_n(r) = [1+\exp((r-R)/a)]^{-1}$$

and varied R and a to optimise the fit to the measured (p,n) cross section. The best fit accounted well for the angular variation of the crosssection, which confirms the reliability of the model. The optimum values of the parameters R and a correspond to a ratio of the root mean square radii $\langle r_n^2 \rangle^{1/2} / \langle r_p^2 \rangle^{1/2} = 1.07 \pm 0.03$, thus confirming previous work indicating a slightly larger neutron distribution. Since $\langle r_p^2 \rangle^{1/2}$ is 5.30 fm, the value of $\langle r_n^2 \rangle^{1/2}$ is 5.68 fm.

The stability of this result with respect to small changes in the assumed potentials was investigated, and this accounts for part of the suggested 3% uncertainty. Studies of the effect of the difference between the unbound proton wave function in the final state of ^{208}Bi compared with the corresponding bound neutron wave function in ^{208}Pb showed that the longer tail of the former reduces the ratio of root mean square radii by about 1%. The effects of two step contributions to the reaction was also studied and found to be small.

Plant messenger RNA and poly(A)

from our
Plant Cell Physiology Correspondent

THE discovery that many animal messenger RNA molecules contain regions of poly(adenylic) acid is of considerable interest (see *Nature*, **247**, 426 and 512; 1974). One reason for this is that the presence of poly(A) confers specific properties on mRNA molecules which can be exploited during RNA purification. Many workers have used columns of oligo(dT) cellulose or poly(U) sepharose which retain the mRNA by specifically base pairing with the poly(A) region. Another approach has been to use unmodified cellulose or nitrocellulose to purify the mRNA.

A recent report shows that presumptive mRNA from plants, long known for its high content of adenylic acid, contains poly(A) tracts (Higgins *et al.*, *Nature new Biol.*, **246**, 68; 1973). Sagher *et al.* (*Biochim. biophys. Acta*, **349**, 32; 1974) now suggest that the length of poly(A) in higher plants is of the order of 150–250 nucleotides which contrasts with the 50 or so residues found attached to yeast and fungal mRNA (McLaughlin *et al.*, *J. biol. Chem.*, **248**, 1466; 1973; Reed and Wintersberger, *FEBS Lett.*, **32**, 213; 1973; Silver and Horgen, *Nature*, **249**, 252; 1974). At present only the poly(A)-containing RNA from polyribosomes of plants which synthesise a large number of proteins has been analysed and not surprisingly it is heterodisperse. Individual mRNAs of known function have yet to be isolated but exploitation of the poly(A) tag may speed up the process. Although this approach to the study of mRNA metabolism holds out great promise it should not be accepted

without reservations. For example, it is by no means certain what proportion of mRNAs contain poly(A). In animals only the histone mRNAs have been shown to lack poly(A) but others may be added to this list and it is too early to assume that all plant mRNAs contain poly(A). It is also probably true that some nuclear RNA molecules that contain poly(A) do not function as mRNAs.

The most favourable situations for the isolation of a single mRNA exist where cells devote a large part of their protein synthesis to the production of one or a small number of specific proteins. One can anticipate that already in a number of laboratories there is a search under way for the mRNAs for the storage proteins of legume cotyledons and the larger and smaller subunits of the fraction I protein.

Nuclear magnetic order in solid ^{3}He

from a Correspondent

THE long predicted nuclear magnetic ordering transition in solid ^{3}He seems to have been observed at a temperature of 1.17 mK in an experiment carried out at Cornell University by Halperin, Archie, Rasmussen, Burhman and Richardson, and reported in a recent issue of *Physical Review Letters* (**32**, 927; 1974).

The compressional cooling technique used in this work, which has been developed during the past few years by the low temperature group at Cornell, has already been extraordinarily fruitful in that it has led to discovery of the two new and apparently superfluid states of liquid ^{3}He. The technique, known as Pomeranchuk cooling after the Russian physicist who originally suggested it, is based on the fact that for temperatures below about 0.3 K, ^{3}He has a negative latent heat of solidification so that, unlike other materials, it absorbs heat while changing phase from liquid to solid.

In practice, after being precooled to about 15 mK by means of a dilution refrigerator, the liquid is then steadily compressed by the application of external pressure. At a pressure of about 34 atmospheres it starts to solidify and, because heat is thus being absorbed, it also starts to cool. The cooling effect can be maintained until, eventually, the cell is entirely filled with solid. The final temperature depends on a number of factors including the starting temperature, the rate at which heat leaks into the system or is developed by mechanical inefficiencies in the compression mechanism itself, and the rate at which the liquid is compressed, but is usually in the range 1–2 mK. The ^{3}He, which is the subject of the present experiments, is thus able to act as its own refrigerant from which accrues the enormous benefit that the usual formidable problems of thermal transfer and equilibrium in the low mK temperature range are automatically minimised.

Unlike ^{4}He, the ^{3}He nucleus possesses a magnetic dipole moment and it is to be expected that, at some sufficiently low temperature T_c, a transition will occur in which the nuclei become aligned in some ordered fashion relative to each other. Because the nuclear moments are so tiny the ordering temperature is bound to be exceedingly small and, just considering the direct magnetic interaction of the nuclei, it may be calculated that $T_c \sim 10^{-6}$ K. More refined calculations, however, taking account of the so-called exchange forces, suggest $T_c \sim 10^{-3}$ K, which is just within the experimental range made accessible by Pomeranchuk cooling.

In essence the basic Cornell experiment was extremely simple: the volume occupied by the ^{3}He was reduced at a steady rate while its pressure was continuously monitored. The pressure, with a mixture of liquid and solid in the cell, was necessarily always that of the melting curve at the relevant temperature and, since the melting pressure increases with falling temperature, the pressure automatically gave a measure of the temperature. Under these conditions a plot of pressure P against t is almost linear, corresponding to cooling of the sample at a constant rate, provided that no phase changes occurred in either liquid or solid apart, of course, from the continuous solidification-action process responsible for the refrigeration. In practice, the original experiment showed one change of slope and one discontinuity in the $P(t)$ plot, and these were eventually proved to be caused by two superfluid transitions in the liquid phase. In the present work a third anomaly in the $P(t)$ graph was observed at the even lower temperature of 1.17 mK.

The interpretation of these experiments, where both liquid and solid are simultaneously present, is of course extremely difficult. Through a series of ingenious additional experiments, however, in which heat pulses were applied to the helium while compression was continued at a steady rate, the authors seem to have demonstrated unambiguously that the $P(t)$ anomaly at 1.17 mK corresponds to a phase change within the solid, which must presumably represent the onset of magnetic ordering, and they have been able to deduce that the entropy of the solid falls by a factor of 5 within a temperature range of only 100 μK.

It has not been possible from these experiments to deduce the nature of the magnetic order below T_c. Measurements of the nuclear susceptibility at higher temperatures had led to the development of a detailed model which predicted a transition at 2.2 mK to an antiferromagnetic state, in which neighbouring nuclei point in opposite directions. The fact that the actual transition has now been found to occur at only half this temperature is in itself strong evidence of the inadequacy of this model. A great deal of further work will certainly be required before a proper understanding of magnetic ordering in solid ^{3}He can be attained, but it is very encouraging to know that the phenomenon occurs in a temperature range which has now become accessible to experiment.

Chemical heat at oceanic ridges

from Peter J. Smith
Geomagnetism Correspondent

NEAR the crests of active oceanic ridges the mean heat flow is generally higher than the global average, and measurements are considerably more scattered than those in, for example, typical ocean basins. Both of these observations are explicable in general terms, for hotter mantle-derived material rises beneath ridge crests to form new ocean floor, and a combination of rugged ridge topography and uneven sediment cover tends to produce both high measurement errors and a distortion of the heat flow pattern (for example, by thermal refraction). Nevertheless, in view of the importance of testing the seafloor spreading hypothesis as rigorously as possible, such loose explanations are not entirely satisfactory; and it is not unreasonable to suspect that the rather messy heat flow picture may be concealing important information about physical and chemical processes in mid-ocean regions.

Because of the large scatter of data, for example, there may be legitimate doubt about the validity of the mean heat flow values themselves, all the more so as McKenzie (*J. geophys. Res.*, **72**, 6261; 1967) has shown that they are inconsistent with the formation of new oceanic crust at a reasonable temperature. Mean heat flow as measured over ridge crests may indeed be high, but it is not as high as might be reasonably expected.

Recognising the scope for investigation here, Lister (*Geophys. J.*, **26**, 515; 1972) made an important series of heat flow measurements on the Juan de Fuca ridge and near the Explorer trough in which he took care to assess topographic effects and the thermal influence of recent sediment disturbances. The conclusion he came to was that the thermal balance of a ridge crest zone can be interpreted in terms of a

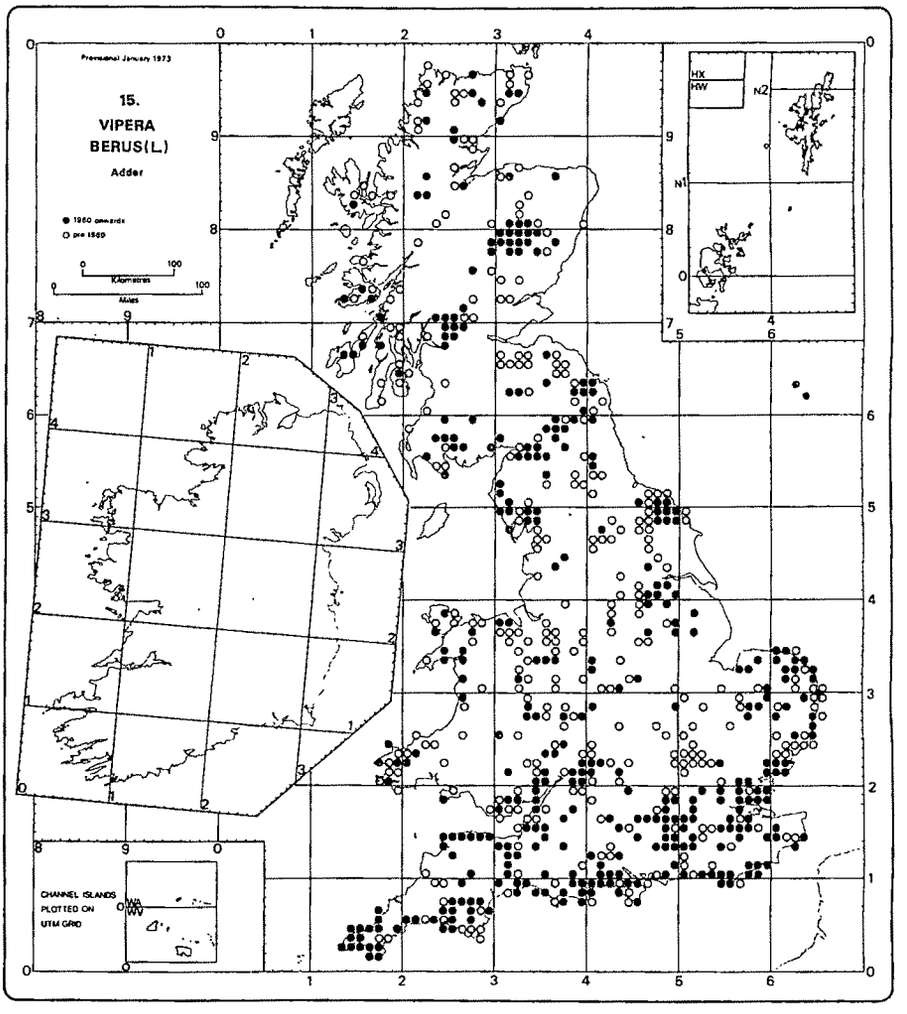
simple model of seafloor spreading, but only if a considerable part of the surface heat loss at the crest is non-conductive. He thus proposed that in addition to conduction, a significant proportion of crestal heat is carried away by convection in the form of open hydrothermal circulation within the upper 5–10 km of the rock material at the ridge crest. The critical requirement here is that the oceanic crustal rocks should be permeable enough for the circulation to take place; and although relevant data are sparse, there seems little doubt that this is the case. Accepting hydrothermal flow, therefore, it follows that the (conducted) heat measured will be less than the total heat actually generated, thus explaining the discrepancy noted by McKenzie.

The existance of hydrothermal circulation may also explain other things, including the long-time puzzle of why anomalously low heat flow is commonly found on ridge flanks. Moreover, in a rather different field, and as Spooner and Fyfe (*Contr. Mineral. Petrol.*, **42**, 287; 1973) have discussed at some length, "vigorous convective circulation of brine within the oceanic crust" seems to be the phenomenon most likely to account for the pattern of metamorphic rocks found on the ocean floors. In other words, hydrothermal flow is "a significant process at axes of seafloor spreading". Just how significant it is in thermal terms is indicated by a simple calculation. One cubic kilometre of liquid basalt at 1,200° C could heat 3 km^3 of sea water to 300° C (lower than the boiling point at a depth of 1 km under a hydrostatic pressure gradient). This means that at the present rate of magma production all oceanic water could be so heated during circulation through oceanic crust in about 100 million years. As Spooner and Fyfe pointed out, all this suggests that convection within the crust constitutes the major process by which the cooling of the intrusive material in the crust takes place.

But Fyfe (*Geophys. J.*, **37**, 213; 1974) has now drawn attention to another implication of hydrothermal flow. The chemical processes induced by such circulation are oxidation, hydration, carbonation and the fixation of sulphur, all of which are exothermic reactions. Thus magmatic heat induces large-scale convection and the convection then induces further heat-producing reactions. To estimate just how much heat is likely to be generated in this way, Fyfe assumes that the upper 15 km of the oceanic crust cal and hydration of basaltic pyroxene (a thicker section than Lister suggested, to "allow for some tectonic mixing of the surface layers as the conveyor belt moves") and that the crust comprises 10 km of basalt and 5 km of peridotite. He then calculates that, in a 1 cm^2 column, serpentinisation of the peridotite would produce 10^8 cal, oxidation of 10% FeO in basalt would give 2×10^8 cal, hydration and addition of CO_2 to basaltic anorthite would yield 2×10^8 cal and hydration of basaltic pyroxene would give 10^8 cal. This gives a total heat source of about 6×10^8 cal —comparable to the radioactive heat generated in an equivalent column of granite over a period of 20 Myr.

Insofar as the chemical reactions considered by Fyfe are probably simpler than those actually taking place, the accuracy of this estimate of heat production is difficult to assess. Nevertheless, it is obvious that there is a potential heat source here which is far from trivial. Fyfe contends, therefore, that such chemically generated heat can hardly be ignored in considering the thermal regime of the ocean floor and oceanic ridges.

Atlas of British amphibians and reptiles



THE number of adders (*Vipera berus*) present in the British Isles seems to be reasonably healthy; as this distribution map shows, records of sitings have been returned from most parts—except, of course, from Ireland. As the new *Provisional Atlas of the Amphibians and Reptiles of the British Isles* (edit. by Arnold, Biological Record Centre, 25p) reports, however, three of the remaining 11 indigenous species—the natterjack toad (*Bufo calamita*), sand lizard (*Lacerta agilis*) and smooth snake (*Coronella austriaca*)—are now extremely uncommon and will need protection if they are going to survive. Staff at the Biological Records Centre at Monks Wood Experimental Station, Abbots Ripton, are aware of the provisional nature of the atlas; there are several areas where no recording of the commoner species has been done and they will be pleased to receive any record that fills in a gap on one of the maps. The atlas includes brief notes on the identification of species.

Solar geomagnetic disturbances and weather

by John Gribbin

ONE of the key links in the developing theory that atmospheric circulation (and hence climate) on Earth is affected by solar activity has been provided by evidence that geomagnetic activity can be connected with a deepening of troughs in the atmosphere. But although evidence presented by Roberts and colleagues (see *J. atmos. Sci.*, **30**, 135–140; 1973) has seemed to show that troughs at the 300 mbar level level are affected in this way, and although other groups have presented evidence that even surface pressure responds to solar geomagnetic disturbances (see Mustel, *Soviet astr.*, **10**, 288–294; 1966) the reality of the claimed effect has been queried by others. In particular, Stolov and Shapiro found no evidence to support the claim that atmospheric circulation is disrupted during disturbed geomagnetic periods, according to a paper published three years ago (*Proc. Symposium on Solar Corpuscular Effects in the Troposphere and Stratosphere*, Moscow, 1971). So the recent publication of another paper by the same two authors, in which they report that a reanalysis of the data does show such an effect, is particularly important (*J. geophys. Res.*, **79**, 2161–2170; 1974).

The reason for the emergence of the effect from the new analysis seems to be that the strong seasonal trend in 700 mbar heights which is introduced by ordinary meteorological factors must be removed before the solar geomagnetic effect can be picked out from the data. The data themselves are comprehensive, covering the period January 1, 1947 to December 31, 1970. They consist of sea level pressures and 700 mbar heights measured at 5° latitude intervals and 10° longitude intervals in a diamond shaped grid over 20° to 70° N. The solar geomagnetic incidents studied in the analysis were chosen from a variety of sources which gave a total of 454 events, some of which were related to one another.

Using a superposed epoch analysis and removing the seasonal effects which dominate the atmospheric variations, Stolov and Shapiro find "firm statistical evidence . . . that strongly suggests the existence of a real relationship between solar geomagnetic storms and the subsequent behaviour of the 700 mbar contour height". The effect is more pronounced in winter, when the zonal flow is increased 4 days following geomagnetic disturbance. In summer, a smaller but still significant effect occurs 2 days after such disturbances. At its maximum (for the quadrant 90° to 175° W in winter) the effect corresponds to a 7% increase in the mean westerly flow. Stolov and Shapiro say that "no acceptable physical observations are available to explain the observations"; however, that no longer seems to be strictly true in the light of King's recent investigation of links between the magnetosphere and atmosphere (*Nature*, **247**, 131–134; 1974). What is certainly true is that further statistical studies are needed, and that these might contribute both to weather forecasting and to an understanding of climatic change.

Vole populations and their diet

from our Animal Ecology Correspondent

ONE of the greatest drawbacks to the theories which purport to explain the factors causing vole population cycles is their relative untestability (for example, Chitty, *Can. J. Zool.*, **38**, 99; 1960; Christian and Davis, *J. Mammal.*, **47**, 1; 1966) and recent attempts to elucidate behavioural or genetic factors have been inconclusive. Batzli and Pitelka (*Ecology*, **51**, 1027; 1970) and Schultz (*Symp. Brit. Ecol. Soc.*, 57; 1964) have suggested that nutritional aspects of forage may play an important part in regulating cycles. These could take the form of potassium or phosphorous deficiencies which bring about specific or general debility.

Another nutritional factor which might be involved is the toxicity of many food species of plant (Freeland, *Am. Nat.*, **108**, 238; 1974). From the huge mass of published work on species preferences of vole food at various stages of the population cycle, Freeland argues that the loss of animals from peak population densities is the direct result of reduced viability induced by the consumption of plants, or parts of plants, that contain toxins. It is known that quite small amounts of toxins imbibed by voles cause inhibition of protein synthesis and growth, cessation of the reproductive processes, occurrence of megalocytes in the liver together with haemorrhagic necrosis, hypertrophy of the spleen, and cause neural and digestive disorders. If a vole eats more than one toxic species the effect of the toxins they contain may be mutually enhanced.

Freeland puts forward a hypothesis with four central tenets: (1) voles must have a preference for non-toxic species; (2) with increasing population numbers these preferred foods should become scarcer with a concurrent increase in availability of non-preferred, toxic species; (3) at high population densities voles ingest quite large quantities of toxic foods leading to a reduction in growth and a slowing of reproduction; and (4) in the absence of grazing, the preferred non-toxic species should compete more strongly for space than the less preferred toxic species. The replenishment of favoured food so produced following a 'crash' of population allows another cycle to start.

In 1965 Thompson (*Amer. Midl. Natur.*, **74**, 75) examined the toxicity level of many food species of the voles *Microtus pennsylvanicus* and *M. californicus* in relation to their preferences of selection. A plant was ranked as toxic only if it was known to contain a compound or compounds that cause death or severe physiological damage. Bitter tasting or foul smelling species were not rated as toxic unless a truly toxic compound occurred as well. The result of Thompson's laboratory trials was that voles prefer non-toxic plants like *Trifolium repens* and *Lolium multiflora* to toxic ones like *Ranunculus* and *Rumex*. Batzli and Pitelka have found that during one cycle of *Microtus* abundance the amount of toxic species available becomes relatively more abundant in the face of a diminishing supply of preferred species. The establishment of exclosures indicated that non-toxic species out-compete toxic ones. In areas grazed by voles there is a reciprocal fluctuation of the relative abundances of these two species types.

Krebs *et al.* (*Ecology*, **50**, 587; 1969) have noted that during population decline the rates of disappearance of different age and sex classes of voles are not the same. There is evidence that male sex hormone increases the activity of liver microsomal enzymes which bring about the degradation of toxins (Parke, *The Biochemistry of Foreign Compounds*, Pergamon, Oxford; 1968). So the differential mortality rates frequently observed by field workers is a phenomenon not incompatible with Freeland's hypothesis.

The crux of the hypothesis is that numerical change must come about sufficiently slowly for there to be a compositional change in the vegetation. Even though *Microtus* and similar rodent species have a high capacity for population increase, natural wastage to predation keeps the numbers low enough to avoid a hasty overgrazing of the vegetation. The slow build-up of rodent numbers produces a slow increase in availability of toxic species which, in time, decreases rodent abundance and allows the whole cycle to start again.

This hypothesis has merit and warrants close scrutiny. At the same time could population ecologists consider not so much how the cycle works as much as why it happens at all? Are cyclical rodents really in such a bad state of balance with their ecosystems, or is there another reason?

How history has blended

H. V. Wyatt

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The experiments of Hershey and Chase, giving evidence that DNA is the genetic material, have been greatly simplified and modified in subsequent descriptions. Students are in danger of receiving a quite misleading impression of the process of scientific discovery.

IT was the classic paper of Hershey and Chase[1] which marked the psychological watershed after which DNA was rapidly accepted as the genetic material of living organisms. Looking back, it is difficult to see why this work was accepted whereas Avery's demonstration that transformation was effected by DNA took longer to be appreciated (see for example refs 2–5). Moreover, whereas Avery's 1944 paper[6] still holds, the conclusions of Hershey and Chase have since been modified in detail.

The tightly constructed and interlocking experiments of Hershey and Chase have in most cases been reduced to only one experiment—the Blender experiment—which can be vividly portrayed by diagrams and is therefore popular in the textbooks. In the process of canonisation, their experiments are remembered 'with advantages'.

In this account, I attempt to review the Hershey and Chase experiments in the context of the discovery of transformation and the identification of the genetic role of DNA and to show how they have since been admitted to the Pantheon.

In their paper[1], Hershey and Chase describe the experiments in the following order. (1) Plasmolysis produces phage ghosts of protein and releases DNA into solution. (2) Phage DNA becomes sensitive to DNase after adsorption to heat-killed bacteria. (3) Phage DNA is liberated when phage adsorbs to bacterial fragments. (4) Removal of phage coats from infected bacteria (the Blender experiment). (5) Only 1% of the sulphur from the infecting phage is transferred to the progeny (including a modified Blender experiment). (6) Thirty per cent of the phosphorus from infecting phage is transferred to the progeny. (7) Formalin-inactivated phage can attach to, but cannot inject their DNA into bacteria.

The experiments formed a logical sequence. The first three provided further evidence for the functional differentiation of the protein coat from the enclosed DNA and continued the work of Herriott[7] and Northrop[8]. The Blender experiment (a term used by Hershey[9]) showed this elegantly. Experiments 5 and 6 were altogether different and showed differential transfer of protein and DNA to phage progeny. The most important experiments, 4 and 5–6 are therefore complementary: alone, none is convincing. The two sets of experiments originated from two sources[2,9]. The Blender experiment followed from Herriott's idea[7] of a microsyringe the genesis of which lay in Avery's work and that of Hotchkiss. The progeny experiments followed those of Watson and Maaløe[10].

The Blender experiment has since achieved so much fame that it is almost the only part of the Hershey and Chase paper which is still quoted. The novel use of the Waring Blender has perhaps stirred the imagination. Davis *et al.*[11] for instance give an account of experiment 2 and of the Blender experiment, adding that these showed that "the phage DNA carries the genetic information and that infection consists of the penetration of the viral DNA into the cells". The results of the Blender experiment varied throughout the paper (Table 1), doubtless because a number of different experiments were reported in different contexts. The experiments showed that 65 to 85% of the ^{32}P and 25 to 18% of the ^{35}S was not stripped from the bacteria. Hershey and Chase inferred from both types of experiment only that the protein coat "probably has no function in the growth of intracellular phage. The DNA has some function." They added "Further chemical inferences should not be drawn." In the discussion they stated that the following questions remain unanswered. (1) Does any sulphur-free phage material other than DNA enter the cell? (2) If so, is it transferred to the phage progeny? (3) Is the transfer of P to progeny direct or indirect?" At the Cold Spring Symposium, Hershey[9] spelt out the limitations of the Blender experiment. (1) Does the 20% of the virus DNA which is strippable mean that some of the DNA of the virus is dispensable or that some fails to inject? (2) Does the 20% of the protein that is not strippable have any function in the growth of the intracellular virus? (3) Does the non-strippable protein consist of the stubs of the tails only, or is some protein injected along with the DNA?

Acceptance of the Blender experiment

Mirsky suggested[12] that the purity of DNA—the transforming principle—might include 2 to 3% protein even in a so-called pure preparation. It was this degree of contamination which made it seem possible that protein might still be the genetic carrier because Mirsky calculated that transformation required about 10^9 molecules of transforming principle.

Hotchkiss had meanwhile shown that the amino acid recovered from acid hydrolysates of purified transforming principle was glycine, arising from hydrolysed adenine—not from contaminating protein. This work was originally published in France[13] and virtually lost[14]. Hotchkiss, however, later gave it at a symposium attended mainly by biochemists and probably only Herriott from the phage groups[2]; the contamination of transforming DNA by protein was less than 0.02%, if at all[15].

Yet the contamination in the Blender experiment was of the order of 25%; why was this ignored by those who accepted the phage work? First, in the Blender experiment, almost all the infected bacteria survived the treatment and produced infectious phage and almost all had been infected by only one phage (ratio of phage to bacteria was 0.28). The contamination of protein (^{35}S) in the Blender experiment was shown in a non-blender experiment to be associated with the bacterial cell wall and to be mainly coats of patental phage particles, presumably identical with the coats which could be sheared off. Hershey and Chase concluded that this protein probably never entered

Table 1 Published data of the Blender experiment

	% Isotope eluted*	
Hershey and Chase	^{32}P	^{35}S
Table 5	21–24	81–82
Figure 1	35†	80†
Text on page 48	21–35	75–80
Discussion	("most")‡	at least 80
Summary	15	75
Hershey (1953)	20	80
Limits of figures	15–35	75–82

*Phage material stripped from infected bacteria.
†My calculations.
‡Item in bracket refers to isotope not eluted.

Table 2 How the Hershey and Chase experiments have been cited

	% Isotope eluted*	
	^{32}P	^{35}S
Garen and Kozloff[31]	20	80
Stent[22]	<20	>80
Herskowitz[24]	0	97
Cohen[32]	20	>80
Beadle and Beadle[33]	very little	{viral coats contained ^{35}S}
Hershey[34]	(most with cells)	most
Hotchkiss[14]	15 or less	80
Clowes[25]	(most transferred)	mostly
Fraser[26]	little	70
Davis *et al.*[11]	{essentially all remained in cell}	80
Hayes[21]	20	80
Pollack[30]		>99 protein
Stent[5]	20	90
Stent[23]	20–35	75–80
Stent[35]	20 or less	at least 80
Joklik and Smith[36]	(remained with cells)	{small amount with bacterium}
Levy *et al.*[27]	(most in cells)	mainly in fluid
Kreuger *et al.*[29]	20	80

*Phage material stripped from infected bacteria.
Quotes in brackets refer to % isotope remaining with the bacterium, not eluted.

the infected cell. In the next experiments they found that < 1% of the ^{35}S could be recovered from progeny phage: this moreover was divided among an average of > 30 phage progeny per parent phage. This would not be very convincing if replication was of a non-conservative type but thinking about replication was necessarily vague at this time.

This might have implied that less than 0.03% of the phage protein was necessary for the transfer of genetic information. The most difficult objection would have been if the genetic material had been protein and that this protein had contained little or no sulphur-containing amino-acids. An experimental technique similar to that used by Meselson and Stahl[16] might have resolved this difficulty. The experiment of Meselson and Stahl is itself a blend of Avery's isolation of DNA and of Hershey and Chase's use of isotopic recognition.

How Hershey and Chase have been quoted

The paper has been reprinted in full in reprint collections[17,18] and, with the omission of pages 39–44 and Tables 1–3 in other collections[19,20]. It is therefore widely available. Most authors quote only the Blender experiment with its results as shown in Table 2. Hayes[21], Stent[22,23] and Davis *et al.*[11] give details of other experiments in the Hershey and Chase paper. Some authors give accounts of subsequent experiments. Herskowitz[24] gives not the results of the experiment he describes but an idealised interpretation and attributes the account in a footnote "this account follows the work of A. D. Hershey and M. Chase". Diagrams of the experiment are given by Clowes[25] and by Fraser[26]: a diagram in Levy *et al.*[27] is however confusing as it seems that the same phage is labelled with both ^{32}P and ^{35}S although the text is clear. Ravin[28] is also confusing, saying that Hershey and Chase "... grew the viruses on bacterial hosts in a medium containing sulphur (^{35}S) and phosphorus (^{32}P)". A flow diagram of the experiment in Kreuger *et al.*[29] not only obscures the elegance of the original but confuses the reader.

The only reproduction of the figure from the original[1] paper is in Stent[23]—although a figure showing "representative results of the Hershey–Chase 'blender' experiment" is given in Kreuger *et al.*[29]. Stent[22] says that the "Blender experiment ... consists of infecting bacteria with ^{32}P, ^{35}S or ^{14}C labelled phage particles" and details the results as "more than 80% of the viral sulphur and amino-acid carbon ... more than 80% of the viral phosphorus or purine-pyrimidine carbon". The isotope ^{14}C was used by Watson and Maaløe[10] to label purines in their experiments when they found equal transfer of phosphorus and purines to phage progeny. In the conversations recorded from a conference, Stent[5] quotes the results of the Blender experiment as "the Hershey–Chase experiment", saying that the experiment was rather weak. This is probably true of that experiment in isolation but it was surely the combination of experiments that made the paper[1] so convincing.

Pollack[30] stated that "with Hershey and Chase's demonstration that bacteriophage left all (that is, experimentally more than 99%) of their protein coat and other superficial 'apparatus' behind on infection of the cell, it became very difficult still to maintain that nucleic acid was not the key substance in the maintenance of genetic continuity". This is a blending of the Blender experiment with the results of the transfer of parental phage radioactive sulphur to progeny phage (1%). Although this is the furthest from accuracy, it nevertheless conveys the general impression of the entire paper more cogently than the quotations of the Blender experiment alone.

A comparison of the figures in Tables 1 and 2 shows that only Stent[23] could have taken his figures from the original (text on page 48). A number of the figures are outside the limits of the results given in the paper from which they are supposed to be taken. The figures of 20% ^{32}P and 80% ^{35}S eluted are the most common and are given by Hershey in his summary of the work[9]. It is difficult to believe that so many authors have consulted this paper and not the original. It is more probable that these figures, now part of the myth, stem from an original quotation, now forgotten.

Effects of idealising experiments

Original experiments are not quoted in textbooks to give authority to the statements and general principles or facts which follow. Rather they are quoted to give a sense of historical perspective to the matter and to relate it to other material. Quoting a name provides a handy label for recall of the matter and perhaps unconsciously a handle for examination questions. Authors may also feel they are sugaring the pill with human interest. Where an experiment is quoted in detail, it is surely to show an example of an elegant economical experiment—a model to strive towards. It should illustrate the clarity of the experimenter's aims and of his reasoning and it should show how far the results meet those required for a definitive answer; it usually shows how a single experiment or a single experiment as the culmination of others, can set scientists on a new path. The very existence of books of selected reprints and their use for teaching[19] shows how important these experiments are considered.

It is therefore of concern that so many textbooks are guilty of trivialising the experiments of Hershey and Chase. By reducing the complementary experiments to only the Blender experiment these textbooks diminish the elegance of the work and destroy the intricacy of the interlocking evidence: each step is in itself inconclusive, but *in toto* they are overwhelmingly convincing. If students accept these textbook accounts they are being taught not science but magic, which can be seen even more clearly in many practical textbooks. A favourite experiment, shown, for example, on a film currently in academic use, consists of precipitating a polymer (presumed to be DNA) and showing that addition of this substance to bacteria increases the number of mutants which can be isolated by selection. It is alleged that this experiment shows that DNA is the specific chemical substance which carries genetic information. As Avery *et al.*[6] showed, the scientific demonstration that DNA is the carrier of genetic information is difficult and laborious (ref. 3 and my unpublished work). We are educating a generation of scientists who may well believe that crucial experiments need no controls and give only rather vague results.

The other aspect of the quotations in Table 2 is evidence of the rapid conversion of scientists to a new dogma. The original evidence is soon forgotten and a gradual process of improvement ensues. The cracks in the edifice are easily overlooked because they are not present in the idealised model. This faith may be necessary so that the majority of scientists can proceed unworried with their work. But it is the young

scientists who can see the cracks and do the next elegant, economical experiment who will found the new dogma.

1 Hershey, A. D., and Chase, M., *J. gen. Physiol.*, **36**, 39–56 (1952).
2 Wyatt, H. V., *Nature*, **235**, 86–89 (1972).
3 Wyatt, H. V., *New Scient.*, **56** (814), 29–30 (1972).
4 Luria, S. E., *Proc. Conf. History of Biochemistry and Molecular Biology*, 22–28; 32 (American Academy of Arts and Sciences, Brookline, 1970).
5 Stent, G. S., *Proc. Conf. History of Biochemistry and Molecular Biology*, 29–45 (American Academy of Arts and Sciences, Brookline, 1970).
6 Avery, O. T., MacLeod, C. M., and McCarty, M., *J. exp. Med.*, **79**, 137–158 (1944).
7 Herriott, R. M., *J. Bact.*, **61**, 752–754 (1951).
8 Northrop, J. H., *J. gen. Physiol.*, **34**, 715–735 (1951).
9 Hershey, A. D., *Cold Spring Harb. Symp. quant. Biol.*, **18**, 135–139 (1953).
10 Watson, J. D., and Maaløe, O., *Biochim. biophys. Acta*, **10**, 432–442 (1953).
11 Davis, B. D., Dulbecco, R., Eisen, H. N., Ginsberg, H. S., and Wood, W. B. *Principles of Microbiology and Immunology*, (Harper and Row, New York and London, 1967).
12 Mirsky, A. E., *Cold Spring Harb. Symp. quant. Biol.*, **12**, 15–16 (1947).
13 Hotchkiss, R. D., *Colloques int. cent. natn. rech. scient.*, **8**, 57–65 (1949).
14 Hotchkiss, R. D., *Phage and the Origins of Molecular Biology* (edit. by Cairns, J., Stent, G. S., and Watson, J. D.), 100–108, (Cold Spring Harbor, 1966).
15 Hotchkiss, R. D., *Phosphorus Metabolism*, **2** (edit. by McElroy, W. D. and Glass, B.), 426–436 (Johns Hopkins Press, Baltimore, 1952).
16 Meselson, M., and Stahl, F. W., *Proc. natn. Acad. Sci. U.S.A.*, **44**, 671–682 (1958).
17 *Papers on Bacterial Viruses* (edit. by Stent, G. S.) (Little, Brown, Boston, 1960).
18 *Selected Papers on Molecular Genetics* (edit. by Taylor, J. H.) (Academic, London and New York, 1965).
19 *Classic Papers in Genetics* (edit. by Peters, J. A.) (Prentice-Hall, London, 1959).
20 *Selected Papers on Virology* (edit. by Hahon, N.) (Prentice-Hall, London, 1964).
21 Hayes, W., *The Genetics of Bacteria and their Viruses* (Blackwell Scientific, Oxford, 1968).
22 Stent, G. S., *The Viruses* 2 (edit. by Burnet, F. M., and Stanley, W. M.) (Academic, London and New York, 1959).
23 Stent, G. S., *Molecular Genetics* (Freeman, San Francisco and London, 1971).
24 Herskowitz, I. H., *Genetics* (Little, Brown, Boston, 1965).
25 Clowes, R., *The Structure of Life* (Penguin, Harmondsworth, 1967).
26 Fraser, D., *Viruses and Molecular Biology* (Macmillan, London and Basingstoke, 1967).
27 Levy, J., Campbell, J. J. R., and Blackburn, T. H., *Introductory Microbiology* (John Wiley and Sons, New York, 1973).
28 Ravin, A. W., *The Evolution of Genetics* (Academic Press, London and New York, 1965).
29 Kruegar, R. G., Gillham, N. W., and Coggin, J. H., *Introduction to Microbiology* (Macmillan, New York, 1973).
30 Pollack, M. R., *J. gen. Microbiol.*, **63**, 1–20 (1970).
31 Garen, A., and Kosloff, L. M., *The Viruses*, **2** (edit. by Burnet, F. M., and Stanley, W. M.) (Academic, London and New York, 1959).
32 Cohen, S. S., *Viral and Rickettsial Infections of Man* (edit. by Horsfall, F. L., and Tamm, I.) (Lippencott, Philadelphia, 1965).
33 Beadle, G., and Beadle, M., *The Language of Life* (Panther, London, 1966).
34 Hershey, A. D., *Phage and the Origins of Molecular Biology* (edit. by Cairns, J., Stent, G. S., and Watson, J. D.) (Cold Spring Harbor, 1966).
35 Stent, G. S., *Scient. Am.*, December, 84–93 (1972).
36 Joklik, W. K., and Smith, D. T., *Zinsser Microbiology* (Appleton-Century-Crofts, New York, 1972).

Nitrogenase

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The protein components of the enzyme system nitrogenase, responsible for the fixation of nitrogen by microbes, have been purified and characterised from several sources. Examination by physicochemical and kinetic methods is revealing an elaborate reaction mechanism involving electron transport from one protein to the other promoted by reaction with Mg–ATP.

NITROGENASE is the name given to the enzyme system responsible for biological nitrogen fixation, the reduction of dinitrogen (N_2) to ammonia[1]. In the past three years, techniques for handling these O_2-sensitive proteins have improved and sufficient highly purified material has become available for mechanistic studies at the enzyme level. Mössbauer spectroscopy, electron paramagnetic resonance (EPR), rapid reaction kinetics and classical steady state kinetics are converging to advance understanding of the mode of action of the enzyme. The basic action pattern seems to be similar whether the enzyme system is obtained from the strict anaerobe *Clostridium pasteurianum*, the facultative anaerobe *Klebsiella pneumoniae* or the aerobe *Azotobacter vinelandii*. Here we survey the state of the enzymology of nitrogenase at the end of 1973; recent reviews of nitrogen fixation are given in refs 2–5.

Nitrogenase system

Nitrogenase consists of two proteins, one containing molybdenum, iron and acid-labile sulphur (Mo–Fe protein) and the other iron and labile sulphur (Fe protein). Both are essential for activity, together with Mg^{2+} and ATP. ADP is a reaction product; it can inhibit nitrogenase and experimental stratagems are necessary to avoid its accumulation (see ref. 2). High Mg^{2+} concentrations can inhibit, probably by sequestration of ATP as an inactive Mg–ATP complex[6].

In addition to N_2, which is reduced without formation of detectable intermediates, nitrogenase reduces a variety of triply-bonded nitrogen analogues (C_2H_2,HCN,N_2O,$CH_3N{\equiv}C$). If no reducible substrate is supplied, hydrogen ions are discharged as H_2; this reaction is not completely suppressed by other substrates *in vitro*. Carbon monoxide inhibits all but the hydrogen-evolving function of nitrogenase[2–5]. The reduction of C_2H_2 has been adopted as the now famous 'acetylene test' for nitrogen fixation[7]; the reduction products of the other analogues suggest that substrates bind to a transition metal during enzyme function[2,5].

Nitrogenase preparations from a number of organisms have been fractionated into two components both of which are required for activity (Table 1). The Mo–Fe proteins from *C. pasteurianum*[8–10], *K. pneumoniae*[11], *A. vinelandii*[12,13], the bacteroid form of *R. japonicum*[14] from soya bean nodules and the Fe proteins of *C. pasteurianum*[10,15] and *K.pneumoniae*[11] have been highly purified.

Nitrogenase components from one organism will often cross react with components from other organisms to give functional enzymes. Table 2 summarises the position at the end of 1973; many of the tests did not use purified nitrogenase components so the table is expressed in only semi-quantitative form.

Table 1 Organisms from which active nitrogenase has been extracted

	Comments	References
Anaerobic bacteria		
Clostridium pasteurianum	Both components characterised	Table 3
Desulfovibrio desulfuricans	Extracts of low activity	55
Facultative anaerobic bacteria		
Klebsiella pneumoniae	Both components characterised	Table 3
Bacillus polymyxa	Two components separated	56
E. coli C-M74	Constructed hybrid, Mo–Fe protein immunologically identical with *K. pneumoniae* protein	57
Aerobic bacteria		
Azotobacter chroococcum	Components separated	58
A. vinelandii	Mo–Fe protein characterised	Table 3
Mycobacterium flavum 301	Two components separated	59
Photosynthetic bacteria		
Chromatium vinosum	Mo–Fe protein characterised	Table 3
Rhodospirillum rubrum	Presumptive Mo–Fe protein only	59
Blue-green algae		
Anabaena cylindrica	Active extract by sonication	60, 61
Gloecapsa	Active extract by sonication	62
Plectonema boryanum 594	Active extract by sonication	63
Legume bacteroids		
Glycine max + *Rhizobium japonicum*	Mo–Fe protein characterised	Table 3
Lupinus luteus + *R. lupinii*	Active extract by sonication	64
Ornithopus sativus	Active extracts by pressure disruption	65
Phaseolus aureus, *Vigna sinensis* + *Rhizobium* spp	Two components shown	66

Both proteins are irreversibly inactivated by oxygen. The Fe protein is particularly sensitive, therefore purification under an oxygen-free atmosphere is mandatory, and usually $Na_2S_2O_4$ is included as an O_2 scavenger in buffer solutions used during purification. Otherwise purification schemes make use of familiar techniques of protein chemistry[5].

The extreme O_2 sensitivity of nitrogenase poses a special problem for aerobic N_2-fixing organisms, particularly those which are aerobic and/or photosynthetic. Some strains of blue-green algae protect their nitrogenase from oxygen produced during photosynthesis by compartmentation of the enzyme into specialised thick-walled cells termed heterocysts, which lack photosystem II, the O_2-producing photosynthetic system. Species of N_2-fixing algae exist which do not form heterocysts, but they generally fix N_2 only at low pO_2 values or low illumination levels[16]. Azotobacters have an elaborate physiological protection mechanism for their nitrogenase which has been reviewed recently[17].

They have a very high respiratory rate which is closely coupled to prevailing O_2 tension in the culture and which provides a dynamic process for lowering the dissolved oxygen concentration to a level at which nitrogenase can function. Extensive O_2 stress probably causes a 'conformational protection' of O_2-sensitive nitrogenase which gives rise to an inactive but O_2-tolerant form in the intact organism. Such an O_2-tolerant particulate form of nitrogenase is present in crude extracts of *A. vinelandii* and *A. chroococcum*, in which the enzyme proteins are stable in air and become O_2-sensitive only during purification or on the addition of ATP or other nucleoside triphosphates[18]. A soluble O_2-sensitive nitrogenase can be isolated from azotobacters by osmotic shock[19]; O_2-sensitively of nitrogenase components is apparently influenced by association with membranes, NADH oxidase[20] or ATP.

Ammonia, the product of enzymic nitrogen fixation, does not inhibit nitrogenase activity, but represses synthesis of the nitrogenase system. In *K. pneumoniae* this control occurs at the transcriptional level[21]. Other sources of fixed nitrogen such as NO_3^- or urea also inhibit synthesis of nitrogenase if they penetrate the cell. Studies on the regulation of nitrogenase in *A. vinelandii*[22] suggest coordinate control of the level of both nitrogenase components. During repression, activity of the Mo–Fe protein decreases rapidly and at the same rate as its characteristic EPR signal at g=3.7 (below), but immunologically active material persists for much longer.

Nitrogenase proteins

The Mo–Fe proteins. Homogeneous preparations of this protein have been obtained from *C. pasteurianum*, *K. pneumoniae*, soya bean bacteroids, *Chromatium vinosum* and *A. vinelandii*; data are collected in Table 3. It is usually a heteromeric tetramer in free-living bacteria but is homomeric in bacteroids. The protein from *A. vinelandii* has been crystallised[12] but the crystals are too small for X-ray analysis. Crystallisation is an unreliable criterion of purity[13].

The Fe content ranges from 18–34 Fe atoms per mol, and is approximately equivalent to the acid-labile sulphide content. Reported Mo range from 1–2 atoms per mol. A preparation from *C. pasteurianum* containing 1 Mo atom per mol was separated into a protein with increased activity containing 2 Mo atoms per mol[23], and an inactive species with 0.5 Mo atoms per mol[24]; the two species were nearly identical in amino acid composition, subunit structure and in immunological reaction. Zumft and Mortenson[24] suggested that other seemingly homogeneous preparations might consist of a mixture of dimolybdo and demolybdoproteins. Nothing is known about the distribution of metals within

Table 2 Cross-reactions of nitrogenase proteins from various organisms

Physiological status	Source of Fe protein	Source of Mo–Fe protein: Av	Ac	Mf	Kp	Bp	Cp	Rr	Acy	Rj	Dd
Aerobes	*Azotobacter vinelandii* (Av)	+	0	0	+	−	−	0	0	+	0
	Azotobacter chroococcum (Ac)	0	+	‡	+	‡	0	tr	tr	0	0
	Mycobacterium flavum (Mf)	0	+	+	+	+	0	+	0	0	0
Facultative anaerobe	*Klebsiella pneumoniae* (Kp)	+	+	+	+	+	−	+	tr	0	‡
	Bacillus polymyxa (Bp)	+	tr	‡	+	+	−	‡	0	+	‡
Anaerobe	*Clostridium pasteurianum* (Cp)	−	0	−	−	+	+	−	0	−	0
Photosynthetic	*Rhodospirillum rubrum* (Rr)	0	0	0	0	0	0	+	0	0	0
	Anabaena cylindrica (Acy)	0	0	0	0	0	0	0	+	0	0
Symbiotic	*Rhizobium japonicum* (Rj)	+	0	0	0	0	0	0	0	+	0

0 = not tested, tr = trace, ‡ = about 50%, + = positive, − = no activity.
Dd = *Desulfovibrio desulfuricans*. Data from several sources given by Burris[5] and, in addition, refs. 59, 67. Data for Dd from Dr T. H. Blackburn, that for Acy from P. Raynaud (personal communications).

Table 3 Comparison of the properties of nitrogenase components from various organisms

	Mo–Fe protein					Fe protein	
	K.pneumoniae[11]	*C.pasteurianum*[9,23,24,52]	*A.vinelandii*[12,33,69]	*R.japonicum*[14] bacteroids	*Chromatium vinosum*[31]	*K.pneumoniae*[11]	*C.pasteurianum*[15,70]
Molecular weight	229,000	220,000	270,000	200,000		66,700	55,000
Subunit composition	51,300 59,600	50,700 59,500	54,000 60,000	50,000 one type	— two types	34,000	27,500
Metal and sulphide content per g atom per mol							
Mo	1	2	2	1.3	1.3	0	0
Fe	18	22–24	32–36	29	17	4	4
S^{2-}	18	22–24	28	26	14	3.8	4
EPR spectrum	4.32,3.63 2.009	4.27,3.78 2.01	4.3,3.65 2.01	— —	4.3,3.68 2.01	2.053,1.942 1.865	2.05,1.94 1.88
Sensitivity to O_2	+ $t_{\frac{1}{2}}$ 10 min	+	+	+ $t_{\frac{1}{2}}$ 4.5 min	+	+++ $t_{\frac{1}{2}}$ 45 s	+++

the subunits since (i) the metals do not exchange with isotopes and (ii) the treatment with acid or detergent required to dissociate the subunits releases both metals[23].

All common amino acids are present, (see Table 4) with a high proportion (about 20%) of aspartate and glutamate, which are about twice as abundant as arginine and lysine. The protein from *C. pasteurianum* has a very much lower content of tryptophan than the other proteins and has two C termini, consistent with two different polypeptide subunits. When the compositions are used to estimate compositional relatedness[25], and to predict the probable degree of sequence homology[26], the proteins from *A. vinelandii* *K. pneumoniae* and soya bean bacteroids prove to be more closely related to each other than to that from *C. pasteurianum*. It would be premature to speculate how conservatively the amino acid sequence has been preserved within this group of proteins but the inability of the protein from *C. pasteurianum* to produce an active nitrogenase with complementary proteins from the other organisms (Table 2) might well reflect these differences.

Mo–Fe proteins from *C. pasteurianum*[27,28], *K. pneumoniae*[29,30], *Chromatium vinosum*[31] and *A. vinelandii*[12,28] have a unique and characteristic EPR spectrum below 30 K with g=2.01, 3.7 and 4.3 (Table 3). These resonances are most intense in the presence of $Na_2S_2O_4$, and diappear on oxidation with a dye and also in the functioning nitrogen-fixing system. Palmer *et al.*[27] suggest that they originate from an antiferromagnetically coupled system of iron atoms with an effective spin state of S=3/2. Molybdenum is, in principle, more likely than Fe to produce this species, but enrichment with ^{95}Mo (nuclear spin 5/2) produced no hyperfine structure[27,30,31] whereas enrichment with ^{57}Fe (nuclear spin 1/2) produced some line broadening[30]. Hence Fe atoms are the probable source of the resonances. The precise g values and line widths of the protein of *K. pneumoniae* are *p*H dependent, and the substrate analogue C_2H_2 displaces the signal in favour of the high *p*H form[30].

The Mo–Fe protein of *K. pneumoniae* labelled with ^{57}Fe has been investigated by Mössbauer spectroscopy[32] and three redox states of the undamaged protein can be detected. In the presence of $Na_2S_2O_4$, as normally isolated, the spectrum at 195 or 77 K consists of three overlapping quadrupole pairs of absorptions with relative intensities corresponding to iron sulphur clusters containing 2, 8 and 8 iron atoms. One of the groups of 8 atoms has magnetic properties and has been correlated with the EPR active species. In functioning nitrogenase a more highly reduced form of the Mo–Fe protein appears. Oxidation of the protein gives Mössbauer spectra different from any others, particularly from those observed during catalytic activity; a species formed by oxidation with Lauth's Violet retains full enzyme activity. Extensive O_2 damage results in spectra characteristic of ferric oxide or oxyhydroxide and presumably reflects destruction of the chromophore. A Mössbauer spectrum of the protein from *A. vinelandii*[33] has parameters similar to those of the oxidised protein from *K. pneumoniae*, with possible contributions from O_2-inactivated protein.

The ultraviolet and visible spectra of the Mo–Fe proteins from all sources[9,11–13,31] are characteristic of iron–sulphur proteins with more than two Fe and S^{2-} atoms per molecule. Their solutions are brown and have an absorption maximum near 280 nm. There are no maxima in the visible region, but rather a broad absorption extending from about 350 nm to well into the near infrared. The circular dichroism spectra of the proteins from *K. pneumoniae*[11] and *C. pasteurianum*[34] are very similar, consisting of negative troughs near 210 nm and 220 nm associated with α-helical structure. They are exceptional among iron–sulphur proteins in that they do not show the optical activity in the visible region found with ferredoxins and xanthine oxidase[35].

Redox titrations of the proteins from *K. pneumoniae*[36], and *C. vinosum*[37] indicate a difference of two electrons per mol between the dye-oxidised and $Na_2S_2O_4$-reduced forms but a value of four electrons per mol has been reported for *C. pasteurianum*[38]. The protein of *C. vinosum* has two centres with identical EPR spectra which undergo single electron reductions with midpoint potentials of −60 mV and −260 mV; this differs from *C. pasteurianum* where a four electron reduction at −70 mV is thought to occur[38].

The Fe protein. Only the proteins from *C. pasteurianum*[10,15] and *K. pneumoniae*[11] have been obtained in homogeneous form; their properties are shown in Table 3. The extreme O_2-sensitivity of these proteins is a major obstacle in purification. The proteins from *A. vinelandii* and *C. pasteurianum*, but not *K. pneumoniae*, are cold labile and lose activity rapidly below 5° C. Fe-proteins are monomeric dimers with similar Fe and S^{2-} contents. As with the Mo–Fe proteins, there is a high proportion (about 20%) of acidic amino acid residues which are about twice as abundant as basic residues[11,34]. Although there is a general similarity in composition (Table 4) and a characteristic absence of tryptophan, the compositional relatedness of these proteins is not close.

Electron paramagnetic resonance spectra of the proteins from *C. pasteurianum*[28,29], *K. pneumoniae*[30] and *A. vinelandii*[28] in the reduced form show a plant ferredoxin-like signal of the g=1.94 type below 40 K, although the line shape indicates a more complex structure than spinach ferredoxin. They integrate to 0.2–0.8 electrons per dimer depending on the source of the protein. The oxidised forms of these proteins have no EPR signal. When Mg^{2+} and ATP or ADP are added to the reduced proteins, the symmetry changes from a rhombic to an axial type; other nucleoside triphosphates are inactive. Similar changes in signal symmetry occur if the protein conformation is perturbed by 5 M urea[39]. Titration of the EPR spectrum with ATP showed tight binding of 2 ATP molecules to the protein

Table 4 Amino acid compositions of nitrogenase components from various organisms

	Mo-Fe protein				Fe protein	
	K.pneumoniae[11]	*C.pasteurianum*[34]	*A.vinelandii*[33]	*R.japonicum*[14] bacteroids	*K.pneumoniae*[11]	*C.pasteurianum*[68]
Asx	210	192	249	182	60	24
Thr	104	120	115	89	36	44
Ser	110	92	134	109	24	22
Glx	206	196	250	179	84	68
Pro	92	86	101	89	18	16
Gly	152	182	206	171	60	60
Ala	158	140	169	162	60	36
Val	124	148	173	126	42	32
Met	76	60	86	39	36	16
Ile	100	160	134	112	48	32
Leu	182	138	190	136	42	48
Tyr	68	84	79	65	18	16
Phe	96	68	102	80	12	12
His	48	46	55	53	6	4
Lys	102	160	177	122	36	32
Arg	102	62	108	94	24	24
Cys(O_3H)	38	40	41	23	18	12
Trp	56	6	50	29	0	0
Total	2,024	1,980	2,419	1,860	624	498
% of Glx + Asx	20.5	19.5	20.6	19.4	23	18.4
% of Lys + Arg	10.7	11.2	11.8	11.7	9.6	11.2
C termini	—	Ala, Leu	—	—	—	Leu

Values given are residues per molecular weight of 229,000 for *K.pneumoniae*; 220,000 for *C.pasteurianum*; 270,000 for *A.vinelandii* and 200,000 for *R.japonicum* bacteroids Mo–Fe protein and 66,700 for *K.pneumoniae* and 55,000 for *C.pasteurianum* Fe proteins.

from *C. pasteurianum*[39]; equilibrium dialysis also showed two binding sites[40].

ATP increases the sensitivity of the proteins from *A. chroococcum* or *K. pneumoniae* to O_2-inactivation[41]. With *K. pneumoniae* Fe protein it produces marked changes in sedimentation behaviour and also enhances the reactivity of sulphydryl groups with DTNB[42]. The iron of the protein from *C.pasteurianum* reacts faster with the chelating agent α,α'-dipyridyl in the presence of ATP[43]. A conformational change of the Fe protein produced by ATP binding, presumably alters the symmetry of the iron–sulphur chromophore to a strained configuration, with a more reducing standard potential, from which state electrons are transferred to the Mo–Fe protein. The Mössbauer spectrum of reduced ^{57}Fe protein from *K. pneumoniae* is consistent with one type of Fe cluster of spin state of S=1/2 with the spin equally distributed over the four iron atoms[32]. Oxygen damage gives rise to a spectrum very similar to that of reduced high potential Fe protein of *Chromatium vinosum* (Hipip), a protein which contains an Fe_4S_4 cluster[44]. ATP with Mg^{2+} causes little change in the Mössbauer spectrum.

The UV/visible spectra of the Fe proteins from *K. pneumoniae*[11] and *C. pasteurianum*[15] are very similar, being featureless in the visible region. Oxidation of the protein from *C. pasteurianum* with O_2 or dyes produces a maximum increase in absorption at 420 nm[38]. Similar changes occur in the N_2-fixing system when the deductant is exhausted[45], indicating that it becames oxidised under these conditions. The proteins from *K. pneumoniae* and *C. pasteurianum* have troughs associated with tertiary structure in the ultraviolet and no visible circular dichroism[11,34]. As noted above for the Mo–Fe proteins, the absence of visible CD is unusual for proteins with iron–sulphur chromophores.

Electron donors to nitrogenase. Natural reductants of nitrogenase are ferredoxins and/or flavodoxins. Ferredoxins of N_2-fixing bacteria have redox potentials below −390 mV but range in iron and sulphur content from 2 to 8 atoms per mol depending on their source[4]. A ferredoxin from one organism will usually show reactivity with nitrogenase from other organisms; flavodoxins have three oxidation states; the hydroquinone-semiquinone couple of *A. chroococcum* flavodoxin is the active electron-donating species to its homologous nitrogenase and also to nitrogenase from *K. pneumoniae*[46]. In anaerobic organisms, pyruvate metabolised by the phosphoroclastic system provides both reduced ferredoxin and ATP; formulate or H_2 give only reduced ferredoxin[4]. Chloroplasts which have been heat-treated to inactivate photosystem II can be used as a reductant for nitrogenase *in vitro* when coupled through a ferredoxin or flavodoxin[47]. Reduced viologen dyes[48] or $Na_2S_2O_4$ reduce nitrogenase directly and are widely use *in vitro*.

Interaction of the nitrogenase proteins. Though the separated nitrogenase proteins show evidence of interaction with ATP or reducible subtrates, neither has yet been shown to catalyse any partial reactions. When increasing amounts of the Mo–Fe protein are added to a fixed amount of Fe protein the nitrogenase activity increases from zero to a plateau if phosphate release from ATP is measured[36,49], but a region of inhibition is reached for reducible substrates[8,11,50] (Fig. 1) and $Na_2S_2O_4$ oxidation[49]. The shape of the inhibited part of the activity titration curve differs according to the source of the enzyme. Experiments with complementary proteins from *K. pneumoniae* and *A. chroococcum* indicate that the Fe protein determines the character of the inhibition[41]. The peak of activity occurs at an equimolar ratio of the two proteins for reduction of N_2 or C_2H_2 and for H_2 evolution but not for HCN reduction[36]. Activity

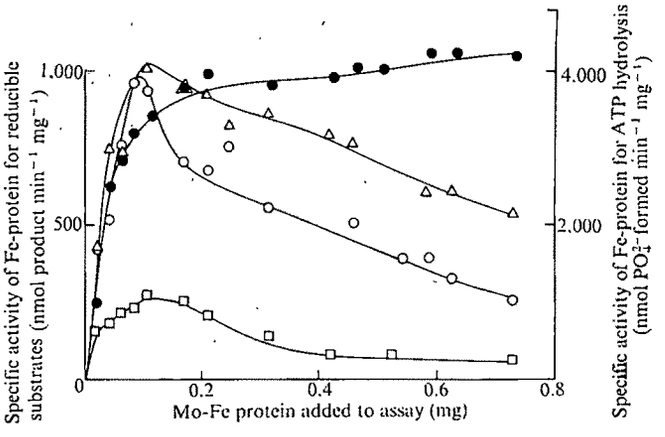


Fig. 1 Nitrogenase activity of *Klebsiella pneumoniae* Fe protein with various concentrations of Mo–Fe protein. Fe protein (42 μg) was assayed with various amounts of Mo–Fe protein (for experimental details see ref. 11). ●——●, ATP hydrolysis; □——□, N_2 reduction; ○——○, C_2H_2 reduction; △——△, H_2 evolution. (After Fig. 10 of ref. 11 but including unpublished data for inorganic phosphate release from ATP.)

titration curves have led to the formulation of the stoichiometry of the nitrogenase complex as 1:1 for *K. pneumoniae*[11] and 1:2 (Fe protein in excess) for *C. pasteurianum*[50]. Ultracentrifuge studies[51] of *K. pneumoniae* components also indicate a 1:1 complex because an equimolar mixture, in the absence of $Na_2S_2O_4$, sediments as a single peak of 12.4S, compared with the 10.4S of the larger Mo–Fe protein. Additional Fe protein sediments independently at its characteristic S value (4.8). $Na_2S_2O_4$ prevents formation of the complex species.

The characteristic Mössbauer and EPR spectra of the two proteins have been used to monitor the oxidation state of both proteins during the pre-steady state and during turnover. EPR experiments have been done with components from *K. pneumoniae*[28,30], *C. pasteurianum*[28,39,50,52,53], *A. vinelandii*[28] and *Chromatium vinosum*[31], but Mössbauer experiments have been made only with *K. pneumoniae*[32,54]. The results obtained with nitrogenase from all four sources are gratifyingly consistent, and an early disagreement whether an oxidative[52] or reductive[28–32] change in the Mo–Fe protein occurs has been resolved[38]. Thus the mechanism schematised in Fig. 2 has a sounder experimental basis than those presented in earlier reviews and articles; essentially it implies that the Mg–ATP complex of reduced Fe protein is the electron donor to the EPR active form (intermediate redox state) of the Mo–Fe protein to which the reducible substrate is bound; it is presented for *K. pneumoniae* nitrogenase because most data are available for these proteins but it is probably substantially true of clostridial and other nitrogenases. A summary of the evidence bearing on the steps in Fig. 2 follows.

(1) The electron donor in *K. pneumoniae* has not been identified but it is probably a flavodoxin or ferredoxin as in other nitrogen-fixing bacteria.

(2) The Fe protein accepts electrons from the donor, and binds Mg–ATP to form a species of altered EPR spectrum[30], tertiary structure[42] and standard potential.

(3) Substrate is bound by the EPR-active form of the Mo–Fe protein at a site which influences the *p*H-dependence of the EPR spectrum. Since it probably involves a transition metal, it is tempting to assign to Mo the substrate binding role[30].

(4) In the steady state, the EPR of the Mo–Fe protein decreases by 90%[30] an the fully reduced species appears in the Mössbauer spectrum, indicating that the reduced Mg–ATP–Fe protein transfers electrons to the Mo–Fe protein-substrate species of intermediate redox state[32]. Consistent with this view, the EPR signal of the Mo–Fe protein returns when reductant ($Na_2S_2O_4$) is exhausted[30].

(5) The Mössbauer spectra of the Fe protein show only slight changes in the functioning enzyme, consistent with some degree of oxidation[32]; the EPR of the reduced form of this protein also persists, though at a lowered intensity[30].

(6) A 1:1 complex with a half-life longer than the half-life of the redox species of its components in the functional enzyme has been assumed in Fig. 2 on the basis of the activity titration[11,36] and sedimentation data[51] indicated earlier.

The oxidised species of the native Mo–Fe protein which has no EPR but a distinctive Mössbauer spectrum, formed *in vitro* by reaction with the dye Lauth's violet, is rapidly reduced by the reduced Fe protein in the presence of ATP[29]. Such oxidised species probably do not participate in enzyme function *in vivo*.

The scheme in Fig. 2 is naturally imperfect: it supplies only a single function for Mg–ATP and accounts for transfer of only one electron from donor to reducible substrate. It also allows only one reductive site on the enzyme complex, when there may be as many as five[71]. But it provides a basis for future hypotheses, which must incorporate the fact that six electrons are transferred to N_2, with the apparent hydrolysis of more than six ATP molecules and without

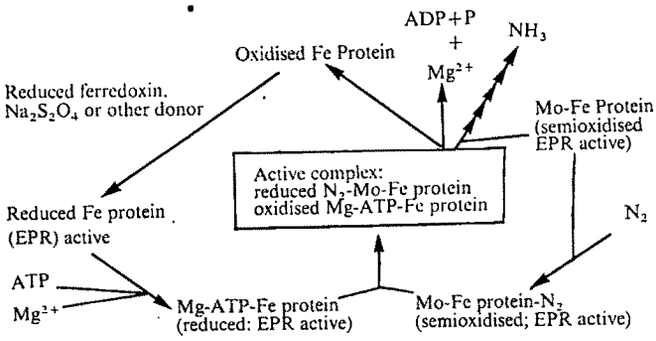


Fig. 2 Schematic representation of events during turnover of nitrogenase. For details see text; (EPR) signifies that the species has an EPR signal; the multiple arrows before NH_3 signify the six electron transfer steps which occur before an identified reduction product is formed.

identified itermediates. Whether the complex, if real, dissociates and reassociates during catalysis: whether the Fe protein is resupplied with ATP or replaced by new Mg–ATP protein are among the many questions that the scheme poses.

1 Postgate, J. R., *Nature*, **226**, 25–27 (1970).
2 Hardy, R. W. F., and Burns, R. C., *A. Rev. Biochem.*, **37**, 331–358 (1968).
3 Dalton, H., and Mortenson, L. E., *Bact. Rev.*, **36**, 231–260 (1972).
4 Streicher, S. L., and Valentine, R. C., *A. Rev. Biochem.*, **42**, 279–302 (1973).
5 Burris, R. H., in *Chemistry and Biochemistry of Nitrogen Fixation*. (edit. by Postgate, J. R.) 105–160 (Plenum Press, London, 1971).
6 Thornley, R. N. F., and Willison, K. R., *Biochem. J.*, **139**, 211–214 (1974).
7 Postgate, J. R., in *Methods in Microbiology*. (edit. by Norris, J. R., and Ribbons, D. W.). **6b**, 343–356 (Academic Press, London, 1972).
8 Vandecasteele, J. P., and Burris, R. H., *J. Bact.*, **101**, 794–801 (1970).
9 Dalton, H., Morris, J. A., Ward, M. A., and Mortenson, L. E., *Biochemistry*, **10**, 2066–2072 (1971).
10 Tso, M-Y., Ljones, T., and Burris, R. H., *Biochim. biophys. Acta*, **267**, 600–604 (1972).
11 Eady, R. R., Smith, B. E., Cook, K. A., and Postgate, J. R., *Biochem. J.*, **128**, 655–675 (1972).
12 Burns, R. C., Holsten, R. D., and Hardy, R. W. F., *Biochem. biophys. Res. Commun.*, **39**, 90–99 (1970).
13 Shah, V. K., and Brill, W. J., *Biochim. biophys. Acta*, **305**, 445–454 (1973).
14 Israel, D. W., Howard, R. L., Evans, H. J., and Russell, S. A., *J. biol. Chem.*, **249**, 500–508 (1974).
15 Moustafa, E., and Mortenson, L. E., *Biochim. biophys. Acta*, **172**, 106–115 (1969).
16 Stewart, W. D. P., *A. Rev. Microbiol.*, **27** (in the press).
17 Postgate, J. R., in *Biological Nitrogen Fixation* (edit. Quispel, A.), (North Holland, Amsterdam, in the press).
18 Yates, M. G., *Eur. J. Biochem.*, **29**, 386–392 (1972).
19 Oppenheim, J., Fisher, R. J., Wilson, P. W., and Marcus, L., *J. Bact.*, **101**, 292–296 (1970).
20 Yates, M. G., *FEBS Lett.*, **8**, 281–285 (1970).
21 Tubb, R. S., and Postgate, J. R., *J. gen. Microbiol.*, **79**, 103–117 (1973).
22 Davis, L. C., Shah, V. K., Brill, W. J., and Orme-Johnson, W. H., *Biochim. biophys. Acta*, **256**, 512–523 (1972).
23 Huang, T. C., Zumft, W. G., and Mortenson, L. E., *J. Bact.*, **113**, 884–890 (1973).
24 Zumft, W. G., and Mortenson, L. E., *Eur. J. Biochem.*, **35**, 401–409 (1973).
25 Metzer, H., Shipiro, M. B., Mosimann, J. E., and Vinton, J. G., *Nature*, **219**, 1166–1168 (1968).
26 Harris, C. E., and Teller, D. C., *J. theor. Biol.*, **38**, 347–362 (1973).
27 Palmer, G., Multani, J. S., Cretney, W. C., Zumft, W. G., and Mortenson, L. E., *Archs. biochem. Biophys.*, **153**, 325–332 (1972).
28 Orme-Johnson, W. H., Hamilton, W. D., Liones, T., Tso, M-Y., Burris, R. H., Shah, V. K., and Brills, W. J., *Proc. natn. Acad. Sci., U.S.A.*, **69**, 3142–3145 (1972).
29 Smith, B. E., Lowe, D. J., and Bray, R. C., *Biochem. J.*, **130**, 641–643 (1972).

[30] Smith, B. E., Lowe, D. J., and Bray, R. C., *Biochem. J.*, **135**, 331–341 (1973).
[31] Evans, M. C. W., Telfer, A., and Smith, R. V., *Biochim. biophys. Acta*, **310**, 344–352 (1973).
[32] Smith, B. E., and Lang, G., *Biochem. J.*, **137**, 169–180 (1974).
[33] Hardy, R. W. F., and Burns, R. C., in *Methods in Enzymology* (edit. San Pietro, A.), **24B**, 480–496 (Academic Press, London, 1972).
[34] Chen, J-S., Multani, J. S., and Mortenson, L. E., *Biochim. biophys. Acta*, **310**, 51–59 (1973).
[35] Tsibris, J. C., and Woody, R. W., *Coordin. Chem. Rev.*, **5**, 417–458 (1970).
[36] Eady, R. R., Smith, B. E., Thorneley, R. N. F., Ware, D. A., and Postgate, J. R., *Biochem. Soc. Trans.*, **1**, 37–38 (1973).
[37] Albrecht, S. L., and Evans, M. C. W., *Biochem. biophys. Commun.*, **55**, 1009–1014 (1973).
[38] Walker, M., and Mortenson, L. E., *Biochem. biophys. Res. Commun.*, **54**, 669–676 (1973).
[39] Zumft, W. G., Palmer, G., and Mortenson, L. E., *Biochim. biophys. Acta*, **292**, 413–421 (1973).
[40] Tso, M-Y., and Burris, R. H., *Biochim. biophys. Acta*, **309**, 263–270 (1973).
[41] Eady, R. R., Smith, B. E., Thorneley, R. N. F., Yates, M. G., and Postgate, J. R., in *Nitrogen Fixation and the Biosphere* (edit. Nutman, P. S., and Stewart, W. D. P.), (*Internat. biol. Programme*, in the press).
[42] Thorneley, R. N. F., and Eady, R. R., *Biochem. J.*, **133**, 405–408 (1973).
[43] Walker, G. A., and Mortenson, L. E., *Biochem. biophys. Res. Commun.*, **53**, 904–909 (1973).
[44] Dus, K., DeKlerk, H., Sletten, K., and Bartsch, R. G., *Biochim. biophys. Acta*, **140**, 291–311 (1967).
[45] Ljones, T., *Biochim. biophys. Acta*, **321**, 103–113 (1973).
[46] Yates, M. G., *FEBS Lett.*, **27**, 63–67 (1972).
[47] Yoch, D. C., and Arnon, D., *Biochim. biophys. Acta*, **197**, 180–184 (1970).
[48] Ware, D. A., *Biochem. J.*, **130**, 301–302 (1972).
[49] Ljones, T., and Burris, R. H., *Biochim. biophys, Acta*, **275**, 93–101 (1972).
[50] Mortenson, L. E., Zumft, W. G., Huang, T. C., and Palmer, G., *Biochem. Soc. Trans.*, **1**, 35–37 (1973).
[51] Eady, R. R., *Biochem. J.*, **135**, 531–535 (1973).
[52] Zumft, W. G., Cretney, W. C., Huang, T. C., and Mortenson, L. E., *Biochem. biophys. Res. Commun.*, **48**, 1525–1532 (1972).
[53] Mortenson, L. E., Zumft, W. G., and Palmer, G., *Biochim. biophys. Acta*, **292**, 422–435 (1973).
[54] Kelly, M., and Lang, G., *Biochim. biophys. Acta*, **223**, 86–104 (1970).
[55] Sekiguchi, T., and Nosoh, Y., *Biochem. biophys. Res. Commun.*, **51**, 331–335 (1973).
[56] Detroy, R. W., Witz, D. F., Parejko, R. A., and Wilson, P. W., *Proc. natn. Acad. Sci. U.S.A.*, **61**, 537–541 (1968).
[57] Cannon, F. C., Dixon, R. A., Postgate, J. R., and Primrose, S. B., *J. gen. Microbiol.*, **80**, 227–239 (1974).
[58] Kelly, M., *Biochim. biophys. Acta*, **171**, 9–22 (1969).
[59] Biggins, D. R., Kelly, M., and Postgate, J. R., *Eur. J. Biochem.*, **20**, 140–143 (1971).
[60] Stewart, W. D. P., Haystead, A., and Pearson, H. W., *Nature*, **224**, 226–228 (1969).
[61] Smith, R. V., and Evans, M. C. W., *Nature*, **225**, 1253–1254 (1970).
[62] Gallon, J. R., LaRue, T. A., and Kutz, W. G. W., *Canad. J. Microbiol.*, **18**, 327–332 (1972).
[63] Haystead, A., Robinson, R., and Stewart, W. D. P., *Arch. Mikrobiol.*, **74**, 235–243 (1970).
[64] Kennedy, I. R., *Biochim. biophys. Acta*, **222**, 135–141 (1970).
[65] Evans, H. J., in *How crops grow a century later* (edit. Horsfall, J. G.), *Conn. Agr. Exp. Stn. Bull.*, **708**, 110–127 (1969).
[66] Phillips, D. A., Howard, R. L., and Evans, H. J., *Pl. Physiol.*, **28**, 248–253 (1973).
[67] Murphy, P. M., Koch, B. L., *Biochim. biophys. Acta*, **253**, 295–297 (1971).
[68] Mortenson, L. E., in *Methods in Enzymology* (edit. by San Pietro, A.), **24B**, 446–456 (Academic Press, London, 1972).
[69] Fleming, H., and Haselkorn, R., *Proc. natn. Acad. Sci., U.S.A.*, **70**, 2727–2731 (1973).
[70] Nakos, G., and Mortenson, L. E., *Biochemistry*, **10**, 455–458 (1971).
[71] Hwang, T. C., Chen, J. S., and Burris, R. H., *Biochim. biophys. Acta*, **292**, 256–270 (1973).

Stratospheric sink for chlorofluoromethanes: chlorine atomc-atalysed destruction of ozone

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Chlorofluoromethanes are being added to the environment in steadily increasing amounts. These compounds are chemically inert and may remain in the atmosphere for 40–150 years, and concentrations can be expected to reach 10 to 30 times present levels. Photodissociation of the chlorofluoromethanes in the stratosphere produces significant amounts of chlorine atoms, and leads to the destruction of atmospheric ozone.

HALOGENATED aliphatic hydrocarbons have been added to the natural environment in steadily increasing amounts over several decades as a consequence of their growing use, chiefly as aerosol propellants and as refrigerants[1,2]. Two chlorofluoromethanes, CF_2Cl_2 and $CFCl_3$, have been detected throughout the troposphere in amounts (about 10 and 6 parts per 10^{11} by volume, respectively) roughly corresponding to the integrated world industrial production to date[3–5,31]. The chemical inertness and high volatility which make these materials suitable for technological use also mean that they remain in the atmosphere for a long time. There are no obvious rapid sinks for their removal, and they may be useful as inert tracers of atmospheric motions[4–6]. We have attempted to calculate the probable sinks and lifetimes for these molecules. The most important sink for atmospheric $CFCl_3$ and CF_2Cl_2 seems to be stratospheric photolytic dissociation to $CFCl_2 + Cl$ and to $CF_2Cl + Cl$, respectively, at altitudes of 20–40 km. Each of the reactions creates two odd-electron species—one Cl atom and one free radical. The dissociated chlorofluoromethanes can be traced to their ultimate sinks. An extensive catalytic chain reaction leading to the net destruction of O_3 and O occurs in the stratosphere:

$$Cl + O_3 \rightarrow ClO + O_2 \quad (1)$$

$$ClO + O \rightarrow Cl + O_2 \quad (2)$$

This has important chemical consequences. Under most conditions in the Earth's atmospheric ozone layer, (2) is the slower of the reactions because there is a much lower concentration of O than of O_3. The odd chlorine chain (Cl, ClO) can be compared with the odd nitrogen chain (NO, NO_2) which is believed to be intimately involved in the regulation of the present level of O_3 in the atmosphere[7–10]. At stratospheric temperatures, ClO reacts with O six times faster than NO_2 reacts with O (refs 11, 12). Consequently, the Cl–ClO chain can be considerably more efficient than the NO–NO_2 chain in the catalytic conversion of $O_3 + O \rightarrow 2O_2$ per unit time per reacting chain[13].

Photolytic sink

Both $CFCl_3$ and CF_2Cl_2 absorb radiation in the far ultraviolet[14], and stratospheric photolysis will occur mainly in the 'window' at 1,750–2,200 Å between the more intense absorptions of the Schumann–Runge regions of O_2 and the Hartley bands of O_3.

We have extended measurements of absorption coefficients for the chlorofluoromethanes to cover the range 2,000–2,270 Å. Calculations of the rate of photolysis of molecules at a given altitude at these wavelengths is complicated by the intense narrow band structure in the Schumann–Runge region, and the effective rates of vertical diffusion of molecules at these altitudes are also subject to substantial uncertainties. Vertical mixing is frequently modelled through the use of 'eddy' diffusion coefficients[10,15–18], which are presumably relatively insensitive to the molecular weight of the diffusing species. Calculated using a time independent one-dimensional vertical diffusion model with eddy diffusion coefficients of magnitude $K \sim (3 \times 10^3)$–10^4 cm^2 s^{-1} at altitudes 20–40 km (refs 10, 15–18), the atmospheric lifetimes of $CFCl_3$ and CF_2Cl_2 fall into the range of 40–150 yr. The time required for approach toward a steady state is thus measured in decades, and the concentrations of chlorofluoromethanes in the atmosphere can be expected to reach saturation values of 10–30 times the present levels, assuming constant injection at current rates, and no other major sinks. (The atmospheric content is now equivalent to about five years world production at current rates.) Lifetimes in excess of > 10 and > 30 yr can already be estimated from the known industrial production rates and atmospheric concentrations[3,5], and so the stratospheric photochemical sink will be important even if other sinks are discovered.

Our calculation of photodissociation rates is modelled after those of Kockarts[19] and Brinkmann[20], and is globally averaged for diurnal and zenith angle effects. The photodissociation rates at an altitude of 30 km are estimated to be 3×10^{-7} s^{-1} for $CFCl_3$ and 3×10^{-8} s^{-1} for CF_2Cl_2, decreasing for each by about a factor of 10^{-2} at 20 km. The appropriate solar ultraviolet intensities at an altitude of 30 km may be uncertain by a factor of 2 or 3 (ref. 21) and we have therefore calculated lifetimes for photodissociation rates differing from the above by factors of 3 or more. The competition between photodissociation and upward diffusion reduces the relative concentration of chlorofluoromethane at higher altitudes and the concentrations should be very low above 50 km. The peak rate of destruction, and formation of Cl atoms, occurs at 25–35 km, in the region of high ozone concentration. The rates of formation of Cl atoms at different altitudes, and the chlorofluoromethane atmospheric lifetimes are sensitive to the assumed eddy diffusion coefficients, as well as to the photodissociation rates.

The major chain processes in the stratosphere involving species with odd numbers of electrons belong to the H (H, OH, HO_2), N (NO, NO_2), and Cl (Cl, ClO) series. (ClO_2 is rapidly decomposed and its concentration is negligible relative to Cl plus ClO.) These odd-electron chains can only be terminated by interaction with one another, although other reactions can convert one series to another. At most altitudes, the first reaction for converting the Cl–ClO odd-electron chain to an even-electron species containing chlorine is the abstraction of H from CH_4, which transfers the odd-electron character to the CH_3 radical:

$$Cl + CH_4 \rightarrow HCl + CH_3 \qquad (3)$$

At stratospheric temperatures the rate constant for Cl atoms[22], for (3) is about 10^{-3} times as fast as (1) and the O_3/CH_4 concentration ratio can make the rate of (3) less than that of (1) by another factor of 10. The Cl atom chain can be renewed by the reaction of OH with HCl (ref. 23):

$$OH + HCl \rightarrow H_2O + Cl \qquad (4)$$

Ultraviolet dissociation by absorption in the range 1,750–2,200 Å can also occur at the higher altitudes. The reaction rate of (4) in the stratosphere depends on the concentration of OH, which is known only roughly. In our estimates, termination of the Cl–ClO chain results from downward diffusion of the longer lived species in the chain (ClO, HCl) and eventual removal by tropospheric processes. The rate of termination thus also depends on diffusion processes and estimates will vary with the choice of eddy diffusion coefficients.

Possible terminations involving the Cl series with itself (for example, $Cl + ClO \rightarrow Cl_2O$) or with one of the others (for example, $Cl + NO \rightarrow NOCl$) normally lead to molecules with appreciable absorption coefficients at longer wavelengths, which are very rapidly dissociated again by the much more intense solar fluxes available there. Thus, even if a molecule which temporarily terminates two chains is formed, at least one of which involves the Cl series, the terminating molecule is rapidly photolysed and both chains are regenerated again.

Under most stratospheric conditions, the slow reactions in both the Cl–ClO and NO–NO_2 chains occur between O atoms and ClO and NO_2 molecules. The two chains are interconnected:

$$ClO + NO \rightarrow Cl + NO_2 \qquad (5)$$

The rate of this reaction in the stratosphere is frequently comparable to that of reaction (2). The overall effect is complex and depends on the relative concentrations of ClO_x, NO_x, O_3, O and OH. Reaction (1) is so rapid that the ClO/Cl ratio is usually > 10, even when Cl is produced by both reaction (2) and reaction (5), so that the overall rate of reaction (2) is not directly affected by the occurrence of reaction (5). As soon as Cl is produced, however, HCl can form by reactions (1) or (3), resulting in the temporary termination of the Cl atom chain. Whether or not the chain is then restarted depends primarily on the concentration of OH. There are substantial ranges of stratospheric altitudes in which neither reaction (3) nor reaction (5) seriously impedes the chain process of reactions (1) and (2).

The initial photolytic reaction produces one Cl atom from each of the parent molecules, plus a CX_3 radical (X may be F or Cl). The detailed chemistry of CX_3 radicals in O_2 or air is not completely known, but in the laboratory a phosgene-type molecule, CX_2O, is rapidly produced and another X atom—probably Cl (or ClO)—is released from $CFCl_2$ or CF_2Cl[21,25]. CX_2O may also photolyse in the atmosphere to give a third and fourth free halogen atom. Thus, each molecule of $CFCl_3$ initially photolysed probably leads to between two and three Cl atom chains, and CF_2Cl_2 probably produces two Cl atom chains when it is photolysed. Initial calculations suggest that F atom chains will be much shorter than Cl atom chains because the reaction of abstraction from CH_4 is much faster for F atoms[26], whereas the reaction between OH and HF is 17 kcalorie mol^{-1} endothermic and will not occur in the stratosphere. We have not yet attempted to analyse the subsequent reaction paths of HF.

Production rates

The 1972 world production rates for $CFCl_3$ and CF_2Cl_2 are about 0.3 and 0.5 Mton yr^{-1} respectively[1,2,5], and are steadily increasing (by 8.7% per year for total fluorocarbons in the United States from 1961–71) (ref. 1). We have not included any estimates for other chlorinated aliphatic hydrocarbons also found in the atmosphere, such as CCl_4 (refs 3 and 4), $CHCl_3$, C_2Cl_4 and C_2HCl_3 for which there is no evidence for long residence times in the atmosphere[27]. If the stratospheric photolytic sink is the only major sink for $CFCl_3$ and CF_2Cl_2, then the 1972 production rates correspond at steady state to globally averaged destruction rates of about 0.8×10^7 and 1.5×10^7 molecules cm^{-2} s^{-1} and formation rates of Cl atoms of about 2×10^7 and 3×10^7 atoms cm^{-2} s^{-1}, respectively. The total rate of production of 5×10^7 Cl atoms cm^{-2} s^{-1} from the two processes is of the order of the estimated natural flux of NO molecules (2.5–15 $\times 10^7$ NO molecules cm^{-2} s^{-1}) involved in the natural ozone cycle[9–12], and of the 5×10^7 NO molecules cm^{-2} s^{-1} whose introduction around 25 km from stratospheric aviation is estimated would cause a 6% reduction in the total O_3 column[10].

Photolysis of these chlorofluoromethanes does not occur in the troposphere because the molecules are transparent to wavelengths longer than 2,900 Å. In fact the measured absorption coefficients for $CFCl_3$ and CF_2Cl_2 are falling rapidly at wavelengths longer than 2,000 Å (ref. 14). The reaction between OH and CH_4 is believed to be important in the troposphere[17,28], but the corresponding Cl atom abstraction reaction (for example, $OH + CFCl_3 \rightarrow HOCl + CFCl_2$) is highly endothermic and is negligible under all atmospheric conditions.

Neither $CFCl_3$ nor CF_2Cl_2 is very soluble in water, and they are not removed by rainout in the troposphere. Details of biological interactions of these molecules in the environment are very scarce because they do not occur naturally (except possibly in minute quantities from volcanic eruptions)[29], but rapid biological removal seems unlikely. The relative insolubility in water together with their chemical stability (especially toward hydrolysis)[30] indicates that these molecules will not be rapidly removed by dissolution in the ocean, and the few measurements made so far indicate equilibrium between the ocean surface and air, and therefore a major oceanic sink cannot be inferred[3].

It seems quite clear that the atmosphere has only a finite capacity for absorbing Cl atoms produced in the stratosphere, and that important consequences may result. This capacity is probably not sufficient in steady state even for the present rate of introduction of chlorofluoromethanes. More accurate estimates of this absorptive capacity need to be made in the immediate future in order to ascertain the levels of possible onset of environmental problems.

As with most NO_x calculations, our calculations have been based entirely on reactions in the gas phase, and essentially nothing is known of possible heterogeneous reactions of Cl atoms with particulate matter in the stratosphere. One important corollary of these calculations is that the full impact of the photodissociation of CF_2Cl_2 and $CFCl_3$ is not immediately felt after their introduction at ground level because of the delay required for upward diffusion up to and above 25 km. If any Cl atom effect on atmospheric O_3 concentration were to be observed from this source, the effect could be expected to intensify for some time thereafter. A lengthy period (of the order of calculated atmospheric lifetimes) may thus be required for natural moderation, even if the amount of Cl introduced into the stratosphere is reduced in the future.

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1 *Chemical Marketing Reporter*, August 21, 1972.
2 *Chemistry in the Economy* (American Chemical Society, Washington, DC, 1973).
3 Lovelock, J. E., Maggs, R. J., and Wade, R. J., *Nature*, **241**, 194 (1973).
4 Wilkniss, P. E., Lamontagne, R. A., Larson, R. E., Swinnerton, J. W., Dickson, C. R., and Thompson, T., *Nature*, **245**, 45 (1973).
5 Su, C.-W., and Goldberg, E. D., *Nature*, **245**, 27 (1973).
6 Machta, L., Proceedings of the Second IUTAM-IUGG Symposium on Turbulent Diffusion in Environmental Pollution (Charlottesville, 1973).
7 Crutzen, P. J., *J. geophys. Res.*, **30**, 7311 (1971).
8 Johnston H., *Science*, **173**, 517 (1971).
9 Johnston, H. S., Proceedings of the First Survey Conference, Climatic Impact Assessment Program, US Department of Transport, 90 (1972).
10 McElroy, M. B., Wofsy, S. C., Penner, J. E., and McConnell, J. C., *J. atmos. Sci.*, **31**, 287 (1974).
11 Bemand, P. P., Clyne, M. A. A., and Watson, R. J., *J. Chem. Soc., Faraday I*, **69**, 1356 (1973).
12 Hampson, R., *et al.*, *Chemical Kinetics Data Survey VI*, National Bureau of Standard Interim Report 73-207 (1973).
13 Stolarski, R. S., and Cicerone, R. J., International Association of Geomagnetism and Aeronomy (Kyoto, Japan, 1973); see also *Can. J. Chem.*, (in the press).
14 Doucet, J., Sauvageau, P., and Sandorfy, C., *J. chem. Phys.*, **58**, 3708 (1973).
15 McConnell, J. C., and McElroy, M. B., *J. atmos. Sci.*, **30**, 1465 (1973).
16 Schütz, K., Junge, C., Beck, R., and Albrecht, B., *J. geophys. Res.*, **75**, 2230 (1970).
17 Wofsy, S. C., McConnell, J. C., and McElroy, M. B., *J. geophys. Res.*, **77**, 4477 (1972).
18 Wofsy, S. C., and McElroy, M. B., *J. geophys. Res.*, **78**, 2619 (1973).
19 Kockarts, G., in *Mesospheric Models and Related Experiments* (edit. by Fiocco, G.), 168, Reidel, Dodrrecht (1971).
20 Brinkmann, R., *ibid.*, 89.
21 Hudson, R. D., and Mahle, S. H., *J. geophys. Res.*, **77**, 2902 (1972).
22 Clyne, M. A. A., and Walker, R. F., *J. Chem. Soc., Faraday I*, **69**, 1547 (1973).
23 Takacs, G. A., and Glass, G. P., *J. phys. Chem.*, **77**, 1948 (1973).
24 Marsh, D., and Heicklen, J., *J. phys. Chem.*, **69**, 4410 (1965).
25 Heicklen, J., *Adv. Photochem.*, **7**, 57 (1969).
26 Homann, K. H., Solomon, W. C., Warnatz, J., Wagner, H. G., and Zetzch, C., *Ber. Bunsenges. phys. Chem.*, **74**, 585 (1970).
27 Murray, A. J., and Riley, J. P., *Nature*, **242**, 37 (1973).
28 Levy, H., *Planet. Space Sci.*, **20**, 919 (1972); **21**, 575 (1973).
29 Stoiber, R. E., Leggett, D. C., Jenkins, T. F., Murrmann, R. P., and Rose, W. J., *Bull. geol. Soc. Am.*, **82**, 2299 (1971).
30 Hudlicky, M., *Chemistry of Organic Fluorine Compounds*, 340 (MacMillan, New York, 1962).
31 Lovelock, J. E., *Nature*, **230**, 379 (1971).

LETTERS TO NATURE

PHYSICAL SCIENCES

X-ray spectrum of NP0532

THERE is a change in the X-ray spectral index of the Crab pulsar NP0532 near 10 keV (refs 1 and 2). We here examine this in a more quantitative fashion than before, using most of the available published data, together with some unpublished data (Table 1). The pulsed fraction is the ratio of the pulsed radiation to total radiation; data published as the ratio of the pulsed to unpulsed components have been duly corrected. The unpulsed reference level has been determined according to Ducros *et al.*[3] and where other workers have used a significantly different convention, these data have been corrected. We believe that all data (31 points), can be validly compared. Other results, not considered here[2,4,5], are generally in agreement with the data we have used. The use of the pulsed fraction as the basic spectral quantity allows the low energy spectrum to be treated independently of interstellar absorption because it is the same for both the pulsed and unpulsed components.

A multiparameter, nonlinear, weighted routine was used to fit least squares (see refs 6 and 7). This program yields values of the unknown parameters of any generalised mathematical model which is applied to input data, together with relevant statistics, including the approximate 95% confidence interval for each parameter and the reduced χ^2 χ^2_{rt}, the value of χ^2 per degree of freedom) for the successful fit.

Any change in spectral slope will probably be gradual, perhaps spanning a decade or more. The precision of the available data is, however, such that a sharp break can be assumed for the source model, at least initially. This is also analytically simple. The assumption of identical spectral indices above the break energy is justified in a similar way (see also ref. 8). The pulsed fraction F as a function of energy E (keV) is thus given by

$$F(E) = \begin{cases} A, & E \geq E_B \\ B \exp(-\beta\lambda^2) E\gamma-\alpha, & E < E_B \end{cases}$$

where A is the fraction (assumed constant) above the break

Table 1 Summary of the data used*

Source	Energy range (keV)	No. of data points
a, Saclay (unpublished)	0.7– 10	11
b, NRL[1]	0.6– 9	6
c, Caltech[12]	0.7– 2.5	2
d, MIT[13]	1.5– 10	1
e, GSFC[14]	2– 10	1
f, Saclay[3]	2.2– 30	4
g, MIT[15]	25–100	1
h, Rice[8]	42–100	3
i, Saclay[16]	15–150	2

*Values are derived for the size and location of the break, assuming identical spectral indices for the pulsar and the nebula above the break energy and taking into account the effect of interstellar dust scattering on the pulsed radiation below approximately 2 keV. *a*, Unpublished data (C. Jehanno, C. Cheron, J. Leloup, R. Rothenflug, and R. Thomas).

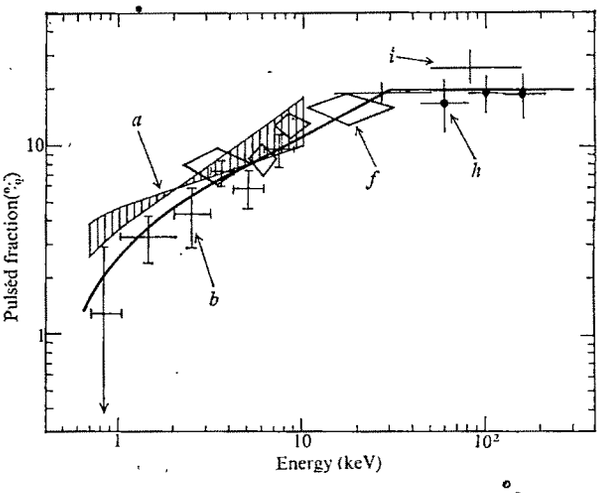


Fig. 1. The X-ray pulsed fraction of NP0532, showing the least squares fit. A selection of the data listed in Table 1 is displayed. Notation as in Table 1. Solid curve, line of best fit.

energy E_B, α and γ are the spectral indices of the pulsar and total emission energy respectively, $\beta(Å^{-2})$ is the interstellar grain scattering coefficient, λ is the photon wavelength (Å), and $B = A \exp(\beta\lambda_B^2)E_B\alpha^{-\gamma}$ where $\lambda_B = hc/E_B$. The effect of scattering is felt only below about 2 keV, and so β is left a fixed parameter. An X-ray measurement of this quantity, and thus of the amount of dust in the line of sight should, however, become possible with further improvement in the precision of 0.5 and 2 keV data. Consequently, two fixed values of β were tried: $\beta = 0$ (no scattering) and $\beta = 2 \times 10^{-3}$ Å^{-2} (as expected in the direction of the Crab)[9]. The quantities α, A and E_B were left as free parameters, with $\gamma = 1.0$. The computational results are summarised in Table 2 and the best fit is drawn through the data of Fig. 1.

The choice of β makes little difference to the quality of the fit, but using $\beta = 2 \times 10^{-3}$ Å^{-2} makes the spectral index and

Table 2 Computational results for parameters used

$\beta(Å^{-2})$	χ_r^2	α	E_B(keV)	A(%)
0	0.75	0.35±0.15	24±13	19± 4
2×10^{-3}	0.72	0.44±0.13	30±17	20± 4

the break energy slightly greater. The large error in E_B, together with the acceptable χ_r^2 value, reflects the lack of precision of the data. A sharp break at 30 ± 17 keV satisfies the data well, although statistically improved data will probably modify this conclusion in the future.

The value $\alpha = 0.44 \pm 0.13$ is derived relative to a total emission index of $\gamma = 1.00$. Taking the error in γ to be ± 0.1 will yield a final result of $\alpha = 0.4 \pm 0.2$. This result is not inconsistent with the suggestion[10] that the pulsar X-ray index may be identical to the Nebula radio index of 0.26, indicating a common parent electron population in regions with different magnetic fields. A spectral index of between 0.4 and 0.5, extrapolated toward the optical region, agrees, however, much better with the known pulsar optical spectrum than does an index of 0.26 or 0.2 (ref. 1).

The present fit shows that there is no necessity to postulate a further sharp change of spectral index in NP0532 between 1.5 and 3 keV as suggested by Rappaport *et al.*[4].

The idea that the decrease in the pulsed fraction below the break is attributable entirely to interstellar scattering (ref. 11) was tested. The resulting fit was unacceptable ($\chi_r^2 = 2.7$) and the derived value of $\beta = (1.6 \pm 0.9) \times 10^{-2}$ Å^{-2} was an order of magnitude higher than optical measurements imply (ref. 9), regardless of the grain model assumed. This model can therefore be rejected (see also refs 3 and 9).

Variations of the total emission spectral index of the Nebula with energy[2], are relevant. Our assumption that the total emission spectral index is given by $\gamma = 1.0 \pm 0.1$ remains valid for energies up to 60 keV (refs. 1 and 2), a fact which lends confidence to the derived value of $\alpha = 0.4 \pm 0.2$ for the pulsar below about 30 keV. The increase in γ to 1.46 ± 0.11 above 60 keV brings into question the assumption that $\alpha = \gamma$ for $E > E_B$, which would imply a second pulsar break at 60 keV, for which there is no evidence. In our opinion, however, the use of the analytically simple model presented here is justified at this time by the relatively poor statistics of the higher energy pulsar data.

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1 Fritz, G., Meekins, J. F., Chubb, T. A., and Friedman, H., *Astrophys. J. Lett.*, **164**, L55–L60 (1971).
2 Laros, J. G., Matteson, J. L., and Pelling, R. M., *Nature phys. Sci.*, **246**, 109–111 (1973).
3 Ducros, G., Ducros, R., Rocchia, R., and Tarrius, A., *Nature*, **227**, 152–154 (1970).
4 Rappaport, S., Bradt, H., and Mayer, W., *Nature phys. Sci.*, **229**, 40–42 (1971).
5 Smathers, H. W., Chubb, T. A., and Sadeh, D., *Nature phys. Sci.*, **232**, 120–121 (1971).
6 Draper, N. R., and Smith, H., *Applied Regression Analysis*, Chapter 10 (Wiley, New York, 1966).
7 Cline, D., and Lesser, P. M. S., *Nucl. Instrum. Meth.*, **82**, 291–293 (1970).
8 Fishman, G. J., Hardnen, F. R., Johnson, W. N., and Haymes, R. C., *Astrophys. J. Lett.*, **158**, L61–L64 (1969).
9 Naranan, S., and Shah, G. A., *Nature*, **225**, 834–836 (1970).
10 Shklovsky, I. S., *Astrophys. J. Lett.*, **159**, L77–L80 (1970).
11 Slysh, V. I., *Nature*, **224**, 159–160 (1969).
12 Garmire, G., and Riegler, G. R., *Astr. Astrophys.*, **21**, 131–138 (1972).

[13] Bradt, H., Rappaport, S., Mayer, W., Nather, R. E., Warner, B., MacFarlane, M., and Kristian, J., *Nature*, **222**, 728–730 (1969).
[14] Boldt, E. A., Desai, N. D., Holt, S. S., Serlemitsos, P. J., and Silverberg, R. F., *Nature*, **223**, 280–281 (1969).
[15] Floyd F. W., Glass, I. S., and Schnopper, H. W., *Nature* **224**, 50–51 (1969).
[16] Boclet, D., Brucy, G., Claisse, J., Durouchoux, Ph., and Rocchia, R., *Nature phys. Sci.*, **235**, 69–72 (1972).

Charge composition of cosmic rays between 4 and 100 GV

SEVERAL groups have reported the energy dependence of the charge composition of cosmic-ray nuclei with $Z \geq 3$ (refs 1–5). The results suggest that the ratio of the numbers of nuclei in groups L and M (defined in Table 1) decreases as the rigidity increases. At 100 GV the ratio is about 25% lower than the value below 10 GV. There is, however, no general agreement about the variation with rigidity of the ratio of VH nuclei to M nuclei; a variable ratio would be evidence for the existence of more than one basic acceleration mechanism, because both charge groups are thought to consist mainly of particles coming directly from the sources. The largest variation in the ratio of VH to M nuclei is reported by Ormes and Balasubrahmanyan[1]. We report here the measurement of the ratios of L nuclei to M

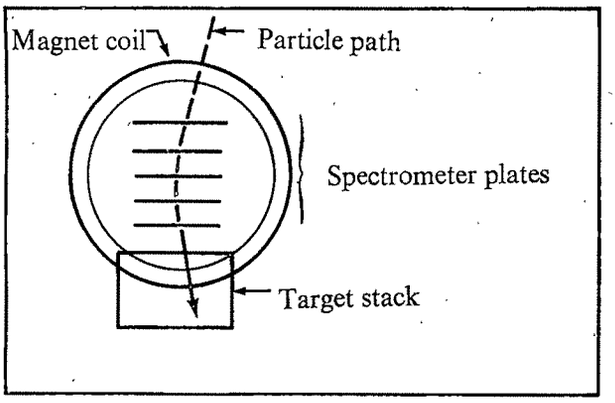


Fig. 1 Schematic representation of the detector system indicating the location of the five spectrometer plates and the target emulsion stack.

nuclei and of VH nuclei to M nuclei using a magnetic spectrometer. Our results are based on two balloon flights, the first from Palestine, Texas, on August 23, 1969, and the second on November 13, 1970, from Parana, Argentina. The combined exposure factor for these flights was 273 cm^2 sr s at an average altitude of 3 g cm^{-2}.

The apparatus[6] consists of an emulsion block and five double-coated glass plates arranged in the field of a superconducting magnet (Fig. 1). Events were found by area scanning the target block and rigidities were measured by tracing particles through the emulsion-coated glass plates. The scanning efficiency was >95%. The process of rigidity measurement has a resolution of

Table 1 Number of nuclei as a function of charge group and rigidity

	Rigidity interval (GV)					
Charge group	4–7	7–10	10–15	15–25	25–50	>50
L ($3 \leq Z \leq 5$)	18	21	18	21	7	8
M ($6 \leq Z \leq 8$)	74	64	81	78	49	35
LH + MH ($9 \leq Z \leq 19$)	22	29	18	26	18	5
VH ($20 \leq Z \leq 27$)	7	7	7	5	1	3

Total no. of events = 625. LH, $9 < Z < 14$; MH, $15 < Z < 19$.

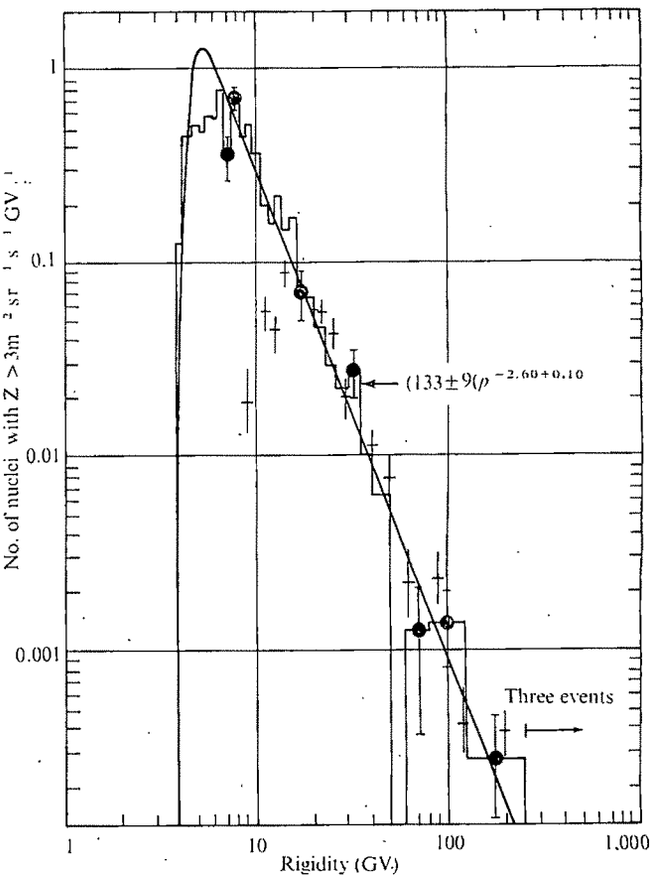


Fig. 2 Rigidity spectrum. The histogram contains data from the Palestine flight; the data bars are from the Parana flight. Both curves are uncorrected for geomagnetic transmission. ●, Palestine flight; + Parana flight.

12% at 5 GV and 35% at 100 GV (ref. 6); it is very straightforward (using a 14-constraint least squares fit to each track) and does not rely on assumptions concerning observed spectrum. Alignment and resolution information are obtained by turning off the magnet in flight to calibrate the system with tracks which are straight except for Coulomb scattering. A computer-controlled microscope with a measuring accuracy of ± 2.5 μm, was used to measure the trajectories. A total of 791 tracks of nuclei with $Z \geq 3$ were located during the area scan. This paper is based on the 625 events which have so far been measured completely. In

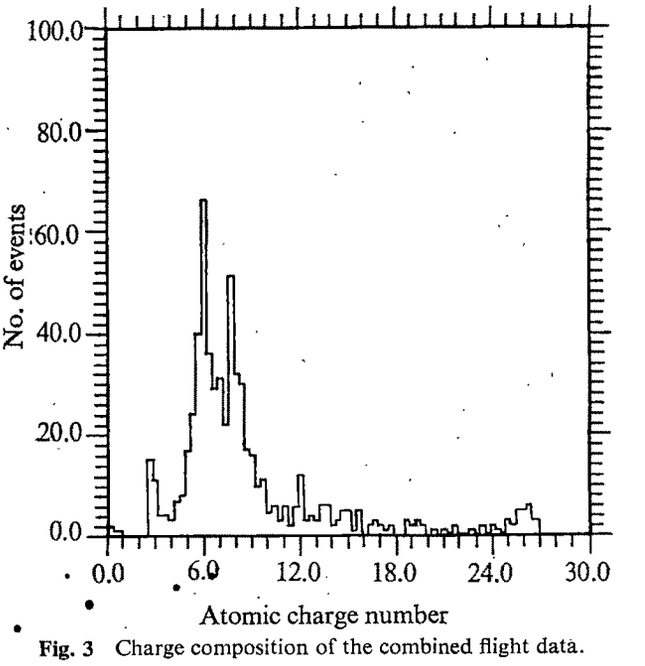


Fig. 3 Charge composition of the combined flight data.

Fig. 2 we present the rigidity spectra of nuclei with $Z \geqslant 3$ gathered during the two flights. There is close agreement between the energy spectra of Texas and Argentina flights at rigidities above 12 GV, where solar modulation should be negligible. The absolute flux value agrees with that of Anand *et al.*[7]. A maximum likelihood analysis gives a spectral index of -2.6 ± 0.1, which is consistent with other observations in this region.

Charge measurement was carried out using a television microscope to measure track area. The charge distribution obtained with the microscope photometer is shown in Fig. 3. The resolution is 0.4 charge units at carbon and ~ 1.0 charge unit at iron. Clearly, there is some contamination of L nuclei from the neighbouring carbon peak. This contamination should be independent of rigidity.

Figures 4 and 5 show the charge composition as a function of rigidity. The data are uncorrected for the 3 g cm^{-2} of air, 3 g cm^{-2} of emulsion and 1 g cm^{-2} of glass above the scan line.

Measurement of spectral indices for the individual charge groups is difficult at rigidities below 12 GV because of very

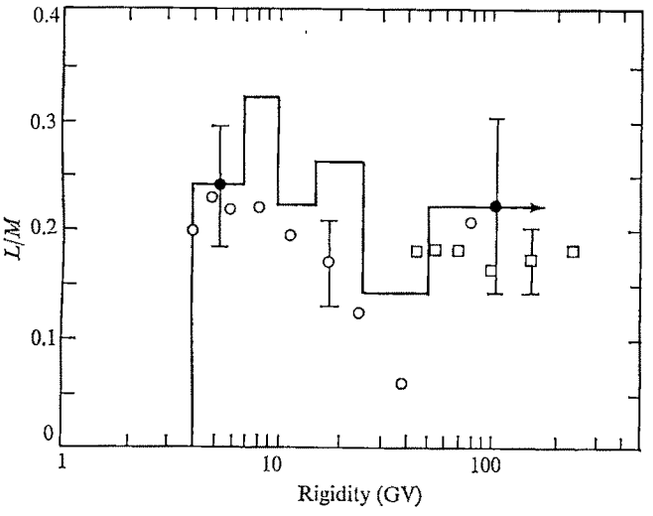


Fig. 4 Ratio of L nuclei to M nuclei as a function of rigidity. ○, Smith *et al.*[4]; □, Juliusson *et al.*[2].

strong solar modulation during the Texas flight and a geomagnetic cutoff at 12 GV during the second flight. Instead of measuring separate spectral indices we have measured the dependence on rigidity of the ratios of the charge groups. This rests on the implicit assumption that effects arising from solar modulation and geomagnetic cutoff do not depend on charge in the range 4–12 GV. The analysis of the dependence on rigidity of the charge ratios was carried out by using the M nuclei at a given rigidity to predict the numbers of VH or L nuclei. For a given charge group (VH, for example) a charge ratio relative to the M group $R = a\rho^{-\Delta K}$ is assumed. The constant a is chosen so that the number of expected VH (or L) nuclei equals the number of observed VH (or L) nuclei. The parameter ΔK is the difference in spectral indices ($\Delta K > 0$ means VH is flatter than M). In each rigidity interval

$$\langle VH \rangle_{\text{expected}} = R(\bar{\rho}, \Delta K)\,(M)_{\text{observed}} \qquad (1)$$

Using the values of $\langle VH \rangle_{\text{exp}}$ and $(VH)_{\text{obs}}$ a pseudo-likelihood function is evaluated for a given value of ΔK by multiplying the Poisson probabilities of all the rigidity intervals.

$$L(\Delta K) = \prod_{\text{rigidity intervals}} P(\langle VH \rangle_{\text{exp}}, (VH)_{\text{obs}}) \qquad (2)$$

where P is the Poisson probability of observing $(VH)_{\text{obs}}$ when VH_{exp} is the expected number of counts.

The effects of mixing of data from adjacent rigidity intervals

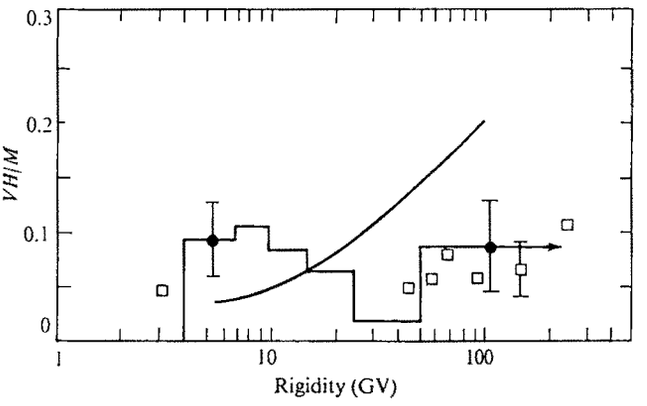


Fig. 5 Ratio of VH nuclei to M nuclei as a function of rigidity. □, Juliusson *et al.*[2]; curve, best fit for VH/M after Ormes and Balasubrahmanyan[1].

are small for the magnetic spectrometer. The uncertainty in measured rigidity is easily determined by least-squares error propagation. The largest correction would be to the highest rigidity bin in the ratio of VH nuclei to M nuclei. The ratio should be lowered by ~ 0.01 which is negligible. Although such corrections may be significant for other less accurate energy-measuring devices they can be neglected in this analysis.

The likelihood analysis yields a spectral index difference $\Delta K = -0.2 \pm 0.2$ for the VH/M ratios. Our result is in strong conflict with that of Ormes and Balasubrahmanyan[1], $+0.57 \pm 0.14$, which has a likelihood of 0.1% relative to our most probable result. This result is not inconsistent with the observations of Juliusson *et al.*[2], Webber *et al.*[3] and Atallah *et al.*[4] as can be seen in Fig. 5. Our experiment itself, however, reveals no evidence of a difference between the VH spectrum and the M spectrum. The L/M analysis gives a spectral index difference $\Delta K = +0.12 \pm 0.4$, consistent with the observations of all other observers but also consistent with zero. It is worth noting again that our L sample is contaminated with 20–25% of carbon nuclei. The (LH + MH)/M ratio gave a spectral index difference of $-0.14^{+0.1}_{-0.16}$.

Since the area scan is 95% efficient, the identification of particles is independent of energy and the rigidity measurement relies only on the solution of the Lorentz-force equation, this experiment can be expected to be exceptionally free of energy–charge biases. We find no evidence that the VH spectrum is steeper than the M spectrum. The difference in spectral indices is -0.2 ± 0.2 (minus meaning VH is steeper than M). Observations of the L and (LH + MH) fluxes relative to the M flux is consistent with the observations of the Berkeley and Chicago groups.

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1 Ormes, J. F., and Balasubrahmanyan, V. K., *Nature*, **241**, 95 (1973).
2 Juliusson, E., Meyer, P., and Muller, D., *Phys. Rev. Lett.*, **29**, 445 (1972).
3 Webber, W. R., Lezniak, J. A., Kish, J. C., and Damle, S. V., *Nature*, **241**, 96 (1973).
4 Smith, L. H., Buffington, A., Smoot, G. F., Alvarez, L. W., and Wahlig, W. A., *Astrophys. J.*, **180**, 987 (1973).

[5] Atallah, K., Cleghorn, T. F., Modlinger, A., and Schmidt, W. K. H., *13th int. Cosmic Ray Conf. Paps*, paper 675 (1973).
[6] Golden, R. L., Adams, J. H., Marar, T. M. K., Boykin, W. R., Marbach, J. R., Neumann, W. R., Deney, C. L., Hintz, R. E., Heckman, H. H., and Lindstrom, P. J., *Nucl. Inst. Meth.* (in the press).
[7] Anand, K. C., Daniel, R. R., Stephens, S. A., Bhowmik, R., Krishna, K. C., Aditya, P. K., and Puri, R. K., *Proc. Ind. Acad. Sci.*, **67**, 138 (1969).

Isotropy of the arrival directions of ultra high energy cosmic rays

THERE has been little success in the search for anisotropies in the arrival directions of ultra high energy cosmic rays. Data on 50 events of very high energy, having an arrival direction which is 'not obviously isotropic', have however, been collected by the Sydney extensive air shower group[1]. The data were recorded over a period of 5 yr and, as the world total of such events is likely to increase only rather slowly, this seems to be the appropriate time to combine these data with comparable data from the Northern Hemisphere arrays operated at Cornell (United States), Haverah Park (United Kingdom) and Volcano Ranch (United States). The arrival directions of 87 showers with primary energies above a few times 10^{19} eV are discussed here. It is shown that there are no large deviations from an isotropic distribution and it is concluded that the ultra high energy cosmic rays are most likely to come predominantly from extra-Galactic sources.

Table 1 Comparison of observed and predicted number of equi-event areas

No. of events per square	Observed no. of squares assuming Poissonian distribution	Expected no. of squares assuming Poissonian distribution
0	285	284.8
1	67	66.5
2	7	7.76
3	1	0.6
4	0	0.04

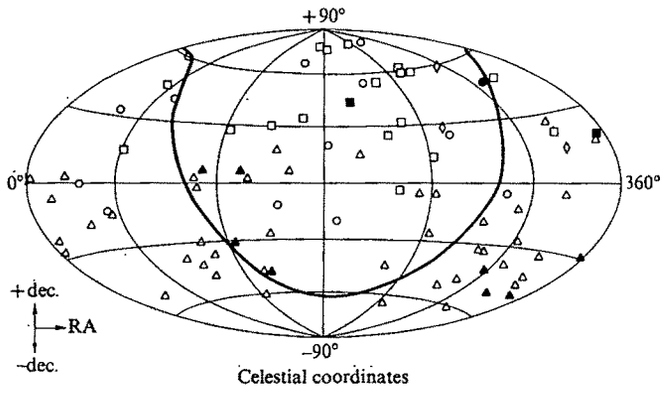


Fig. 1 Aitoff equal area projection of 87 events[1,3-6] in celestial coordinates. Solid line, Galactic plane; triangles, Sydney observations (50); circles, Volcano Ranch observations (14); squares, Haverah Park observations (20); diamonds, Cornell observations (3). Events which may have primary energies above 10^{20} eV have the appropriate symbol filled. Energy assignment has been made using a model of shower development described by Hillas (see ref. 4 for details).

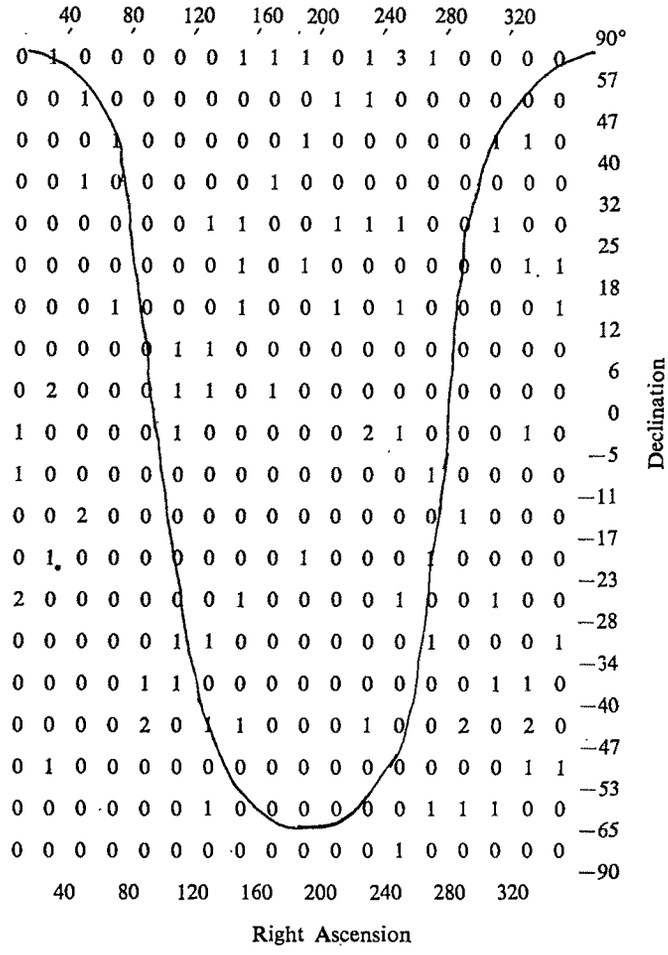


Fig. 2 Equi-event plot in celestial coordinates of 84 events[1,4-6]. Areas of 20° RA × $(\delta_i - \delta_j)$° dec. are expected to contain equal numbers. The values of δ_i, δ_j are listed at the right hand side of the figure and are deduced from the declination distributions[1,5,6] by assuming that the exposure time of each experiment is proportional to the number of events recorded. The solid line represents the Galactic plane.

The events discussed by the Sydney group are all of higher primary energy than a threshold level which is claimed to be 1.5×10^{19} eV. The assignment of energy is subject to the energy conversion which is used to relate the measured number of muons in their showers to primary energy. Less ambiguity in the threshold of selection of data from different arrays is therefore introduced if only those events are included which were recorded at an intensity less than a certain value. Using the energy spectrum given by the Sydney group[2] their threshold intensity is found to be 4.62×10^{-15} m^{-2} s^{-1} sr^{-1}. Events from the Cornell, Haverah Park and Volcano Ranch data banks were therefore selected only if they were recorded at intensities less than this value. Of the additional 37 events recorded by the Northern Hemisphere experiments, three were recorded at Cornell (refs 3, 4 and J. Delvaille, personal communication), 20 were recorded at Haverah Park[5,6] and 14 at Volcano Ranch. The directional uncertainty varies slightly between experiments but is believed to be better than 3×10^{-3} sr for all 87 events.

All 87 events are shown on an Aitoff equal-area projection in celestial coordinates (Fig. 1). The Galactic plane is also indicated. It is clear that there is no evidence of significant clustering of events. In particular the region close to the Galactic plane shows no indication of the excess which could be

expected if the sources of high energy cosmic rays were objects (for example, pulsars) which are strongly clustered on the Galactic plane. Statistical support can be given to the conclusion that the data are consistent with isotropy if a rectangular right ascension (RA)–declination (dec.) plot is divided into areas within which the number of observed events would be expected to be equal. For the Volcano Ranch, Haverah Park and Sydney arrays sufficient data have been published[1,6,7] at low energies (10^{17}–10^{18} eV), where the evidence of isotropy is very strong, to enable such an 'equi-event' diagram to be constructed, and Fig. 2 shows 360 'equi-event' areas. The mean number of events expected per area is 0.234 and the number of areas with 0, 1, 2, 3. . . events is compared with the predicted number in Table 1 assuming that the arrival directions are randomly distributed. The data are clearly consistent with isotropy of arrival directions and contain no evidence for the existence of point sources of ultra high energy cosmic rays.

It seems likely that primary cosmic rays in the energy region in question ($E_p \geq 2 \times 10^{19}$ eV) have atomic masses $1 \leq A \leq 56$, but no experimental data are available to enable this range to be narrowed. Karakula *et al.*[8] have estimated the extended anisotropies which would be likely to arise in various Galactic magnetic field models if the cosmic ray sources were concentrated in the disk or the spiral arms of the Galaxy, and if the primary particles were protons. Comparing the data of Fig. 1 with the distributions predicted by these authors for protons of 10^{19} eV and several reasonable Galactic field models, we conclude that if the primaries are protons then ultra high energy cosmic rays are predominantly of extra-Galactic origin.

If the primary particles at these energies are iron nuclei then the situation is not quite so clear cut. For a model in which the Galactic field is longitudinal but has opposite orientations above and below the Galactic plane, a strong peak is predicted[9] in the direction of the inward spiral arm (RA = 308°, dec. = 35°) for iron nuclei of momentum $> 2.10^{19}$ eV/c, which is clearly in contradiction with the observations. For a simple longitudinal field, which seems to be favoured at the present time[10], some extra-Galactic contribution (probably greater than 30%) seems to be necessary. We therefore conclude that even if the primary particles are iron nuclei a significant fraction of them (> 30%) come from extra-Galactic sources.

The data discussed in this paper include about 11 events with a primary energy of more than 10^{20} eV (Fig. 1) but it is important to note that the absence of a cutoff in the energy spectrum[1,5,6] is not evidence in favour of a Galactic origin for them. Stecker[11] has pointed out that the absence of a cutoff implies only that the lifetimes of these cosmic rays are significantly less than the age of the Universe, and consequently that sources in the local 'super-cluster' of galaxies must be considered possible.

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[1] Bell, C. J., *et al.*, *Proc. thirteenth International Conference on Cosmic Rays, Denver*, **4**, 2525–2528 (1973).
[2] Bell, J. C., *et al.*, *J. Phys.* A (in the press).
[3] Delvaille, J., Kenziorski, F. and Greisen, K., *J. phys. Soc. Japan*, **17**, Suppl. A-III, 76–83 (1962).
[4] Simopoulou, E. P., thesis, Cornell Univ. (1962).
[5] Edge, D. M., *et al.*, *J. Phys.* **A6**, 1612–34 (1973).
[6] Linsley, J., *Proc. eighth International Conference on Cosmic Rays, Jaipur*, **4**, 77–99 (1963).
[7] Hollows, J. D., thesis, Univ. Leeds (1968).
[8] Karakula, S., Osborne, J. L., Roberts, E., and Tkaczyk, W., *J. Phys.* **A5**, 904–915 (1972).
[9] Osborne, J. L., Roberts, E., and Wolfendale, A. W., *J. Phys.* A. 421–433 (1973).
[10] Vallée, J. P., and Kroneberg, P. P., *Nature phys. Sci.*, **246**, 49–51 (1973).
[11] Stecker, F. W., *Phys. Rev. Lett.*, **21**, 1016–1018 (1968).

NO and O_3 measurements in the lower stratosphere from a U-2 aircraft.

AIRBORNE nitric oxide and ozone detectors have been carried on aircraft flights over the west coast of the United States to obtain *in situ* measurements of nitric oxide and ozone at altitudes up to 21 km. Results from two of these flights—December 6, 1973 from 1100 to 1430 local time (1900 – 2230 UT) and December 18. 1973 from 1030 to 1430 local time (1830–2230 UT)—are presented here. The experimental platform was one of two U-2 aircraft used in the NASA Earth Resources Aircraft Project at Ames Research Center, Moffett Field, California.

The nitric oxide instrument is a chemiluminescent detector based on the luminescent reaction of nitric oxide with excess ozone in a flow reactor. The photon count rate signals from the detector are proportional to the atmospheric nitric oxide mixing ratio and to the STP flow rate of the atmospheric sample. Two different calibrator flow rates from an on-board NO/N_2 calibrator bottle provide periodic instrument sensitivity checks. The zero level was periodically measured by mixing the excess ozone with the atmospheric sample upstream of the reactor. Signals were derived from two linear count rate circuits, each having a time constant of about 1 s. An automatic cycle timer sequenced the instrument through the measure, calibrate and zero level modes for 3 min each. The sensitivity of the nitric oxide detector for the measurements reported here is 0.025 parts in 10^9 by volume.

Ozone concentration measurements are based on the absorption of the Hg line at 2,537 Å in the Hartley continuum. The unattenuated mercury lamp intensity at 2,537 Å was determined by routing the atmospheric sample flow over a catalytic reactor which removes all the ozone present. The instrument operates on a cycle of 10 s, 5 s each for the absorbed and unattenuated beam measurements. Beer's law was used to extract the ozone partial pressure using an optical absorption coefficient of 306 cm^{-1} atm^{-1}; the absorption tube is 71 cm long. Pressure and temperature corrections were applied to the instrument readout to derive the partial pressure of atmospheric ozone. The instrument is a commercial unit that was especially adapted for use in the U-2 experimental system. The rated sensitivity is 3 nbar partial pressure of ozone.

A ram inlet port of diameter 1 inch brings the air sample flow into the aircraft. Flexible stainless steel lines connect the instrumentation mainfold to the inlet and outlet ports. Typical in-flight air flow rates are 30 STP min^{-1} to the nitric oxide detector and 0.3 STP min^{-1} to the ozone detector. Motorised valves in the manifold isolate the instruments from the atmosphere and from each other up to an altitude of 12 km, at which point the valves are opened by the pilot and measurements commence. Thereafter the system operation is fully automatic. The environment in the experimental bay of the aircraft during data gathering was maintained at approximately 45° F and 4 pound $inch^{-2}$ absolute. The true air speed of the aircraft was 400 knots.

Data from the two instruments were recorded on magnetic tape once a second and the outputs of various pressure, temperature and flow sensors were recorded every 10 s. Graphic

displays of the raw nitric oxide and ozone data were prepared from the magnetic tape. Analysis of the data was carried out from the graphic outputs.

Figure 1 shows results obtained on the flights of December 6 and December 18, 1973 at longitude 122°W and latitudes as indicated. Conversion to nitric oxide number densities was done using the measured sample air flow, calibrator flow and calibrator nitric oxide mixing ratio. The nitric oxide number densities given in the legend to Fig. 1 are average values observed at 21 km over the entire flight segment at that altitude for each date. The average nitric oxide count rates are typically 600, 750, and 1,200 counts s^{-1} over the 3-min zero, measure and calibrate

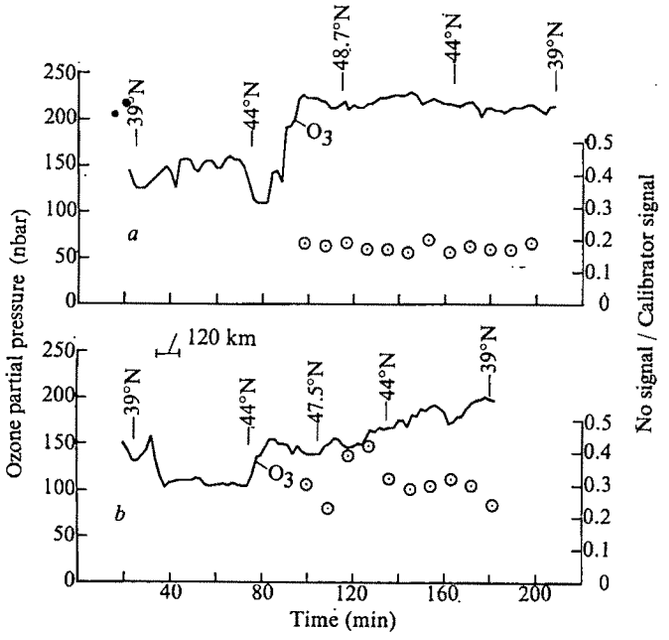


Fig. 1 Measurements *in situ* of nitric oxide and ozone from a U-2 aircraft at 18 and 21 km altitude at longitude 122° W. *a*, December 18, 1973, $[NO]_{ave} = 1.3 \pm 0.6 \times 10^8$ cm^{-3}; *b*, December 6, 1973, $[NO]_{ave} = 2.2 \pm 1.1 \times 10^8$ cm^{-3}. Lower part of each curve (left) corresponds to 18 km altitude, upper part (right) to 21 km.

cycles, respectively. The statistical errors, due to the photon count rates, in the nitric oxide data points of Fig. 1 amount to only about ± 6%. The larger part of the error limits given for the nitric oxide number densities are from estimated uncertainties of ± 5% in the sample and ± 20% in the calibrator flow rates and ± 20% in the calibrator mixing ratio.

The measured loss of nitric oxide in the inlet flow lines is less than 5%; no correction has been made for this. We also measured the loss of ozone in the inlet lines in the laboratory as a function of time from the start of flow of an ozone sample through the lines. The loss is 33% of the input ozone at 30 min decreasing linearly with time to 16% at 210 min. After the appropriate correction for the measured ozone line loss, the estimated uncertainty in the ozone data is ± 20%, of which ± 15% is due to systematic errors.

Data taken at 18 km for nitric oxide are not shown. A system conditioning, indicated by changing calibrator levels, occurred during the first hour of flight. After 1 h the two calibrator levels and their ratio stabilised at values close to those observed in the laboratory before and after the flight. Modifications are in progress to correct this difficulty.

Our measurements of nitric oxide concentrations are in agreement with another chemiluminescent measurement made in the same altitude range[1]. But our measured concentrations are lower, by a factor between three and ten, than recently reported optical measurements[2,3].

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1 Ridley, B. A., Schiff, H. I., Shaw, A. W., Bates, L., Howlett, C., LeVaux, H., Megill, L. R., and Ashenfelter, T. E., *Nature*, **245**, 310 (1973).
2 Ackerman, M., Frimout, D., Muller, C., Nevejans, D., Fontanella, J.-C., Girard, A., and Louisnard, N., *Nature*, **245**, 205 (1973).
3 Toth, R. A., Farmer, C. B., Schindler, R. A., Raper, O. F., and Schaper, P. W., *Nature*, **244**, 7 (1973).

Temperature, fossil CO_2 accumulation and carbonate ion concentration of the oceanic mixed layer

Several authors[1-4] have recently suggested that there will be a dramatic accumulation of fossil CO_2 in the oceanic mixed layer by the end of this century. If these calculations are correct the predicted increases in the atmospheric partial pressure of carbon dioxide (p_{cd}) and in the total concentration of carbon dioxide species in the mixed layer ($C\tau$) do not imply catastrophic decreases in the carbonate ion concentration[1,3] at 25° C (ref. 5). Two important points remain unresolved, however, and they require further clarification.

The first point concerns the relative merits of the various mixing models. The earlier calculations[5] raise doubts about the validity of the more pessimistic mixing models[1-4] because they indicate that a system reasonably close to equilibrium can only accommodate the predicted $C\tau$ and p_{cd} values if the carbonate alkalinity ($CA = (HCO_3^-) + 2(CO_3^{2-})$) is allowed to increase. This implies either that the reaction

$$CO_2 . H_2O + CO_3^{2-} \rightleftharpoons 2HCO_3^- \quad (1)$$

is not the only process involved in the assimilation of fossil CO_2 in the mixed layer, or that the mixed layer itself is grossly out of equilibrium with the atmosphere. Neither conclusion is consistent with the assumptions of the mixing models[1-3].

A more realistic approach is to explicitly confine the assimilation process to reaction (1) so that CA remains constant and to assume a reasonable measure of equilibrium between the atmosphere and the mixed layer. The concentrations of the carbonate and bicarbonate ions in the mixed layer can then be calculated from the equations derived by Park[6]:

$$c_1 = (CO_2.H_2O) = \alpha p_{cd} \quad (2)$$

$$c_2 = (HCO_3^-) = (X - K_3' c_1)/4 \quad (3)$$

$$c_3 = (CO_3^{2-}) = (4CA + K_3' c_1 - X)/8 \quad (4)$$

$$\text{where } X = ((8CA + K_3' c_1) K_3' c_1)^{1/2} \quad (5)$$

and K_3' is the equilibrium constant for reaction (1). The values of p_{cd} predicted by various workers are in good agreement with one another[3] as they must take due account of the increases observed to the present day. Calculations based on these equations (Skirrow and Whitfield, in preparation) confirm that those models[1-4] which suggest that 25% of the injected fossil CO_2 will reside in the mixed layer, represent systems grossly out of equilibirum with the atmosphere. The model of Broecker

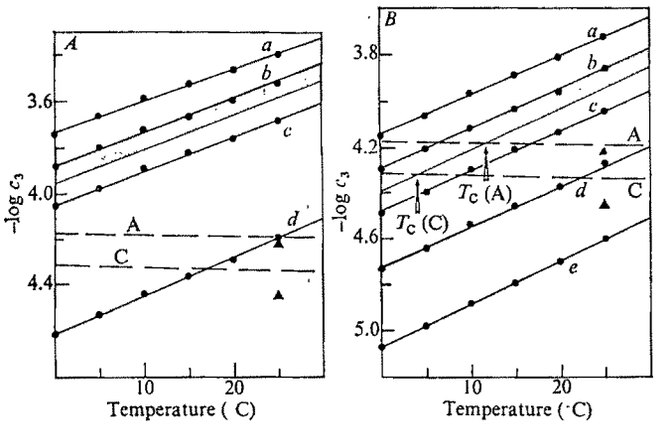


Fig. 1*A*, Variation of carbonate ion concentrations with temperature in 1974 when $p_{cd} = 330 \times 10^{-6}$ atm. Solid lines, seawater values calculated from equations (2) and (4) with the carbonate alkalinities (meq l^{-1}): *a*, 3.0; *b*, 2.5; *c*, 2.0; *d*, 1.0. Shaded area, typical oceanic range. Dashed lines, the carbonate ion concentrations in equilibrium with aragonite (A) and calcite (C) calculated from equations (8) and (7) respectively. ▲, Carbonate ion concentration in equilibrium with aragonite[7] (upper point) and with calcite[7] (lower point). *B*, Variation of carbonate ion concentrations with temperature in 2050 when $p_{cd} = 996 \times 10^{-6}$ atm (ref. 3) with carbonate alkalinities (*CA* meq l^{-1};*a*, 3.0; *b*, 2.5; *c*, 2.0; *d*, 1.5, *e*, 1.0. T_c is the critical temperature at which the carbonate ion concentration of the sea water equals that in equilibrium with aragonite, T_c (A); or calcite, T_c (C); at the saturation point. The values indicated are for $CA = 2.25$ meq l^{-1}, the lowest value normally encountered in the oceans. Other features are as defined for Fig. 1A.

et al.[7], on the other hand, gives an accurate picture of the surface waters in equilibrium with the atmosphere, and predicts that only about 5% of the accumulating fossil CO_2 remains in the mixed layer. Since this layer, by definition, equilibrates fairly rapidly with the atmosphere the model defined by equations (2) to (5) should give the more realistic picture of the effect of increases in atmospheric CO_2 on the chemistry of the mixed layer. This model will be used to discuss the second point: the influence of temperature and carbonate alkalinity on the concentration of carbonate ions in ocean surface waters.

So far, calculations concerned with the dissolution of calcium carbonate have either made no specific mention of the ambient temperature[1-3] or they have been confined to 25° C, a temperature typical of tropical surface waters[6,7]. This bias has arisen primarily because higher latitude waters only represent a relatively small proportion of the Earth's ocean surface. In addition the prospect of calcium carbonate dissolution naturally focuses immediate attention on the fate of the tropical coral reefs. The solubilities of both carbon dioxide and of calcium carbonate in seawater increase, however, as the temperature falls, so that temperate waters will be more sensitive to increases in the value of p_{cd}.

Since the salinity effect is small over the range typically encountered in the open oceans (30–35‰) the values of α given by Murray and Riley[8] at a chlorinity of 19‰ (approximately 35‰ salinity) can be used to calculate c_1 from equation (2) for the appropriate temperature and p_{cd}. This value can then be used in equation (4), together with the appropriate value of K_3' calculated from the data of Lyman[9], to give the carbonate ion concentration at any particular temperature and carbonate alkalinity. In previous calculations[6] (Skirrow and Whitfield, in preparation) the carbonate alkalinity has ranged from 2.2–2.9 meq l^{-1} although the span 2.2–2.5 meq l^{-1} is more typical of the open ocean[10].

The carbonate ion concentration has been calculated from equations (2) and (4) as a function of temperature and carbonate alkalinity for the present day (Fig. 1*A*), and for the year 2050 (Fig. 1*B*) when, according to Zimen and Altenhein, p_{cd} will attain its maximum value.

The concentration of carbonate ions in equilibrium with calcite or aragonite at the saturation point can be calculated from the relationship

$$(CO_3^{2-}) = K_{sp}'/(Ca^{2+}) \quad (6)$$

where K_{sp}' is the apparent solubility product of the relevant carbonate mineral in sea water. According to Ingle *et al.*[11]

$$K_{sp}'\text{ (Calcite)} = (1.390 - 3.096 \times 10^{-3}\, T^\circ\text{C}) \times S \times 10^{-8} \quad (7)$$

As the solubility product of aragonite is pure water is approximately 0.14 logarithmic units higher than that of calcite[12] the corresponding equation for aragonite would be

$$K_{sp}'\text{ (Aragonite)} = (1.919 - 3.096 \times 10^{-3}\, T^\circ\text{C}) \times S \times 10^{-8} \quad (8)$$

Carbonate ion concentrations at saturation, derived from these equations assuming $(Ca^{2+}) = 10$ mmol l^{-1}, are shown in Fig. 1*A* and *B*. Seawaters with carbonate ion concentrations lying above these lines will remain supersaturated with respect to the appropriate mineral, whereas those lying below the line will approach saturation. Although the calculations confirm earlier statements[5] that tropical waters will remain supersaturated throughout, they do indicate that by the year 2050 temperate waters with a mean annual temperature of 10° C (in general[13] those north of latitude 50° N and south of latitude 50°S) will have reached the saturation point for aragonite (Fig. 1*B*). The concentration of carbonate ions will not fall below this level, because the dissolution of carbonate minerals will absorb any additional carbon dioxide[7]:

$$CaCO_3(S) + CO_2\,H_2O \rightleftharpoons Ca^{2+} + 2HCO_3^- \quad (9)$$

This reaction increases both the carbonate alkalinity and the calcium ion concentration and so the system is effectively buffered. These parameters would also increase as the result of enhanced weathering of terrestrial rocks which should become noticeable as the value of p_{cd} rises in the atmosphere.

Using the data of Zimen and Altenhein[3] the calculations used in constructing Fig. 1*B* can be repeated and the critical saturation temperature (T_c, Fig. 1*B*) can be calculated at the end of each decade for seawaters with a particular carbonate alkalinity. All waters with a mean annual temperature less than T_c are likely to approach the saturation level for the mineral specified. The resulting curves (Fig. 2) indicate that, for $CA = 2.25$ meq l^{-1}, waters with mean annual temperatures greater than 5° C are likely to remain supersaturated with respect to calcite and those with temperatures greater than 12° C will remain super-

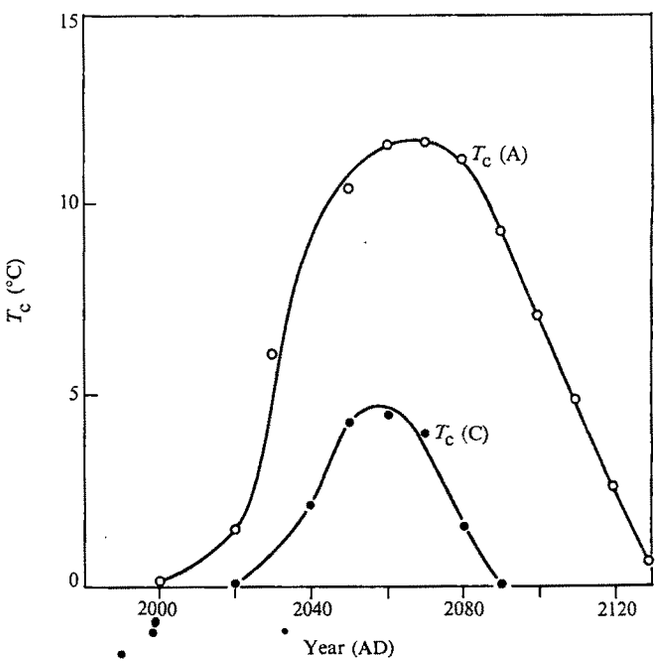


Fig. 2 The variation of the critical saturation temperature for aragonite, T_c (A); and calcite, T_c (C); over the next one and a half centuries calculated from predicted variations in p_{cd} (ref. 3) for seawater with a carbonate alkalinity of 2.25 meq l^{-1}.

saturated with respect to aragonite. The effect is transient, with the time at saturation point increasing as the temperature falls. The detailed picture is of course critically dependent on the solubilities of the various carbonate minerals. Higher[14] and lower[7] values than those given by equations (7) and (8) have been reported for the solubility of calcite and aragonite. A full analysis will become possible only when we have a clearer idea of the values of these parameters at low temperatures.

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1 Fairhall, A. W., *Nature*, **245**, 20–23 (1973).
2 Zimen, K. E., and Altenhein, F. K., *Naturwissenschaften*, **4**, 198–199 (1973).
3 Zimen, K. E., and Altenhein, F. K., *Z. Naturf.* (in the press).
4 Machta, L., *The Changing Chemistry of the Oceans* (edit. by Dyrssen, D., and Jagner, D.), 121–145 (Almqvist and Wiksell, Stockholm, 1972).
5 Whitfield, M., *Nature*, **247**, 523-525 (1974).
6 Park, P. K., *Limnol. Oceanogr.*, **14**, 179–186 (1969).
7 Broecker, W. S., Li, Y-H, and Peng, T-H., *Impingement of Man on the Oceans* (edit. by Hood, D. W.), chapter 11 (Wiley, New York, 1971).
8 Murray, C. N., and Riley, J. P., *Deep Sea Res.*, **18**, 533–541 (1971).
9 Lyman, J., thesis, Univ. California (1957).
10 Riley, J. P., and Chester, R., *Introduction to Marine Chemistry* (Academic Press, London, 1971).
11 Ingle, S. E., Culberson, C. H., Hawley, J. E., and Pytkowicz, R. M., *Mar. Chem.*, **1**, 295–307 (1973).
12 Sillén, L. G., and Martell, A. E., *Stability Constants of Metal-ion Complexes* (Chemical Society, London, 1964).
13 Sverdrup, H. U., Johnson, M. W., and Fleming, A. H., *The Oceans* (Prentice-Hall, New York, 1942).
14 Edmond, J. M., and Gieskes, J. M. T. M., *Geochim. cosmochim. Acta*, **34**, 1261–1291 (1970).

Hot spot and trench volcano separations

WE suggest that the distribution of separations between trench volcanoes located along subduction zones reflects the depth of partial melting, and that the separation distribution for hot spot volcanoes near spreading centres provides a measure of the depth of mantle convection cells.

It has been argued that frictional heating along slip zones between subducting and overriding plates provides the heat for partial melting[1,2]. At hot spots the suggested sources of heat are more controversial, ranging from shear heating in the asthenosphere[3] to more vague sources associated with deep thermal plumes originating at the core–mantle interface[4]. The depth of partial melting under volcanoes along slip zones is 100 ± 20 km, estimated[5] from considerations of the distance of volcano chains behind trenches and the seismically determined dip angles of the subducting plate.

There is a break in the distribution of separations between trench volcanoes at separations of $\sim$ 100 km, and we suggest that this indicates the depth of partial melting beneath these volcanoes. For each volcano along the various trenches we have calculated the separation, S, from its nearest neighbours[6–10]. The cumulative distribution of the number of volcanoes with nearest neighbours within a distance S is plotted against separation (Fig. 1) for volcanoes along major trenches and for the sum of these volcanoes. These distributions all rise rather uniformly up to separations of about 100 km and then flatten, indicating that there are relatively few trench volcanoes with nearest neighbours more than 100 km away.

Assuming that the region of partial melting is a belt along the slip zone at a depth of $\sim$ 100 km and that all along this belt the magma migrates upwards along random paths to the surface to form volcanoes, it is reasonable to suggest that the separation between the volcanoes would not generally exceed the depth of the magma source.

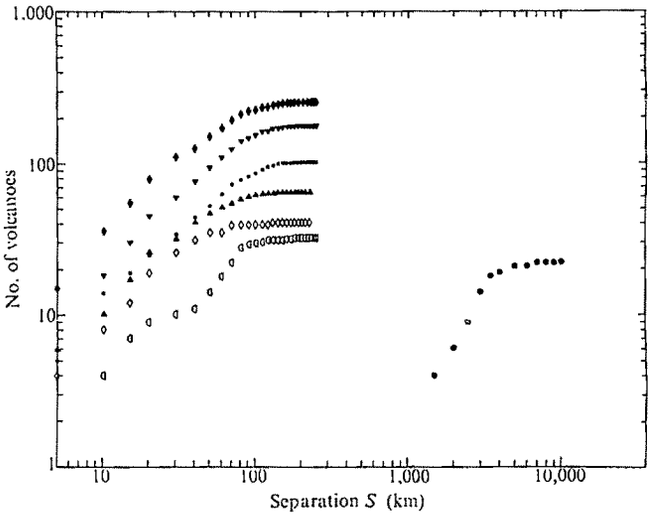


Fig. 1 The cumulative distribution of separation distances for trench and hot spot volcanoes, that is, the number of volcanoes separated from their nearest neighbours by less than S. Solid diamonds and solid inverted triangles are the sums of all trench distributions (from the International Association of Volcanology and ref. 10, respectively); solid circles, Indonesia[8]; solid upright triangles, Kamchatka-Kuriles[6,9]; open diamonds, central America[10]; open semicircles, Aleutians[10]; solid circles, hot spots[4].

Considering hot spot volcanoes located essentially along the oceanic ridge system[4,11], we have found that the distribution of separations between each hot spot volcano and its nearest neighbour shows a similar break or flattening (Fig. 1), but at a separation of $\sim$ 3,000 km, indicating that most hot spots have nearest neighbours within 3,000 km. This distance is the same as the depth to the core–mantle boundary, where deep thermal plumes may originate[4].

We propose that the lateral dimensions of mantle convection cells are also represented by the hot spot separations, rather than by ridge–trench distances[12,13], and that the break in the distribution of hot spot separations at 3,000 km is evidence for both whole mantle convection and a deep thermal plume origin of hot spots. We do not suggest that the magma extruded from hot spot volcanoes comes from the core–mantle boundary. The magma source is at the top of the convective plume just under the oceanic plate, and is consistent with a break at < 100 km in the distribution of separation distances[16] between the volcanoes which make up the Hawaiian chain of islands and guyots.

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1 Oxburgh, E. R., and Turcotte, D. L., *Nature*, **216**, 1041 (1968).
2 McKenzie, D. P., and Sclater, J. G., *J. geophys. Res.*, **73**, 3173 (1968).
3 Shaw, H. R., and Jackson, E. D., *J. geophys. Res.* (in the press).
4 Morgan, W. J., *Bull. Am. Ass. Petrol. Geol.*, **56**, 203 (1972).
5 Turcotte, D. L., and Schubert, G., *J. geophys. Res.*, **78**, 5876 (1973).
6 Gorshkov, G. S., *Catalogue of the Active Volcanoes of the World*, Part VII (1958).
7 Mooser, G., Meyer-Abich, H., and McBirney, A. R., *Catalogue of the Active Volcanoes of the World*, Part VI (1958).

[8] Neumann van Padang, M., *Catalogue of the Active Volcanoes of the World*, Part I (1951).
[9] Vlodavetz, V. L., and Piip, B. L., *Catalogue of the Active Volcanoes of the World*, Part VIII (1959).
[10] MacDonald, G. A., *Volcanoes*, 430–450 (Prentice-Hall, Englewood Cliffs, 1972).
[11] Wilson, J. T., *Phil. Trans. R. Soc.*, **A258**, 145 (1965).
[12] Tozer, D. C., in *The Earth's Mantle* (edit. by Gaskell, T. F.), 325–353 (Academic Press, New York, 1967).
[13] Knopoff, L., in *The Earth's Mantle* (edit. by Gaskell, T. F.), 171–196 (Academic Press, New York, 1967).
[14] Eaton, J. P., and Murata, K. H., *Science*, **132**, 925 (1960).
[15] Jackson, E. D., and Wright, T. L., *J. Petrology*, **11**, 405 (1970).
[16] Chase, T. E., Menard, H. W., and Mammerickx, J., *Bathymetry of the North Pacific*, (Scripps Institution of Oceanography, La Jolla, California, 1970).

Massive sulphides and plate tectonics

SEVERAL authors have considered the genesis of various ores in terms of plate tectonic theory[1–6]. Most have discussed various possibilities for the origin of the ore solutions of massive sulphides and, with the exception of Guild[6], have stated or implied a fairly close genetic relationship between ore deposition and the processes of seafloor spreading and subduction. The nature of this relationship is, however, by no means clear and I suggest here that it may be very tenuous.

The deposits included in the term 'massive sulphide' are stratiform and wholly or largely composed of sulphides of iron with varying proportions of the sulphides of copper, lead and zinc, and minor gold and silver. Stratiform, massive oxide deposits (largely magnetite and/or haematite) are related ore types. The term includes 'volcanogenic massive sulphides'[7], but as used here it also covers very similar ores in sedimentary or sedimentary/volcanic successions (for example, Sullivan, Rammelsberg, Cobar, and Mount Isa).

Many geologists have discussed the possibility that massive sulphide deposits may be derived from heated seawater, or from meteoric water that has become an ore solution because of a hot water–rock interaction below the surface[8–11]. The principal requirements of this model are: a thermal anomaly sustained for sufficient time to precipitate an ore deposit; a system allowing long term downward penetration of water (probably to depths of several kilometres) and then upwards to the surface through major fractures; the presence of a suitable depositional site, which is commonly on the sea floor[7,12].

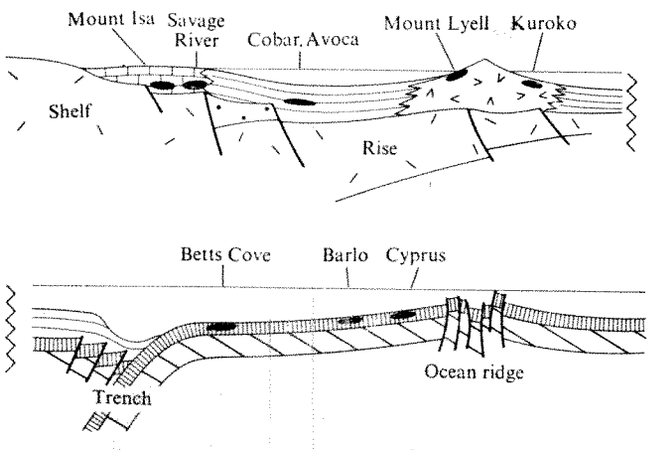


Fig. 1 Diagrammatic cross section of a continental margin to illustrate the range of tectonic environments of massive sulphides.

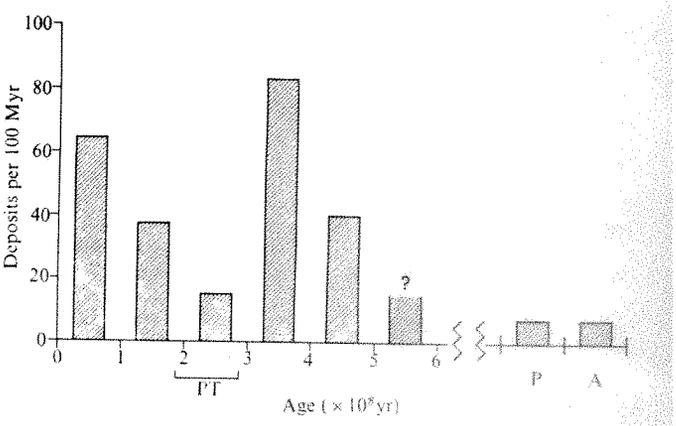


Fig. 2 Variation in the number of massive sulphide deposits per 100 Myr throughout the geological column. The Proterozoic (P) and Archaean (A) are each assumed to be 1,500 Myr long. PT, Permo-Triassic.

This geothermal model seems to account for the salient features of massive sulphides.

The principal host rocks to massive sulphides are volcanics of varying composition (mainly calc-alkaline), but some deposits occur in thick sediments (mainly shales and greywackes) with little or no evidence of volcanism (for example, Meggen,

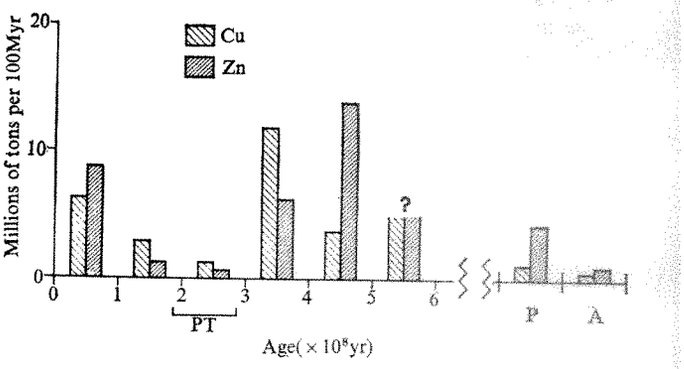


Fig. 3 Variation in the tonnages per 100 Myr of copper and zinc in massive sulphides through the geological column. The tonnages include production and reserves and are very approximate. A, Archaean; P, Proterozoic; PT, Permo-Triassic.

Rammelsberg, Cobar). There seems to be no evidence to support the statement[13] that massive sulphides are associated with mainly mafic volcanics. Deposits in volcanic successions do not show any correlation between the ore type (in terms of the Cu:Pb:Zn ratios) and the nature of the host rocks[14]. This seems to be true for all massive sulphides, except that where the host rocks are dominantly or entirely basaltic (as in Cyprus) the lead content is very low. If ore solutions derive their metal content through water–rock interactions several kilometres below the site of deposition, then correlations involving rock walls will be of little value. But a comparison between ore compositions and the nature of the rock successions for several kilometres below the deposits also showed no correlation, although the quality of the available data was poor. Copper and zinc are relatively abundant in most igneous and sedimentary rocks and the low degree of correlation between rock type and ore type is reflected in the geothermal model. Wide variations in the Cu:Zn ratio may be brought about by chemical factors during transport and at the site of deposition. Also predictable is the absence of lead ores in thick sequences of basic rocks, as these contain only a few parts per million (p.p.m.) of that metal. Another important feature of the host rocks is the lack of coeval plutons.

Massive sulphides tend to occur at distinct stratigraphic

horizons[7,14] which may be unconformities (Mount Lyell), sediment lenses within volcanics (Rosebery, Captains Flat), tops of volcanic sequences (Rio Tinto, Cyprus), or at changes in the composition of volcanics (Noranda area) or the nature of the volcanism (dacite domes of northern Honshu). Thus, the timing of ore deposition does not, apparently, relate to any clearly recognisable, unique event but to periods of hydrothermal activity brought about by various agencies such as faulting, pauses in volcanism, and so on.

Phanerozoic massive sulphides occur in a wide variety of tectonic environments[15], particularly in environments associated with orogenic belts (Fig. 1). Most occur in calc-alkaline island arcs and Andean-type volcanic belts (Mount Morgan, Rosebery, Mount Lyell, Rio Tinto, Hopa-Murgul, Shakanai), but some occur in the flysch and shelf sequences of the continental margin (Cobar, Avoca?), or in oceanic crust in ridge and ocean floor environments (Betts Cove, Skouriotissa, Besshi?). In the Palaeozoic of south-eastern Australia most massive sulphides occur in Andean-type volcanics, and formed fairly late in the development of the volcanic belts during the 'paratectonic' phases of the main tectonic cycles[16], when the volcanics were undergoing faulting, folding and granitic intrusion. Massive sulphides commonly occur in groups aligned along major features that provide deep seated plumbing systems.

Sulphide–sulphur in massive sulphides is enriched in ^{34}S relative to the meteoritic standard and generally shows only small variations within deposits. The values for some deposits may reflect mixing and re-equilibration between sulphur in magmatic water rising from depth and sulphate in seawater[17,18]. The same results can be achieved by mixing sulphur derived from the leaching of rocks with sulphate-bearing seawater in geothermal conditions.

Unlike several ore-types associated, and apparently genetically linked, with plutonic rocks (for example, tin–wolfram veins, nickel sulphide deposits and porphyry coppers), massive sulphides are well represented throughout the geological column (Fig. 2). Figure 2 is not very reliable because there are little adequate data from many areas, particularly from the Cambrian. The main features include the high number of deposits in the Carboniferous and Devonian, and the low number in the Permo-Triassic and the Archaean. The Carboniferous high reflects the large number of deposits in the Iberian pyrite belt, and the low value for the Precambrian (and the Cambrian?) may well be a function of the low preservation rate of these rocks[19]. The plot of tonnages (production plus reserves) of zinc and copper every 100 Myr (Fig. 3), though based on limited data, seems to confirm the Archaean and Permo-Triassic lows, and indicates the existence of several very large Proterozoic deposits (Sullivan, McArthur River, Mount Isa).

The principal features of massive sulphides do not conflict with, and are to some extent predicted by, the geothermal model outlined. In particular the model accounts for the lack of related plutons, the poor correlation between host rock and ore type, and the wide range of tectonic environments in which the deposits occur. The grouping along faults and the S-isotope data and overall chemical composition, are also compatible with the model.

If massive sulphides are essentially of superficial origin, with the entire ore forming process extending no more than a few km below the surface, it is difficult to see why there should be any close relationship with plate tectonic processes and related magmatism. The tendency for ore to be concentrated in marine volcanic zones in orogenic belts may simply be because such areas are the most favourable for developing convective hydrothermal systems and providing plumbing systems and depositional sites. The Permo-Triassic low in the distribution graphs may reflect the relatively low output of volcanic rocks at this time[20], and the large size of the few Proterozoic deposits may be a function of relatively stable conditions in large basins. Though the model seems to account for most of the data it does not necessarily explain the genesis of every deposit. The model needs to be tested on individual cases, possibly using isotope tracers[21].

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1 Sawkins, F. J., *J. Geol.*, **80**, 377 (1972).
2 Sillitoe, R. H., *Trans. Instn Min. Metall.*, **B81**, 141 (1972).
3 Mitchell, A. H. G., *Nature phys. Sci.*, **245**, 49 (1973).
4 Mitchell, A. H. G., and Garson, M. S., *Trans. Instn Min. Metall.*, **B81**, 10 (1972).
5 Tarling, D. H., *Nature*, **243**, 193 (1973).
6 Guild, P. W., *Proc. twenty-fourth int. geol. Congr.*, **4**, 17 (1972).
7 Sangster, D. F., *Can. geol. Surv.*, Paper 72-22 (1972).
8 Craig, H., in *Hot Brines and Recent Heavy Metal Deposits in the Red Sea* (edit. by Degens, E. T. and Ross, D. A.), (Springer-Verlag, Berlin, 1969).
9 Ellis, A. J., in *Geochemistry of Hydrothermal Ore Deposits* (edit. by Barnes, H. L.), (Holt, Rinehart and Winston, 1967).
10 Corliss, J. B., *J. geophys. Res.*, **76**, 8128 (1971).
11 Henley, R. W., *Trans. Instn Min. Metall.*, **B82**, 1 (1973).
12 Anderson, C. A., *Econ. Geol.*, **64**, 129 (1969).
13 Hutchinson, R. W., and Hodder, R. W., *Trans. Can. Inst. Min. Met.*, **75**, 16 (1972).
14 Stanton, R. L., *Econ. Geol.*, **50**, 681 (1955).
15 Mitchell, A. A. G., and Garson, M. S., *Trans. Instn Min. Metall.*, **B82**, 43 (1973).
16 Solomon, M., and Griffiths, J. R., *Geol. Soc. Aust.*, Spec. Pub. 4 (in the press).
17 Lusk, J., *Econ. Geol.*, **67**, 169 (1972).
18 Sasaki, A., *Geochem. J.*, **4**, 41 (1970).
19 Garrels, R. M., and Mackenzie, F. T., *Evolution of Sedimentary Rocks* (Norton, New York, 1971).
20 Ronov, A. B., *Sedimentology*, **10**, 25 (1968).
21 Cooper, J. A., and Richards, J. R., *Geochem. J.*, **3**, 1 (1969).

Late glacial site in the central Grampian Highlands

The last British ice sheet probably reached its greatest extent 17,000–18,000 radiocarbon yr ago, when it covered much of the British Isles. We suggest here that this ice mass had disappeared 5,000 yr later. Significant new evidence comes from Loch Etteridge in the heart of the Grampian Highlands of Scotland (Fig. 1).

Loch Etteridge (grid ref. NN688929) lies in a deep kettle hole bordered by eskers and kame terraces at an altitude of 300 m, in upper Strathspey. The deepest accessible part of the kettle infill was sampled using a piston corer with a chamber 50 cm long and 5 cm in diameter. Seven metres of peat and gyttja overlie a typical late glacial sequence comprising organic deposits between minerogenic deposits (Fig. 2). Pollen analyses of the late glacial and basal postglacial deposits were carried out at 2.5-cm intervals. Samples for radiocarbon dating were obtained by combining material from four bores put down at the corners of a 30-cm square. This was possible because of the well defined stratigraphy, and was facilitated because the full sequence (Fig. 2) was obtained in a single chamber in each bore.

A date of 9,405 ± 260 yr BP (SRR-301) was obtained for the basal 4 cm of the postglacial organic deposits. The pollen spectrum is dominated by *Artemisia*, which exceeds 30% of the total land pollen, whereas values for woody taxa are less than 10%. Immediately above the dated sample, open habitat taxa decline markedly and pollen of woody plants increases. The radiocarbon date is much later than the date of approximately 10,300 yr BP which is conventionally assigned to the end of the Loch Lomond Stadial. In

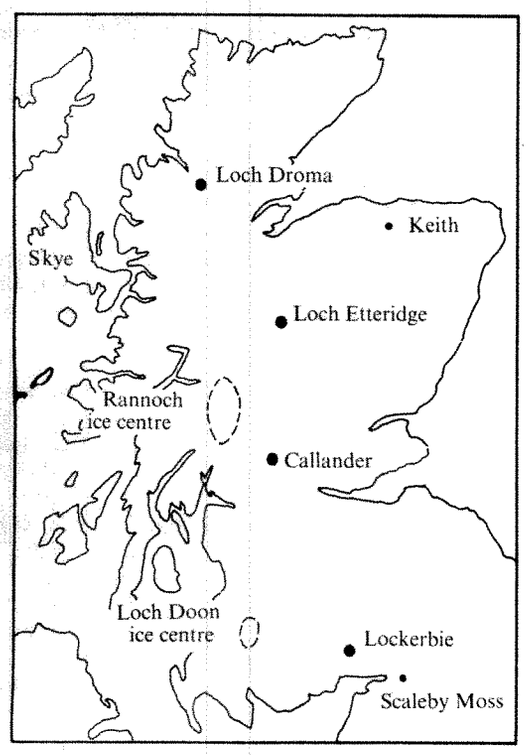


Fig. 1 Locations referred to in the text.

Skye, however, the cessation of minerogenic deposition varies considerably in different lochs, and the youngest date relating to this event is 9,420 ± 150 yr BP (ref. 1). Protracted minerogenic deposition at Loch Etteridge may be related to its altitude and to its location 8 km from the margin of a plateau ice cap which covered nearly 300 km², and which is considered to have developed during the Loch Lomond Readvance[2].

At the top of the Late Glacial interstadial deposits the pollen spectrum is characterised by woody plants, especially *Empetrum* and *Betula* c.f. *nana*, but *Artemisia*, Saxifragaceae and *Rumex* are also significant. In the overlying clay, pollen of woody plants declines and that of open habitat taxa increases. The top 1.5 cm of the interstadial deposits gave a date of 10,764 ± 120 yr BP (SRR-302). This accords with the value of 10,800 yr BP generally accepted for the beginning of the Loch Lomond Stadial, indicated, for example, by dates from Keith in Banffshire and from Scaleby Moss in northern England[3]. It has been inferred that at about 10,800 yr BP minerogenic deposition (implying solifluction on surrounding slopes) began at sites in Skye[1]. It therefore seems that at this time temperatures fell, probably rapidly, over a large area. It does not follow, however, that the glaciers involved in the Loch Lomond Readvance began to build up at this time.

Studies of Coleoptera at sites in England, Wales and the Isle of Man, suggest that a fall of temperature began before 12,000 yr BP and continued until the end of the Late Glacial interstadial[4], by which time mean July temperatures were probably 4°–5° C below present values. The interstadial climate may have been moderately continental, with winter temperatures depressed even more below present values[4]. For glaciers to develop on Ben Nevis a fall of more than 2° C in the present mean summer temperature is required[5]. Combining these figures it seems that the Loch Lomond Readvance began before, perhaps well before 10,800 yr BP. It can also be inferred, in view of the varied relief of Scotland and the marked gradients of former snow lines, that glaciers began to develop at different times in different places.

At the base of the more organic interstadial deposits, the pollen spectrum is dominated by *Empetrum* (over 45%), but *Betula* c.f. *nana* and *Juniperus* are also significant. A sample 1.5 cm thick yielded a radiocarbon date of 11,290 ± 165 yr BP (SRR-303).

The pollen spectrum at the base of the organic deposits is characterised by high values of *Rumex* (over 20% of total land pollen) with *Salix* (many of which resemble *Salix c.f. herbacea*) and Saxifragaceae also important. Values for woody plants increase progressively towards the upper part of the dated horizon.

The basal organic deposits gave a radiocarbon date of 13,151 ± 390 yr BP (SRR-304). The most likely source of any error is the hard water effect, which can give apparent ages that are almost 3,000 yr too great, although the error is rarely as large as this[6,7]. Limestone does not crop out in the area draining to Loch Etteridge, however, and glacial erratics of limestone have not been found. No shells were found in the lake deposits. The possibility cannot be excluded, however, that inert carbon from the complex metamorphic rocks of the surrounding area was incorporated in the dated bulk sample. On the other hand, the date of 10,764 ± 120 yr BP from similar (though organically richer) material higher in the sequence agrees with expectations, and there can be no hard water error here.

Because a thickness of 3–4 cm of the deposits was used for dating, the date obtained, if valid, is younger than the age of the very bottom of the organic layer. It is particularly significant that the site is a deep kettle hole, in which dead ice may have remained for hundreds (or even thousands) of years after deglaciation of the surrounding area[8]. Thus the basal date, if valid, is a minimum date of deglaciation.

The Loch Etteridge date is not unique in Scotland. Wood from lake deposits near Lockerbie in Dumfriesshire has

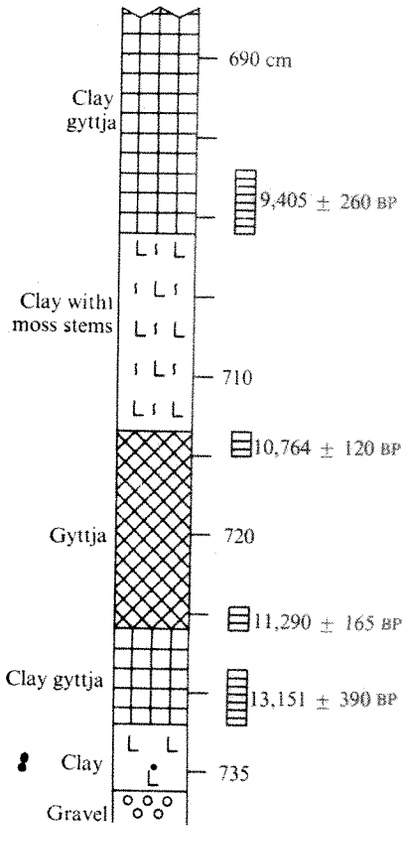


Fig. 2 Stratigraphy and radiocarbon dates at Loch Etteridge.

been dated at 12,940±250 yr BP (ref. 9). Lockerbie is only 60 km from the major centre of icesheet accumulation in the Southern Uplands (Loch Doon Basin). In the heart of the mountains of NW Scotland a date of 12,810±155 yr BP has been obtained for deposits from Loch Droma[10]. Near Callander the basal organic deposits in a kettle hole have provided a date of 12,750±120 yr BP. This site is only 45 km from the centre of the area of greatest ice sheet accumulation in the British Isles (around the Rannoch basin). The Loch Etteridge basal date adds significantly to this evidence, because the site is in the middle of the Grampian Highlands.

Together, the four dates (all of which, if valid, are minimum dates of deglaciation) show that by about 13,000 yr BP the last ice sheet had disappeared from most of Scotland. At about this time two significant, probably interrelated, events are reported to have occurred. Coleoptera and radiocarbon dates from a site in North Wales, indicate that at about 13,000 yr BP temperatures rose very rapidly, with summer temperatures becoming at least as warm as those of the present day[11]. Seafloor sediments indicate that deglacial warming of the North Atlantic Ocean adjacent to the British Isles occurred at about 13,500 yr BP, polar waters having retreated far to the west by 13,000 yr BP (ref. 12). In view of these profound changes we find it difficult to believe that any remnants of the British ice sheets which may have remained at about 13,000 yr BP could have survived for much longer. Total deglaciation by 12,500 yr BP seems a conservative suggestion.

The last British ice sheet, which probably reached its greatest extent at about 17,000–18,000 yr BP, had therefore wasted away by 12,500 yr BP at the latest. Subsequently, glaciers built up again in many mountain areas, and were especially extensive in the Scottish Highlands[13] where they began to accumulate before the climatic deterioration which occurred at about 10,800 yr BP. The glaciers probably reached their maximum extent about 10,300 yr BP, but it is not yet known how long they took to decay. The fact that minerogenic deposition, which implies solifluction and sparse vegetation, continued locally till approximately 9,400 yr BP may be significant in this context.

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1 Birks, H. J. B., *The past and present vegetation of the Isle of Skye: a palaeoecological study* (Cambridge, 1973).
2 Sissons, J. B., *Trans. Inst. Br. Geogr.* (in the press).
3 Godwin, H., *Proc. R. Soc.*, **B153**, 287 (1961).
4 Coope, G. R., Morgan, A., and Osborne, P. J., *Palaeogeogr. Palaeoclimatol. Palaeoecol.*, **10**, 87 (1971).
5 Manley, G., *Geogr. Annlr.*, **31**, 179 (1949).
6 Donner, J. J., Jungner, H., and Vasari, Y., *Soc. Sci. Fenn. Comm. Phys-Math.*, **41**, 307 (1971).
7 Shotton, F. W., *Nature*, **240**, 460 (1972).
8 Porter, S. C., and Carson, R. J., *Quaternary Res.*, **1**, 410 (1971).
9 Bishop, W. W., *Trans. J. Proc. Dumfries. Galloway nat. Hist. Antiq. Soc.*, **40**, 117 (1963).
10 Kirk, W., and Godwin, H., *Trans. R. Soc. Edinb.*, **65**, 225 (1963).
11 Coope, G. R., and Brophy, J. A., *Boreas*, **1**, 97 (1972).
12 Ruddiman, W. F., and McIntyre, A., *Quaternary Res.*, **3**, 117 (1973).
13 Sissons, J. B., Lowe, J. J., Thompson, K. S. R., and Walker, M. J. C., *Nature phys. Sci.*, **244**, 75 (1973).

Late Pleistocene American obsidian tools

We have dated some of the oldest examples of obsidian use yet known in America. The obsidian originates from a new location, the Mostin site, which contains about a dozen burials and is located near the Borax Lake site in northern California. There it is thought that man lived as long ago as 10,000 yr and perhaps even at the end of the Pleistocene[1].

During test excavations in the summer of 1973 human bones and obsidian artefacts[2,3] were found at the new Mostin site (SDA-66), located 16 km north-west of the Borax Lake site. The Mostin site (39°N; 122°50′W) is situated along Kelsey Creek about 2.5 km south of Clear Lake and 5 km from the nearest obsidian source south at Mt Konocti[4-6] (Fig. 1). The Mostin site is now being exposed by the erosive action of Kelsey Creek which is cutting into lake sediments 6 m high deposited during an earlier higher level stage of Clear Lake[7].

The samples sent to us for dating and for tracing their origin included the left fibula, left tibia and the diaphysis of the right femur of burial 4 and seven associated obsidian artefacts. The bone samples were combined and dated by the radiocarbon content of their collagen fraction according to established procedures[8] and yielded a date (UCLA-1795A) of 10,260 ± 340 yr (ref. 9). The statistical error encompasses any deviation due to correction for isotopic fractionation of $^{13}C/^{12}C$ in case the resident human population had been heavily dependent on freshwater fish.

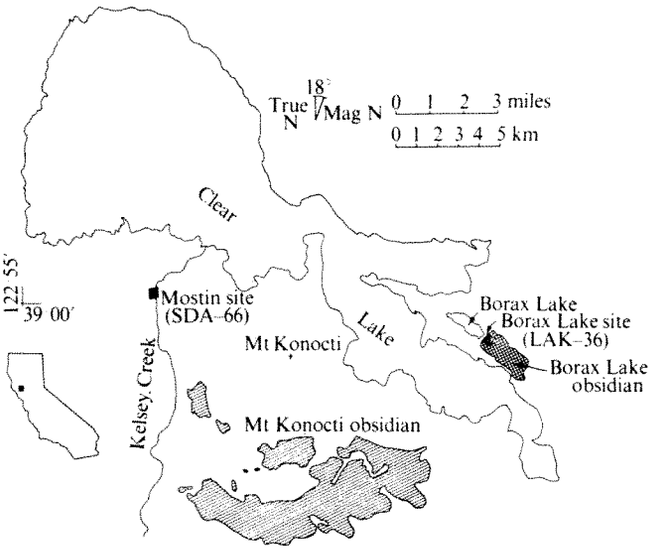


Fig. 1 Location of obsidian sources and archaeological sites, near Clear Lake, California.

The obsidian artefacts were classified into a functional typology based on their morphology and microscopic wear patterns (Table 1). Artefacts M3 (burin) and M6 (scraper) were used as tools, whereas M1 was a biface blank and M4, 5 and 7 primary decortication flakes removed from nodular cobbles of obsidian. Artefacts M1 and 6 showed fractures indicative of percussion flaking with a soft instrument from a prepared core. M2 has the appearance of a blade but its precise classification is uncertain.

All obsidian pieces were subjected to hydration rim thickness measurements and source characterisation by neutron activation and X-ray fluorescence analysis. From the differences in hydration values it became apparent that probably two types of obsidian had been utilised. Artefacts M1, 3 and 6 show a clustering of hydration rind values between 6.2 and 6.8 μm, whereas M4, 5 and 7 are all 4.6 μm. The appearance of artefacts 1, 3 and 6, and 4, 5 and 7 are the same respectively within each group, whereas both groups are distinctly different in visual appearance.

Table 1 Functional typology of obsidian artefacts

Artefact sample no.	UCLA obsidian hydration no.	Hydration measurement (μm)	Origin by X-ray fluorescence*	Functional typology	Notes
M1	3754	6.4	Borax Lake	Biface blank	Partially finished preform
M2	3755	5.7/12.6	Borax Lake	Blade	Unretouched irregular blade with a single medial ridge and convergent edges
M3	3756	6.2	Borax Lake	Thick flake burin	Appearance (and probable function) of an oblique truncation burin—a European Palaeolithic form
M4	3757	4.6	Mt Konocti	Primary decortication flake	Broken from M7, no retouch
M5	3758	4.6	Mt Konocti	Primary decortication flake	No retouch
M6	3759	6.8	Borax Lake	Flake scraper	Three edges exhibit even, regular wear pattern of small scalar flakes
M7	3760	4.6	Mt Konocti	Primary decortication flake	Broken from M4, no retouch

* Source discrimination based on results of a ternary diagram of the intensity of the elements zirconium, rubidium and strontium.

Neutron activation analysis was too indistinct in this case to separate two suspected sources of obsidian, Mt Konocti and Borax Lake, but additional X-ray fluorescence measurements revealed by comparison with known source materials that the slower hydrating obsidian originated from Mt Konocti and the faster from the Borax Lake area.

Comparison of hydration and source data with a hydration rate curve for Borax Lake over a time span of the past 4,000 yr suggests that the rate over the entire 10,000 yr available now is at least partially exponential in nature (unpublished work of F. J. Findlow, S. P. De Atley, and J. E. Ericson). Moreover, this work shows that some two dozen obsidian artefacts found at Borax Lake may be much older than had been anticipated.

The Borax Lake assemblage also contains obsidian originating from Mt Konocti about 5 km away. The reason for this exploitation may lie in the fact that obsidians from the two sources differ in hardness: Mt Konocti material has a hardness of 500 to 600 kg mm^{-1} whereas that from Borax Lake is 734 ± 12 kg mm^{-1}.

This work suggests that obsidian was used in Northern California at least as early as the interface of the Late Pleistocene/Holocene and the extent of early settlement may indeed have been much more extensive than hitherto thought.

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1 Meighan, C. W., and Haynes, C. V., *Science*, **167**, 1213 (1970).
2 King, R. and Berg, G., *Soc. Calif. Archaeol. Archive Ser.*, **51**, 1 (1973).
3 King, T., *Newsl. Soc. Calif. Archaeol.*, **7**, (6) 1 (1973).
4 Anderson, C. A., *Bull. geol. Soc. Am.*, **47**, 165, 629 (1936).
5 McNitt, J. R., *Calif. Div. Mines and Geol., Mapsheet* 9 (1968).
6 Ericson, J. E., Hagan, T. A., and Chesterman, C. W., in *Advances in Obsidian Glass Studies: Archaeological and Geochemical Perspectives* (edit. by Taylor, R. E.), (Noyes, Park Ridge, New Jersey, in the press).
7 McNitt, J. R., *Calif. Div. Mines and Geol., Mapsheet* 10 (1968).
8 Protsch, R., thesis, Univ. California, Los Angeles (1973).
9 Berger, R. and Libby, W. F., *Radiocarbon*, **16** (in the press).

BIOLOGICAL SCIENCES

Particle containing uncleaved major head protein (P23) which is maturable into the head of phage T4

PREVIOUS results[1] suggested that τ-particles produced by a phage T4 temperature-sensitive (*ts*) mutant in gene 24 can be matured into T4 phage heads after a shift to a permissive temperature (temperature shift-down). The experimental evidence which supports this hypothesis can be summarised as follows: (1) the number of τ-particles per bacterium observed after temperature shift-down of a 24^- infected culture decreases concomitantly with an increase in the number of normal T4 phage particles; (2) preshift (under nonpermissive conditions) 24^- radioactively pulse-labelled cultures produce radioactively labelled normal phage after temperature shift-down; (3) after temperature shift-down, preshift radioactively labelled major head protein P23 (molecular weight 59,000) is cleaved into P23* (molecular weight 47,500) both molecular weights determined by A. Tsugita from the amino acid composition, (personal communication)[2-5].

But we also know that τ-particles produced in 21^- infected bacteria cannot be further matured after temperature shift-down, although they are morphologically identical to those produced in 24^- infected cultures[1,6].

Here we shall demonstrate that the uncleaved P23 is organised in the form of a precursor particle, in which the later conversion of P23 to P23* is conservative. Our complete set of experiments will be published elsewhere[16]. With the previous observations, these new results provide very strong evidence that 24^-–τ-particles are maturable into phage heads.

Our previous results[1] did not rule out the possibility that the phage capsids observed after temperature shift-down are built from subunits drawn from a pool containing subunits built before as well as after shift. (In the following we shall distinguish them as 'preshift subunits' and 'postshift subunits'.) An important part of preshift subunits could stem from the breakdown of τ-particles (see discussion in ref. 1). If capsids of phages made after shift were composed of a mixture of preshift and postshift subunits, then a dispersive mode of transfer would have taken place. The transfer would be conservative when the preshift subunits already formed a particle which, after shift, was transformed as a whole into a capsid. We shall demonstrate (Figs 1 and 2) a true conservative transformation of a P23-capsid into a P23*-capsid, by density labelling the preshift subunits as 'heavy' and the postshift subunits as 'light'. By supplementary radioisotope pulse labelling the fate of both types of subunits can be followed independently.

We used T4.10(amB255).24(tsL90). The tail-less heads of mutants in gene 10 have the advantage of being easily emptied by DNase treatment[7]. This allowed us to analyse the density of emptied heads without having to take into account the full DNA complement of a phage head and the density variability due to mixing of preshift and postshift phage tails. After a partial purification by sucrose gradient velocity sedimentation, the emptied heads were looked at in the electron microscope, their protein composition was analysed by SDS-polyacrylamide gel electrophoresis[2,8] and their density was measured by CsCl gradient centrifugation.

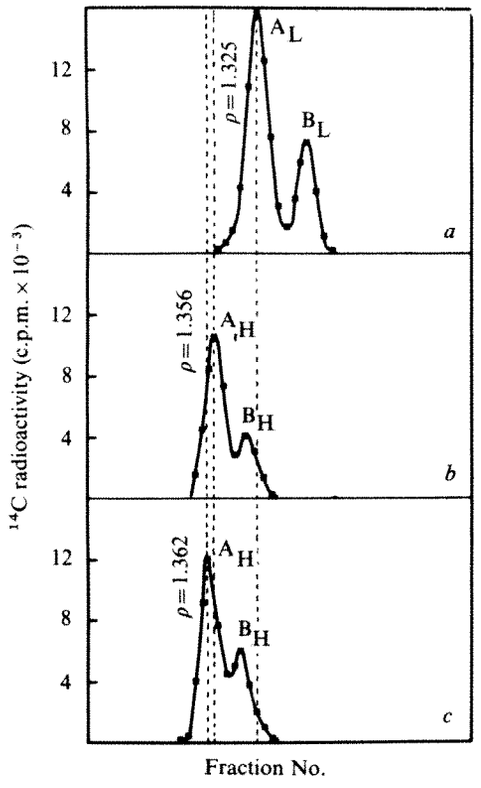


Fig. 1 Density distribution of phage heads treated with DNase obtained in heavy medium alone, after transfer from heavy into light medium and in light medium alone. DNase treated phage heads were purified by a sucrose gradient of which the 300S fractions containing emptied head were collected. These fractions were then analysed by density equilibrium centrifugation. *a*, Emptied heads obtained from 10⁻ infected cells grown in normal light medium; *b*, emptied heads obtained from 10⁻.24⁻ infected cells at 30 min after the culture was shifted from completely heavy medium at 40.5° C into normal light medium at 30° C (permissive temperature); *c*, emptied heads obtained from 10⁻ infected cells grown in completely heavy medium. The explanation for the existence of two peaks is discussed in Fig. 2. Peaks A and B refer to partially emptied and completely emptied heads respectively. The subscripts L and H refer to light and heavy medium.

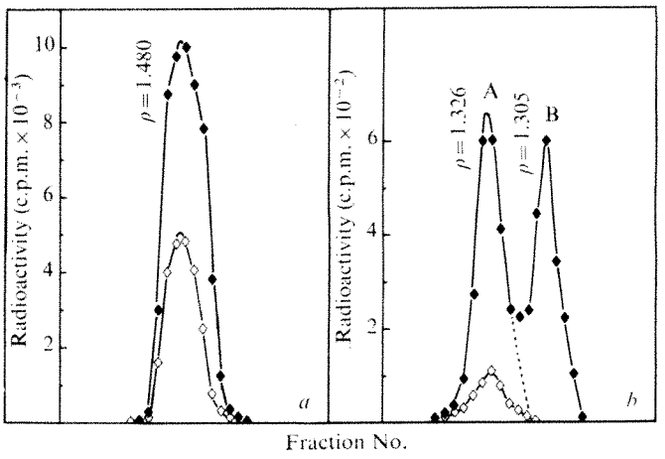


Fig. 2 DNA content of 10⁻ DNase-treated phage heads compared with the DNA content of wild-type phage. Cultures of *Escherichia coli* B^E and *Escherichia coli* CR63 were infected with T4.10 (am B255). At 16 min after infection 16 μCi ml^{-1} of ^{32}P and 200 μCi ml^{-1} of ^{3}H-amino acids were added. At 90 min the bacteria were lysed with chloroform in the presence of DNase. The lysates were centrifuged on a 15–40% sucrose gradient for 45 min at 30,000 r.p.m. in an IEC SB 405 rotor. The 1100S peak (phage *a*) and the 300S peak (emptied heads *b*) were then layered on CsCl with density ρ = 1.48 and ρ = 1.32 (phage and emptied heads respectively). Radioactivity distribution of ^{3}H-labelled proteins (◆ ◆ ◆) and ^{32}P-labelled DNA (◊ ◊ ◊). Labelling conditions for phage and emptied heads were identical.

The density profile for emptied heads obtained with 10⁻ infected cells in light medium is shown in Fig. 1*a*, and that of 10⁻ emptied heads in heavy medium in Fig. 1*c*. In both cases there are two peaks A and B which differ in density by 0.021 g ml^{-1}. The two peaks occur normally and are due to two types of emptied heads: those which are completely emptied (peak B_L; density = 1.304 g ml^{-1}) and those still containing about 20% of DNA (peak A_L; density = 1.325 g ml^{-1}). The incomplete removal of DNA from all of the DNase-treated 10⁻ heads has been observed by Hosoda and collaborators (personal communication). We have repeated an experiment to demonstrate the presence and absence respectively of DNA in the denser and less dense types of emptied heads. The results of this experiment are shown in Fig. 2. Clearly, peak A contains DNA while peak B does not. Although both types of emptied head were nearly always found after DNase treatment, their relative amounts varied with every experiment.

From Fig. 1 we deduce that the two emptied head peaks A and B (double peak) are shifted by 0.037 g ml^{-1} when grown in heavy medium (compare peaks A_L and A_H). The density profile of Fig. 1*b* was obtained from emptied heads which arose after temperature shift-down and simultaneous transfer to light medium with the double mutant 24(tsL90).10(amB255). This double peak is 0.031 g ml^{-1} denser than the light one. This result is incompatible with a dispersive mode of transfer and suggests instead that the transfer is conservative.

It is, however, possible that the infected bacteria do not immediately synthesise structural components after shift into light medium. This would mean that preshift built subunits cannot mix with postshift newly synthesised light subunits. To test this possibility we labelled preshift built material with ^{14}C-labelled amino acids and postshift built material with ^{3}H-labelled amino acids. These results shown in Fig. 3 demonstrate that light subunits are synthesised immediately after temperature shift and that most of the ^{14}C-preshift labelled emptied heads remain in a peak which is considerably denser than the ^{3}H-labelled emptied heads. Furthermore, the ^{14}C-labelled emptied heads remain from 15 to 60 min after temperature shift.

Our previous results together with these experiments give good evidence that 24⁻–τ-particles can be transformed into phage heads in a conservative mode. We have, however, no proof that this τ-particle is also a precursor in the normal pathway of phage head maturation as proposed by Simon[9]. Among the P23-containing particles, only the 24⁻–τ-particle can be further matured; 21⁻–τ-particles, which are morphologically indistinguishable, cannot be transformed[1,6,10]. The same is true for the so-called "crummy heads"[11] produced by *ts* mutants in gene 23[16]. Laemmli and Favre[12] have shown that in pulse-chase experiments in wild type a precursor particle called prohead I which contains predominantly uncleaved P23 and possibly P22 and IP III can be converted into a particle called prohead II, which contains essentially P23*, P22 and IP III. Other results[6,10] show that the 21⁻–τ-particles and the prohead I particles have similar properties (S value, protein composition). These results suggest that the 24⁻–τ-particles may be a true precursor in the normal pathway of phage head maturation.

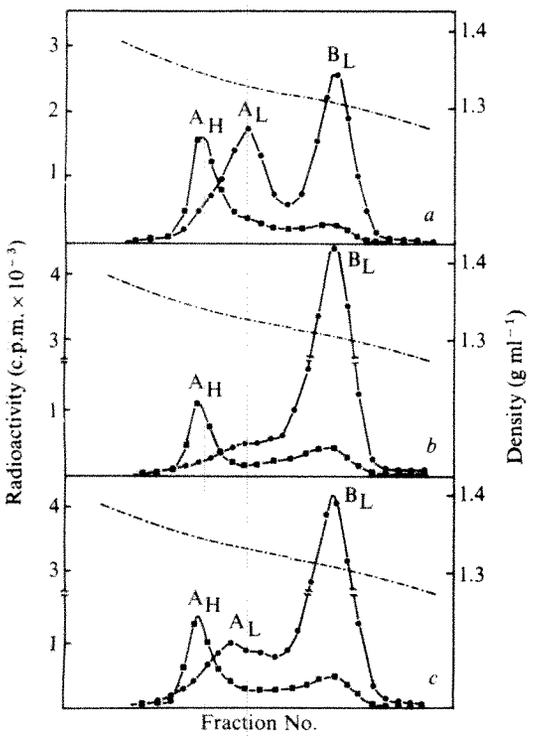


Fig. 3 Density distribution of pre- and postshift labelled emptied heads of 10⁻.24⁻ infected cells at different times after temperature shift-down. *a*, Density distributions of preshift ^{14}C-labelled empty heads (■ - ■ - ■) and postshift ^{3}H-labelled empty heads (● - ● - ●) of a sample taken 15 min after temperature shift-down; *b*, *c*, similar density distributions but of samples taken 30 and 60 min after temperature shift-down respectively. Peaks A_L and B_L refer to partially emptied and completely emptied light heads. Peak A_H refers to partially emptied heavy heads.

Assuming that these precursor particles containing P23 undergo cleavage *in situ* (that is, without breaking down and reassembling in a compartmentalised space), then the number of P23 subunits must remain identical throughout the transformation. This is highly interesting, since the overall size of the phage head is about 20% larger than that of a τ-particle[13]. In this context, it is interesting to note that other results[17] of our laboratory indicate that the lattice constant of the capsids of giant phages[14] is different from that of polyheads. From previous considerations on polymorphism[15] it is likely that τ-particles have the same lattice as polyheads and similarly that the giants are not different from normal phage in this respect.

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1 Bijlenga, R. K. L., Scraba, D., and Kellenberger, E., *Virology*, **56**, 250–267 (1973).
2 Laemmli, U. K., *Nature*, **227**, 680–685 (1970).
3 Kellenberger, E., and Kellenberger-van der Kamp, C., *FEBS Lett.*, **8**, 140–144 (1970).
4 Hosoda, J., and Cone, R., *Proc. natn. Acad. Sci. U.S.A.*, **66**, 1275–1281 (1970).
5 Dickson, R. C., Barnes, S. L., and Eiserling, F. A., *J. molec. Biol.* **53**, 461–474 (1970).
6 Luftig, R. B., and Lundh, N. P., *Proc. natn Acad. Sci. U.S.A.*, **70**, 1636–1640 (1973).
7 Granboulan, P., Séchaud, J., and Kellenberger, E., *Virology*, **46**, 407–425 (1971).
8 Shapiro, A. L., Viñuela, E., and Maizel, J. V., jun., *Biochem. biophys. Res. Commun.*, **28**, 815–820 (1967).
9 Simon, L. D., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 907–911 (1972).
10 Laemmli, U. K., and Johnson, R. A., *J. molec. Biol.*, **80**, 601–611 (1973).
11 Favre, R., Boy de la Tour, E., Segre, N., and Kellenberger, E., *J. Ultrastruct. Res.*, **13**, 318–342 (1965).
12 Laemmli, U. K., and Favre, M., *J. molec. Biol.*, **80**, 575–599 (1973).
13 Kellenberger, E., Eiserling, F. A., and Boy de la Tour, E., *J. Ultrastruct. Res.*, **21**, 335-360 (1968).
14 Doermann, A. H., Eiserling, F. A., and Boehner, L., *J. Virol.*, **12**, 374–385 (1973).
15 Kellenberger, E., in *Polymerization in Biological Systems*, Ciba Foundation Symp. 7 (edited by Wolstenholme, G. E. W. and O'Connor, M.), 189–206 (Elsevier, Amsterdam, 1972).
16 Bijelenga, R. K. L., v.d. Broek, R., and Kellenberger, E., *J. supramolec. Struct.*, **2**, (in the press).
17 Aebi, U., Bijelenga, R. K. L. v.d. Broek, J., v.d. Broek, R., Eiserling, F., Kellenberger, C., Kellenberger E., Mesyanzhinov, V., Müller, L., Showe, M., Smith, R., and Steven, A., *J. supramolec. Struct.*, (in the press).

Banding and chromatid separation in Chinese hamster chromosomes

In general, a chromosome exhibiting its banding pattern does not permit any internal helix to be seen, and vice versa. During studies with chromosomes of Chinese hamster cells, cultivated *in vitro*, it was possible to correlate G banding and spiral structure with the spatial relation between sister chromatids. G banding of human chromosomes was first described by Sumner *et al.*[1] and later was demonstrated in the Chinese hamster chromosomes by Radabli and Kriukova[2]. By a hypotonic treatment, the spiralisation of the chromosomes was demonstrated by Ohnuki[3] in man and later by Kato and Yoshida[4] in the Chinese hamster.

Chinese hamster fibroblasts of the DON line were grown for 2–3 d in Puck's medium supplemented with 10% calf serum and 1% glutamine, collected by trypsinisation after 5.5 h of colchicine treatment (10 μg ml^{-1}), and then centrifuged at 1,000 r.p.m. for 10 min. The supernatant was replaced by a mixture of KCl (0.055 M), KSCN (0.062 M), $NaNO_3$ (0.055 M) and CH_3COONa (0.055 M) in the proportion 4:1:2:2, which is the hypotontic saline used by Ohnuki[3] to demonstrate spiralisation in chromosomes. Cells were then washed, fixed for 50 min with methanol and glacial acetic acid (3:1), spread on slides according to the method of Moorhead *et al.*[5], stained with 2% Giemsa R66 for 30 min, washed with tap water, dried at room temperature and mounted in DPX. Metaphase chromosomes were examined and some of them were photographed with a Zeiss × 100 Neofluar objective.

Table 1 The distribution of 106 metaphase figures

		Appearance of chromatids				
		Banded	Intermediate	Visible helix	Homogeneous	Total
Separation of sister chromatids	Separate	0	1	38	0	39
	Partly separate	0	26	1	4	31
	Closely apposed	36	0	0	0	36
	Total	36	27	39	4	106

They showed either banding, a spiral structure, an intermediate appearance, or homogeneity without evident internal structure; the first three are exemplified in Fig. 1. It is of interest that the banding pattern appeared on the chromosomes without postfixation treatment, which is usually used for banding, and was described by Sumner *et al.*[1]. Within any one cell, all chromosomes were of similar appearance. It was also noted whether sister chromatids were closely opposed to each other, or were separated. If they were separated, the outside width of the whole chromosome was about 2.4 μm, but only 0.8 μm if the chromatids were together. Thus, the apparent separation was due to movement apart and was not wholly explicable by loss of material between the chromatids. It is interesting to compare Darlington's correlation of internal change in chromosomes with their external mechanics[6], but he was concerned with the question of an internal split, whereas the results reported here relate to regional affinity for dyes. In some cells the chromatids were separated along only a part of their length. In each of 10 slides, at least 10 metaphase figures were examined and classified, the counts so obtained are presented in Table 1, which shows that closely paired chromatids always exhibited banding, and conversely, but that the internal helices became evident and the banding lost only when the chromatids separated.

A possible interpretation of this is that the manifestation of banding depends on chromosomal material which is also necessary for maintaining the close apposition of sister chromatids and which conceals the helix, but the disappearance of the bands in this experiment is nevertheless also attributable to the withdrawal of interchromatid fibres. (Fig. 1*b*). Kato and Yoshida[4] tried to correlate G bands with coils by comparing the number of bands with the number of coils on the homologous chromosomes of the Chinese hamster. This correlation was only based on the constancy of the number of the coils in each individual chromosome. But they could only show the resemblance of the knobs, which are the relics of bands on the spirals, and the bands. No correlation has been shown between bands and the spirals on the chromosomes of the Chinese hamster. Ford *et al.*[7], by using a modified whole-mount method, observed the spiral structure of human chromosomes distinctly, and found that the number of turns diminishes as mitosis proceeds. They concluded that the number of turns was not constant but varies with the preparation and doubted if the number of gyres would be of any value for the identification of the individual chromosomes, as was proposed by Ohnuki[3]. The diminution of the gyres in number, during mitosis, would necessitate the unwinding of the chromatids around their axis, which could lead to partial or complete disappearance of the bands. (Fig. 1*c*).

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1 Sumner, A. T., Evans, H. J., and Buckland, R. A., *Nature new biol.*, **232**, 31 (1971).
2 Radjabli, S. I., and Kriukova, E. P., *Mammalian Chromosomes Newsl.*, **14**, 112 (1973).
3 Ohnuki, Y., *Chromosoma*, **25**, 402 (1968).
4 Kato, H., and Yoshida, T. H., *Chromosoma*, **36**, 272 (1972).
5 Moorhead, P. S., Nowell, P. C., Mellman, W. J., Battips, D. M., and Hungerford, D. A., *Expl Cell Res.*, **20**, 613 (1960).
6 Darlington, C. D., *Nature* **127**, 709 (1931).
7 Ford, E. H. R., Thurley, K., and Woollam, D. H. M., *J. Anat.*, **103**, 143 (1968).

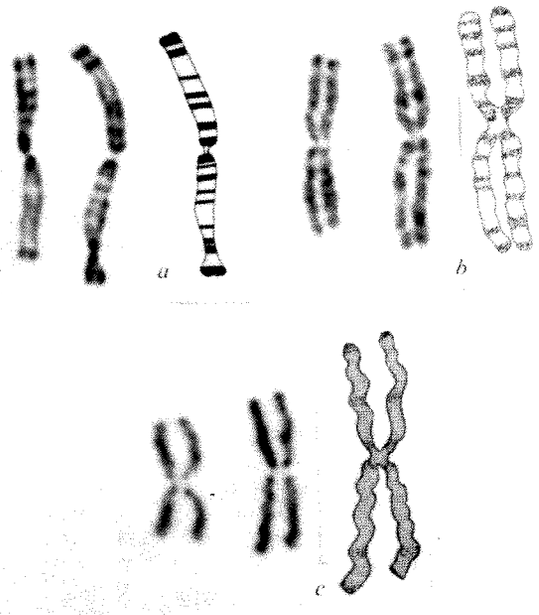


Fig. 1 Chinese hamster chromosomes treated before fixation with the hypotonic saline described in the text and without any postfixation treatment were stained with Giemsa. The chromosomes No. 1 of each group with their diagrammatic representations are shown (× 4200) *a*, Banded chromosomes; *b*, the chromosome intermediate in appearance between *a* and *c*; *c*, the chromosomes show spiralisation without apparent banding.

Virus-like particles induced in guinea pig cells by 5-bromo-2′-deoxyuridine are morphologically similar to murine B-type virus

VIRUS-LIKE particles in tissues of leukaemic and normal guinea pigs have been demonstrated repeatedly[1–5]. Tumour cells contain intracisternal particles resembling mouse intracisternal A particles[3], and extracellular virus-like particles are found in tissues of leukaemic guinea pigs. To the best of our knowledge, a convincing micrograph of a virus-like particle budding from the surface of a guinea pig tumour cell (*in vivo*) has never been published. In a separate study (J.E.D. and K.P., in preparation), it has been concluded that

the guinea pig leukaemia virus[1–3] results from the release of intracisternal A particles from dying tumour cells.

Recently, several groups have induced virus-like particles in tissue cultures of a wide variety of normal and neoplastic guinea pig cells using 5-bromo-2′-deoxyuridine (BrdU) (refs 6–9). Guinea pig cells treated with BrdU contain intracytoplasmic A particles[7,8] and produce extracellular virus-like particles of uncertain morphology. These extracellular particles are formed by a budding process, but it is difficult to determine from published micrographs whether these particles and buds are characteristic of C-type particles.

The categorisation of viruses found in mouse neoplasms as A, B, C, and D, first proposed by Bernhard[10], and later expanded to include two types of A particles[11], has recently been reviewed[12,13]. Intracisternal A particles bud from the rough endoplasmic reticulum into cisternae and seem to be unrelated to any other type of virus-like particles. Intracytoplasmic A particles, more homogeneous in size than intracisternal A particles, occur in the cytoplasmic matrix of the cell, and, in some cases, become the core of B particles. The intracytoplasmic A particles are not precursors of C-type particles. B particles are associated with mouse mammary tumours and can be distinguished from C-type particles by the morphology of the buds, the presence of relatively long spikes on negatively stained particles, and eccentric nucleoids in some of the mature particles. A-type particles are always intracellular, while B and C particles are extracellular.

Virus particles found in mice and other animals should be identified carefully because structural differences may represent important biochemical and genetic differences. In the case of the guinea pig, the virus-like particles induced by BrdU have alternatively been classified as C-type[6,7,9], or as guinea pig leukaemia virus-like[8]. In the study reported here, various cell lines, including tumour cells cultured from leukaemic strain 2 guinea pigs, normal guinea pig embryo cells derived from strain NIH, and 3-methylcholanthrene and benzo[a] pyrene-treated normal guinea pig embryo cells, have been examined for virus-like particles after induction for 5 d with 40 μg ml^{-1} of BrdU. Regardless of the source of the cells, virus particles with a morphology similar to that of the murine B-type virus, as well as intracytoplasmic A particles were the only types found. Although extracellular particles were observed frequently, budding particles were relatively rare (approximately one bud per fifty cell profiles). Figure 1 shows selected examples of thin sections of virus-like particles found in BrdU-treated guinea pig cells. Figure 1*a* shows an intracytoplasmic A particle; both electron dense shells are of approximately equal density. This is also true of the budding particles shown in Fig. 1*b* and *c*. At no time have budding particles been observed that failed to contain a completely formed nucleoid. Figure 1*f* and *g* illustrate eccentric nucleoids in particles occuring in a pellet of purified induced virus, while Fig. 1*h* shows the nucleoid of an immature particle in the same preparation. Because of the density of this pellet, osmium did not penetrate well, and the viral membranes were not well preserved. This had no effect on the nucleoids, which were adequately prefixed with glutaraldehyde. If Fig. 1*a* and *h* are compared, it can be seen that the intracytoplasmic A particle is identical to the nucleoid of a purified extracellular immature particle.

Figure 2 presents micrographs of negatively stained guinea pig particles induced by BrdU, and for comparison with Fig. 1, illustrations of several stages in the development of murine B and C particles. The presence of intracytoplasmic A particles, the ultrastructural characteristics of budding particles, and the presence of eccentric nucleoids in mature extracellular particles are criteria used to define murine type B particles. A further criterion is the presence of spikes in negatively stained particles. Although C-type particles also have spikes[14], they are short and difficult to visualise. The spikes present on the induced guinea pig particles and shown in Fig. 2*a* and *b*, most nearly resemble those found on B-type particles.

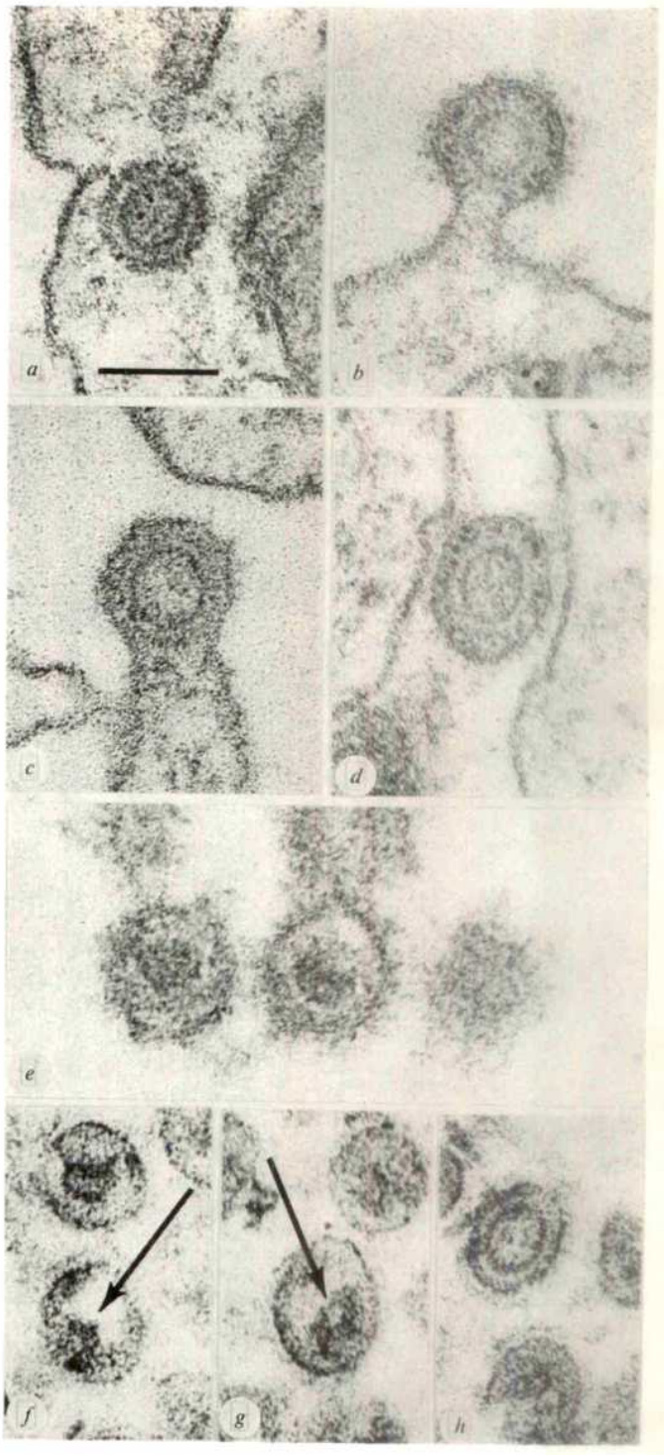


Fig. 1 Virus-like particles induced in guinea pig cells by BrdU. Samples were fixed *in situ* with 3% glutaraldehyde in Millonig's buffer, scraped and pelleted, and post-fixed with 1% osmium tetroxide in 0.1 M cacodylate buffer for 2 h. After dehydration in ethanol and propylene oxide, the pellets were embedded in Epon-Araldite. Thin sections were stained with uranyl acetate and lead citrate and examined at 80 kV in a Siemens 101 equipped with a 50 μm objective aperture. The bar represents 100 mm, and all figures are at the same magnification (approximately 160,000). *a*, Intracytoplasmic A particle; *b*, *c*, budding particles; *d*, an apparently extracellular immature particle; *e*, extracellular mature particles; *f*, *g*, particles occurring in pellets of purified induced virus. Note the eccentric nucleoids (arrows); *h*, nucleoid of immature particle in the same pellet shown in *f* and *g*.

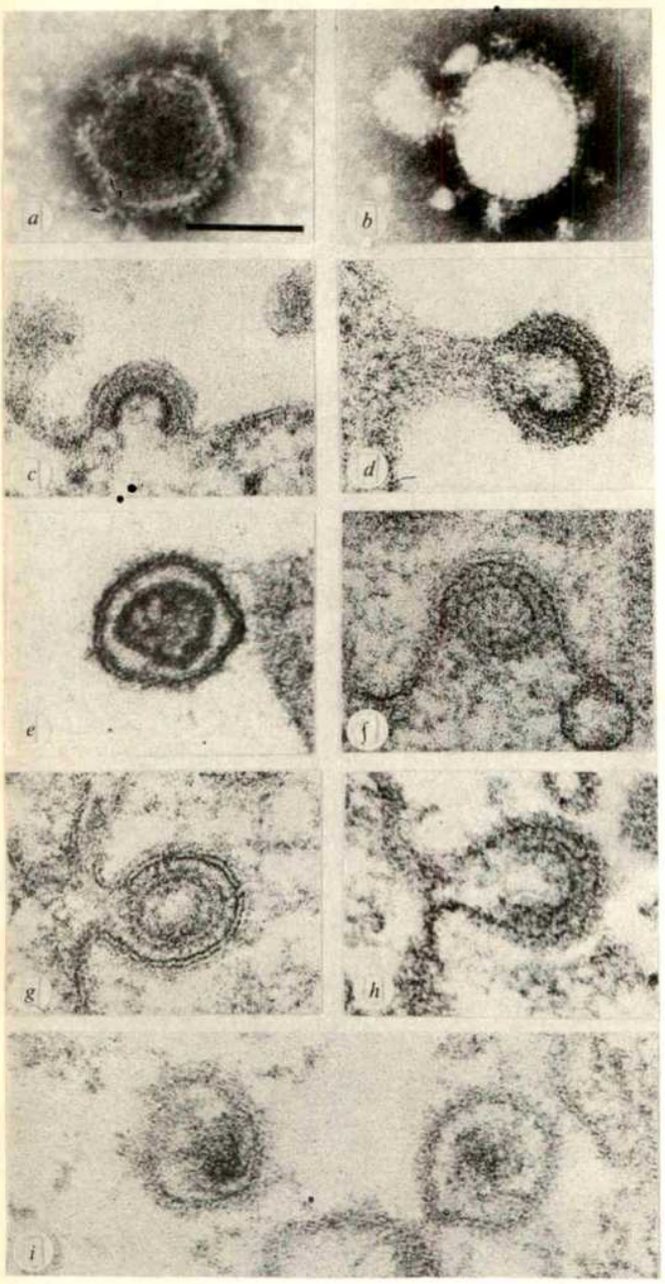


Fig. 2 *a*, *b*, BrdU-induced guinea pig particles, negatively stained with 0.5% neutral phosphotungstic acid following brief fixation with 3% glutaraldehyde; *c*, *d*, buds of murine C-type particles (*c*, Rauscher leukaemia virus in human embryo kidney cells; *d*, the C-type particle present in L cells); *e*, mature C-type particle (RLV in HEK cells); *f*, *g*, *h*, buds of mouse mammary tumour virus (B-type virus, in mammary tumour); *i*, mature murine B-type particles (in mammary tumour). The bar represents 100 nm; all figures are at the same magnification (approximately 160,000).

Figure 2*c* and *d* illustrate buds of murine C-type particles, and show how the inner layers form concomitantly with the bud. The electron density of the inner component is significantly greater than that of the intermediate layer. Figure 2*e* shows the centrally positioned nucleoid of a mature C-type particle. Buds of murine B-type particles may either contain preformed nucleoids (intracytoplasmic A particles; Fig. 2*f* and *g*) or the nucleoid may form in synchrony with the bud (Fig. 2*h*). In either case the electron densities of the inner component and the intermediate layer are approximately equal. This is essentially what is seen in the induced guinea pig particles, although buds form only by incorporating intracytoplasmic A particles. Thin sections of mature murine B-type particles may exhibit either eccentric or central nucleoids (Fig. 2*i*), although eccentric nucleoids usually predominate. In the induced guinea pig particles, eccentric nucleoids are less frequent, and are only very noticeable in older preparations, such as the purified pellet illustrated in Fig. 1*f* and *g*.

The particles induced in guinea pig cells resemble the murine B-type particle in several important ways. Although the induced guinea pig particles may not be morphologically identical to murine B-type particles, it is evident that they are not typical C-type particles, as has been reported[6,7,9].

There is a clear need for caution in categorising newly observed virus-like particles. Without high resolution micrographs of budding particles, it is extremely difficult to characterise accurately oncornavirus-like particles. Efforts are now under way to analyse biochemically particles and induced virus and the guinea pig leukaemia particles and to test their biological activities. If the preliminary diagnosis of this particle as a B-like virus is substantiated, the phenotype of a C-type virus genome has not yet been demonstrated in guinea pigs.

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1 Opler, S. R., *J. natn. Cancer Inst.*, **38**, 797 (1967).
2 Nadel, E., Banfield, W., Burstein, S., and Tousimis, A. J., *J. natn. Cancer Inst.*, **38**, 979 (1967).
3 Feldman, D. G., and Gross, L., *Cancer Res.*, **30**, 2702 (1970).
4 Ma, B. I., Swartzendruben, D. C., and Murphy, W. H., *Proc. Soc. exp. Biol. Med.*, **130**, 586 (1969).
5 Andersen, H. K., and Jeppersen, T. H., *J. natn. Cancer Inst.*, **49**, 1403 (1972).
6 Hsiung, G. D., *J. natn. Cancer Inst.*, **49**, 567 (1972).
7 Gross, P. A., Fong, C. Y. K., and Hsiung, G. D., *Proc. Soc. exp. Biol. Med.*, **143**, 367 (1973).
8 Rhim, J. S., Duh, F. G., Cho, H. Y., Wuu, K. D., and Vernon, M. L., *J. natn. Cancer Inst.*, **51**, 1327 (1973).
9 Nayak, D. P., and Murray, P. R., *J. Virol.*, **12**, 177 (1973).
10 Bernhard, W., *Cancer Res.*, **20**, 712 (1960).
11 Committee, *J. natn. Cancer Inst.*, **37**, 395 (1966).
12 Dalton, A. J., *J. natn. Cancer Inst.*, **49**, 323 (1972).
13 Dalton, A. J., and Haguenau, F., *Ultrastructure of animal viruses and bacteriophages*, 255 (Academic Press, New York, 1973).
14 Nermut, M. V., Frank, H., and Schafer, W., *Virology*, **49**, 345 (1972).

Cancer, autoimmunity and IgA-deficiency related by a common antigen–antibody system

WORKERS[1-5] have reported IgG-precipitating antibodies against predominantly high molecular weight ruminant immunoglobulins to be present in the sera of certain patients with IgA deficiencies. The most frequently identified antigen is bovine IgM but we have also found a high frequency of precipitating antibodies to bovine associated mucoprotein (BAMP) (Table 1). Immunochemical studies have demonstrated that the antibodies to these two proteins are entirely distinct and that antibodies to BAMP in human sera can only precipitate with a fraction of the antigenic determinants detected by antisera raised in rabbits against BAMP.

Bovine associated mucoprotein is a constituent of the fat globule membrane which is formed by vesicular secretion from

the alveolar epithelial cells of the bovine mammary gland. Jackson *et al.*[6] determined a molecular weight of 123,000 for BAMP by analytical ultracentrifugation, although lyophilised and dissolved BAMP elutes in the void volume from Sephadex G-200. BAMP contains 25% carbohydrate of which one-fifth is sialic acid, probably as a terminal residue[6].

Because of the reported occurrence of autoantibodies in IgA-deficient patients[7,8] and because of the epithelial origin of BAMP, routine indirect immunofluorescence screening of human biopsy material was undertaken to determine whether human or rabbit antibodies to BAMP could react with human epithelial tissue. The results of this study are summarised in Table 2 and representative data shown in Fig. 1. Characteristics of the homologous system are the fluorescence of the entire cell membrane of the secretory epithelial cells, and in the mammary gland, an accumulation of BAMP on the secretory surface (lumenal) of these cells (Fig. 1*a*). All normal human biopsies were negative but striking cell membrane fluorescence was seen on all diagnosed human epithelial cell tumours, 12% of the renal transplant biopsies, many foetal epithelial cell membranes and in systemic lupus erythematosis (SLE) skin biopsies. The positive human cells (Fig. 1*b–d*) were always epithelial and showed the same characteristic 'rim' fluorescence seen in bovine tissues (Fig. 1*a*). When the sera from patients with precipitating antibodies to BAMP were tested on sections of human mammary carcinoma or normal bovine tissue in the indirect assay, positive fluorescence was obtained with both human and bovine tissues although it was markedly more pronounced with the human tissue.

The specificity of the fluorescence for a specific antigen related to BAMP on human and bovine tissues was confirmed by four different inhibition experiments.

The occurrence of positive pathological biopsies prompted our investigations of serum from these patients. Of those sera available, the correlation between the presence of serum antibodies to BAMP and BAMP-positive biopsies in patients with SLE was (2/2), mammary carcinoma (3/3), melanoma (1/3) and positive kidney biopsies (1/5). The occurrence of precipitating antibodies in the sera of patients suffering from similar disorders as those with BAMP-positive biopsies, sera from patients with related disorders and normal serum donors is summarised in Table 1.

Data were obtained by immunodiffusion assays with or without the addition of polyethylene glycol (PEG)[9]. All positive precipitin reactions could also be detected in the absence of PEG using cross-immunoelectrophoresis. No evidence was obtained for heterogeneity of antibodies to BAMP among patients with different disorders. The greatest apparent amount of precipitating antibodies occurred in patients with mammary carcinoma.

No clear-cut evidence for a BAMP-related tumour antigen in the sera of patients was obtained by: (1) precipitation in agar gel using rabbit antiserum to BAMP and (2) inhibition of capillary agglutination[10]. The sensitivity of the latter was 8 ng of BAMP.

The data presented here are the first to relate previous clinical and certain experimental evidences which correlate IgA-deficiency, incidence of neoplasms, transplantation and autoimmunity through a common immunochemical system—BAMP and anti-BAMP. The best interpretation of our data suggests that BAMP is a bovine protein that is antigenically related to a carcinoembryonic antigen in man. Hence, BAMP can be called a phylo-carcinoembryonic antigen. In

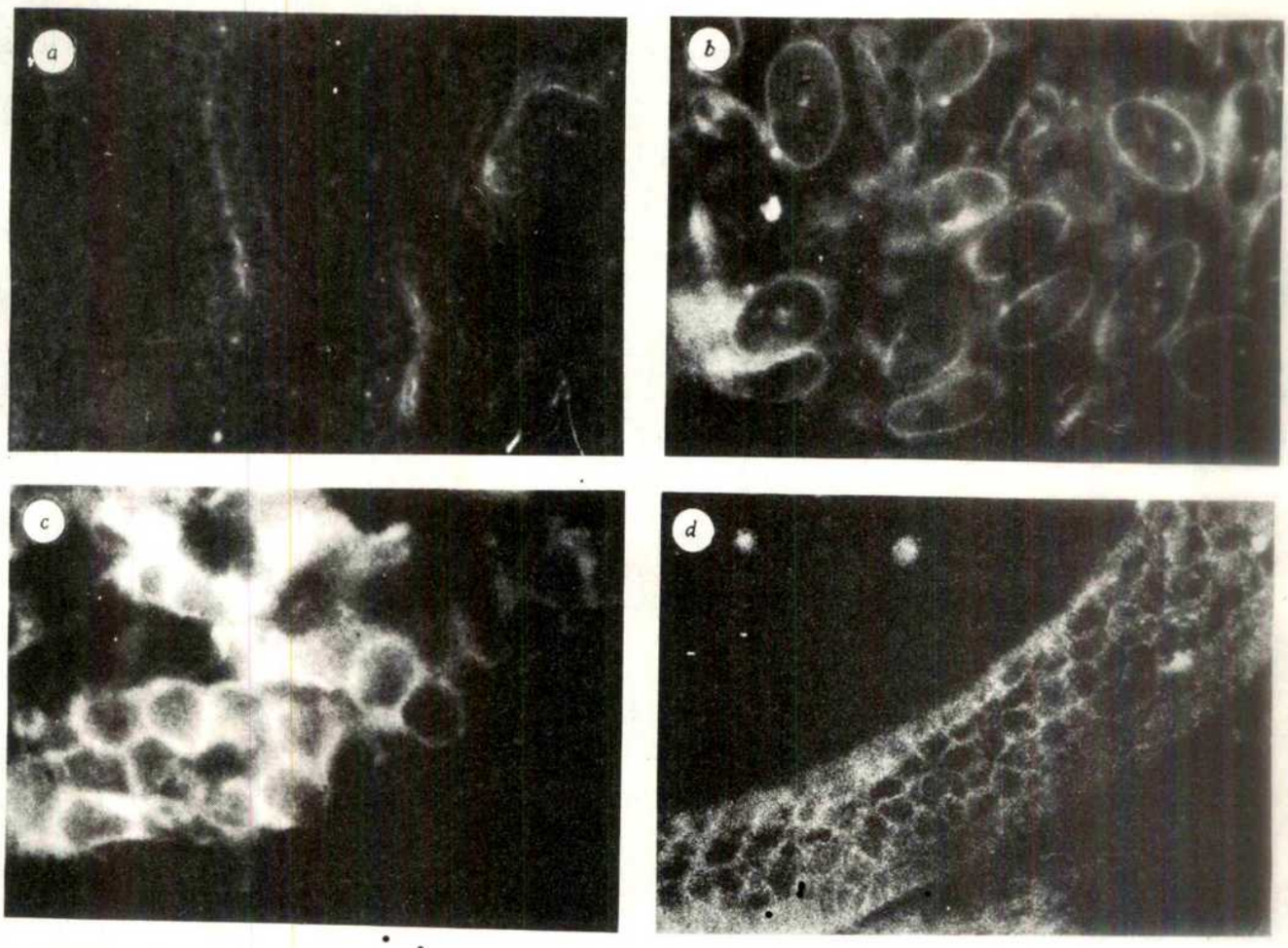


Fig. 1 Indirect fluorescence localisation. *a*, Bovine mammary gland treated with rabbit anti-BAMP (homologous system); *b*, human optic melanoma treated with rabbit-anti-BAMP; *c*, human mammary carcinoma treated with rabbit-anti-BAMP; *d*, human SLE skin biopsy treated with rabbit anti-BAMP.

Table 1 Occurrence of precipitating antibodies to BAMP

	No. tested	No. positive	Group %
IgA deficiencies			75
Antibodies to cow's milk immunoglobulins	11	8	
Nasal-pharyngeal carcinoma	1	1	
Autoimmune phenomenon			90
Systemic lupus erythematosis	9	8	
Rheumatoid arthritis	1	1	
All cancers			45
Nasal-pharyngeal carcinoma	5	2	
Mammary carcinoma	3	3	
Optic melanoma	6	1	
Metastatic islet cell tumour	1	1	
Prostatic cancer	1	1	
Adenocarcinoma	6	2	
All other pathologies			22
Serum from kidney patients	5	1	
Gastritis	2	1	
Crohns disease	8	2	
Alcohol liver disease	3	0	
Normal blood	25	2	8

contrast to the previously described carcinoembryonic antigen (CEA) and α-foetoprotein (AFP), the diagnostic feature of our system is the occurrence of precipitating antibodies to BAMP, whereas the reverse is true for CEA and AFP. The absence of BAMP on 127 normal biopsies and seven lymphomas and its presence on all diagnosed epithelial cell neoplasms, argues against the possibility of BAMP being a histocompatibility antigen.

The glycoprotein nature of BAMP is not unlike previously described tumour antigens and the immunochemical and physicochemical properties of BAMP are similar to those reported for CEA[11-13].

Our findings show that anti-BAMP also occurs in conditions not generally regarded as neoplastic. SLE and rheumatoid arthritis (RA) are characterised by autoimmune phenomena and share certain clinical and pathological features[14]. IgA deficiency is not uncommon in patients with SLE[15] and one-third of the BAMP-positive SLE patients in this study had IgA levels lower than 70 mg%. The occurrence of precipitating antibodies to a variety of undefined homologous and heterologous antigens has also been reported in SLE and RA[16]. A correlation between IgA deficiency and autoimmunity has been reported[17] and the association between thymus function and autoimmune disease seems genuine[18]. The presence of BAMP and anti-BAMP in renal transplant patients is not inconsistent with the reported correlation between neoplastic tissue and transplantation[19] or with reports of heterophile antibodies in transplant patients[20].

The data presented here also offer a new interpretation of reports on the occurrence of antibodies to ruminant immunoglobulins in patients with IgA deficiency[1-5]. The presence of these antibodies has generally been ascribed to the greater absorption of macromolecules by the digestive tract of such patients. The reports that IgA-deficient patients also have autoantibodies to human IgA and human IgM[7,8] instead suggests that the occurrence of antiruminant antibodies could be a reflection of an autoimmune phenomenon or immune surveillance defect. In this study, (1) the failure of human sera to precipitate with only a portion of BAMP detected by anti-rabbit BAMP and (2) the greater degree of fluorescence of human carcinoma tissue compared with bovine mammary tissue obtained using human antibodies to BAMP, both suggest that the antibodies in human serum were formed in response to the human BAMP-related antigen and only cross react with BAMP. An antigen has been partially purified from a mammary carcinoma which cross reacts with BAMP and the serum of this patient contains antibodies to the human antigen.

Cell-mediated immunity is generally regarded as most important in the body's elimination of neoplastic tissue[21] and the specificity control at the T-cell level has been suggested to be of great importance in the prevention of autoimmune responses[18,22]. Reports have suggested that complete development of the IgA system depends on a functioning thymus[23-25]. From this, the occurrence of BAMP or anti-BAMP may be correlated with pathological conditions in which a thymic malfunction is probable.

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[1] Buckley, R. H., and Dees, S.C., *New. Engl. J. Med.*, **281**, 465 (1969).

Table 2 Occurrence of BAMP-positive biopsies

Nature of tissue	No. tested	No. positive	Localisation site	Relative intensity
Bovine mammary gland, adult ileum, lacrimal and parotid glands and lungs	18	18	Secretory, mucosal or glandular epithelial cell membrane	****
Normal human skin, lung, ileum, colon, liver, oesophagus, adrenal and mammary gland	127	0		
Human foetal ileum, colon, prostate, kidney, thymus and tonsil	7	6	Epithelial cell membrane	**
Human foetal pharynx, lip, tongue, skin, lung, oesophagus, stomach, brain, spleen, spinal cord, muscle, testes, placenta, umbilical stump and liver	20	0		
All diagnosed epithelial cell cancer				
Human breast carcinoma	3	3	Secretory epithelial cell membrane	***
Human optic melanoma	3	3	Cell membrane	***
Human retinal balstoma	1	1	Cell membrane	***
Thyroid carcinoma	1	1	Small epithelial membrane	***
All other				
Human renal transplants	39	5	Tubular epithelial cell membrane	***
Skin from SLE patients	2	2	Epithelial cell membrane	***
Human prostatic tumour	1	0		
Various lymphomas	7	0		
Human adenocarcinoma	2	1	Epithelial cell membrane	**
Normal mouse tissue (epithelial)	12	0		
Normal rat tissue (epithelial)	23	0		

[2] Hyslop, N. E., jun., Kern, K., and Butler, J. E., *Fedn Proc.*, **31**, 932 (1972).
[3] Tomasi, T. B., jun., and Katz, L., *Clin. exp. Immun.*, **9**, 3 (1971).
[4] Ammann, A. J., and Hong, R., *Medicine (Baltimore)*, **5**, 223 (1971).
[5] Leikola, J., and Vyas, G. N., *J. Lab. clin. Med.*, **77**, 629 (1971).
[6] Jackson, R. H., Coulson, E. J., and Clark, W. R., *Archs Biochem. Biophys.*, **97**, 373 (1962).
[7] Wells, J. V., Bleumers, J. F., and Fudenberg, H. H., *Clin. exp. Immun.*, **12**, 305 (1972).
[8] Ammann, A. J., and Hong, R., *Lancet*, June, 1264 (1971).
[9] Herrington, J. C., Trenter, J. W., II, and Pert, J. H., *Immunochemistry*, **8**, 413 (1971).
[10] Caldwell, J. L., Severson, C. D., and Thompson, J. S., (in the press).
[11] Krupey, J., Gold, P. and Freedman, S. C., *Nature*, **215**, 67 (1967).
[12] Mach, J. P., and Pusztaszeri, G., *Immunochemistry*, **9**, 1031 (1972).
[13] Colligan, J. E., Lautenschleger, J. T., Egan, M. L., and Todd, C. W., *Immunochemistry*, **9**, 377 (1972).
[14] Vaughan, J. H., and Christian, C. L., *Immunological Disease*, **2**, (edit. by Samter, M.) 997, 1014 (Little, Brown and Co., Boston, 1971).
[15] Heremans, J. F., and Crabbé, P. A., *Birth Defect Orig., Article Series* 4 (edit. by Bergsma, D., and Good, R. A.), 298 (1967).
[16] Anderson, J. R., Gray, K. G., Beck, J. S., and Buchanan, W. W., *Ann. Rheum. Dis.*, **21**, 360 (1965).
[17] Nell, P. A., Ammann, A. J., Hong, R., and Stiehm, E. R., *Pediatrics*, **49**, 72 (1972).
[18] Anderson, R. R., Buchanan, W. W., and Goudie, R. B., *Autoimmunity*, (Charles C. Thomas, Springfield, Illinois, 1967).
[19] Good, R. A., *Proc. natn. Acad. Sci., U.S.A.*, **69**, 1026 (1972).
[20] Svehag, S. E., Olander, R., and Sundquist, K. G., *Clin. exp. Immun.*, **13**, 191 (1973).
[21] Smith, R. T., and Landy, M., *Immune Surveillance* (Academic Press, New York, 1970).
[22] Katz, D. H., and Benacerraf, B., *Adv. Immun.*, **15**, 1 (1972).
[23] Perey, D. Y. E., Frommel, D., Hong, R., and Good, R. A., *Lab. Invest.*, **22**, 212 (1970).
[24] Perey, D. Y. E., and Bienenstock, J., *J. Immun.*, **111**, 633 (1900).
[25] Clough, J. D., Mims. L. H., and Strober, W. J., *J. Immun.*, **106**, 1624 (1971).

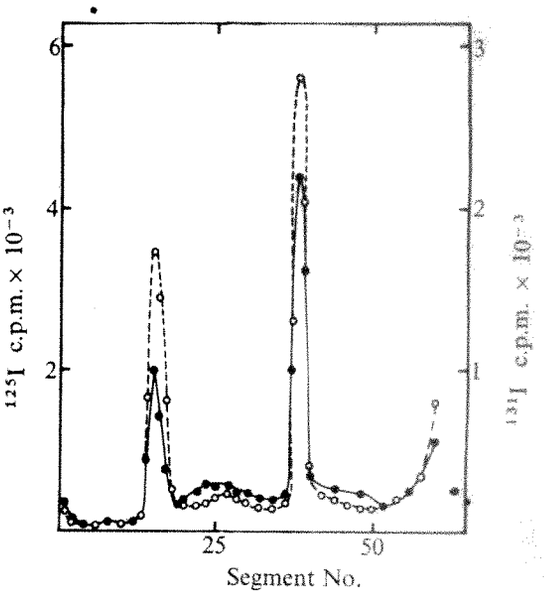


Fig. 1 SDS-polyacrylamide gel electrophoresis of immune complexes between anti-H-2D^a and a ^{131}I-labelled H-2 alloantigen preparation and between anti-H-2K^a and ^{125}I-labelled H-2 alloantigen. The H-2 containing materials were radioactively labelled with iodine by the chloramine T procedure[10]. The immune complexes were isolated by gel chromatography on Sephadex G-200. Gels with an acrylamide concentration of 15% prepared as described by Neville[11] were used. After completion of the electrophoretic separation the gel was divided into segments of 2 mm width and counted for radioactivity in a γ-well type scintillation counter. ●——●, ^{125}I; ○ - - ○, ^{131}I.

Subunit structure of H-2 alloantigens

HISTOCOMPATIBILITY antigens carry the immunological determinants involved in tissue transplant rejection[1]. Recent evience has shown that papain-solubilised HL-A antigens are composed of two polypeptide chains[2,3]. The smaller of the solubilised HL-A polypeptide chains has been shown[4,5] to be identical to β_2-microglobulin and it has also been shown that β_2-microglobulin is physically linked to the HL-A antigens on the cell surface[6–9]. These findings raised the question of whether the major transplantation antigens from other species are composed of subunits. This communication shows that mouse H-2 alloantigens are linked to a small polypeptide chain which is homologous with human β_2-microglobulin.

Papain-solubilised H-2 alloantigens were isolated from 600 A/J spleens essentially as described for HL-A antigens[4]. To achieve further purification, part of the alloantigen containing material was labelled with ^{125}I and ^{131}I (ref. 10) and incubated separately with H-2K^a and H-2D^a alloantisera. The resulting immune complexes were isolated by gel chromatography and subjected to SDS-polyacrylamide gel electrophoresis[11]. From each incubation, only two radioactive bands were apparent after electrophoresis (Fig. 1). Using protein standards of known molecular weight it could be calculated that the two radioactive zones exhibited molecular weights of about 12,000 and 40,000, respectively. Control experiments with normal mouse serum from A/J mice did not give any ^{125}I- or ^{131}I-labelled material appearing in the position of the immune complexes on gel chromatography. Thus, we conclude that highly purified papain-solubilised H-2 alloantigens are composed of two polypeptide chains, irrespective of whether they are coded for by the K or the D end of the histocompatibility locus.

An antiserum against human β_2-microglobulin was incubated with a fraction containing ^{125}I-labelled H-2 alloantigen. The resulting immune complex was isolated and subjected to SDS-polyacrylamide gel electrophoresis. The electrophoretic profile revealed only the presence of the small H-2 alloantigen polypeptide chain which had migrated exactly as human β_2-microglobulin. We have shown that the antiserum used dissociates β_2-microglobulin from the larger HL-A chain[4] and it seems to have the same effect with H-2 antigens. This result strongly suggests that the small H-2 alloantigenic polypeptide chain is highly homologous with human β_2-microglobulin.

The two H-2 polypeptide chains were separated from each other by gel chromatography in 3 M KCl. The smaller polypeptide chain, labelled with ^{125}I, and ^{131}I-labelled human β_2-microglobulin were subjected to acid-urea starch gel electrophoresis[12,13] (Fig. 2). It can be seen that human β_2-microglobulin exhibited an electrophoretic mobility indistinguishable from that of the mouse counterpart. On reduction and alkylation both proteins displayed a retarded mobility compared with the untreated material, signifying that the mouse protein may contain a disulphide loop similar to that of human β_2-microglobulin[14,15].

Further evidence for the great similarity between β_2-microglobulin and the small H-2 alloantigen polypeptide chain was obtained at primary structure level by peptide mapping experiments. Although chymotryptic as well as tryptic digests revealed peptides that were unique for the mouse protein, the majority of the peptides seemed to be shared between the two proteins. These data favour the hypothesis that the small H-2 alloantigen is the mouse homologue of human β_2-microglobulin.

The linkage of the mouse homologue of β_2-microglobulin to the larger H-2 alloantigen polypeptide chain may be a fortuitous result of the solubilisation procedure. Attempts were therefore made to establish a connection between the two polypeptides on the cell surface. It could thus be demonstrated that antibodies against human β_2-micro-

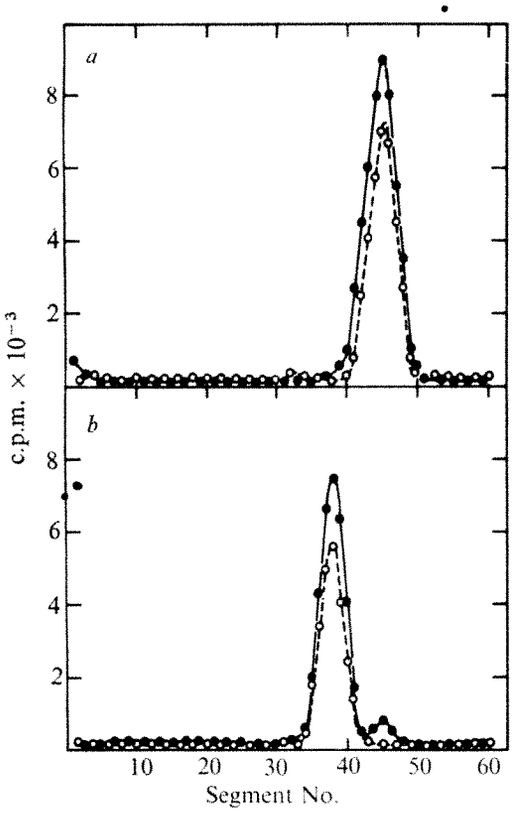


Fig. 2 Urea-starch electrophoresis at *p*H 3.0 according to a modification[13] of the procedure outlined by Edelman and Poulik[12]. *a*, Untreated samples of the small H-2 alloantigen polypeptide chain labelled with ^{125}I and human β_2-microglobulin labelled with ^{131}I. *b*, The same material reduced with 0.1 M mercaptoethanol in 8 M urea for 30 min and alkylated with 0.12 M iodoacetic acid in the dark for 30 min. The radioactive polypeptides were isolated from the labelled antigens by gel chromatography in 3 M KCl. The electrophoresis was run for 2 h at a voltage of 22 V cm^{-1}. After completion of the electrophoresis the gels were divided into segments of 2 mm width and assayed for radioactivity in a γ-well type scintillation counter. ●——●, ^{125}I; O - - O, ^{131}I.

globulin lysed more than 85% of mouse spleen cells in the presence of complement[7]. Fab'-fragments[14] against human β_2-microglobulin could completely abolish the cytotoxic effect of both H-2K^a and H-2D^a alloantisera (compare with ref. 7). Also, antibodies against human β_2-microglobulin cocapped FITC-labelled antibodies directed against H-2K^a or H-2D^a (ref. 6). It is thus conceivable that the mouse β_2-microglobulin counterpart is a true subunit of the H-2 alloantigens coded for both by the K and D ends of the major histocompatibility complex not only when liberated from the cell membrane but also on the intact cell surface.

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[1] Committee Report *Fedn Proc.*, **31**, 1087–1103 (1972).
[2] Cresswell, P., Turner, M. J., and Strominger, J. L., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1603–1607 (1973).
[3] Tanigaki, N., Katagiri, M., Nakamuro, K., Kreiter, V. P., and Pressman, D., *Fedn Proc.*, **32**, Abstr. 1017 (1973).
[4] Peterson, P. A., Rask, L., and Lindblom, B., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 35–39 (1974).
[5] Nakamuro, K., Tanigaki, N., and Pressman, D., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2863–2865 (1973).
[6] Östberg, L., Lindblom, J. B., and Peterson, P. A., *Nature*, **249**, 463–465 (1974).
[7] Lindblom, J. B., Östberg, L., and Peterson, P. A., in *Tissue antigens* (in the press).
[8] Poulik, M. D., Bernoco, M., and Ceppelini, R., *Science*, **182**, 1352–1355 (1973).
[9] Solheim, B. G., and Thorsby, E., in *Tissue antigens* (in the press).
[10] Hunter, W. M., and Greenwood, F. C., *Nature*, **194**, 495–496 (1962).
[11] Neville, D. M., *J. biol. Chem.*, **246**, 6328–6334 (1971).
[12] Edelman, G. M., and Poulik, M. D., *J. exp. Med.*, **113**, 861–871 (1961).
[13] Peterson, P. A., and Berggard, I., *J. biol. Chem.*, **246**, 25–33 (1971).
[14] Peterson, P. A., Cunningham, B. A., Berggard, I., and Edelman, G. M., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1697–1701 (1972).
[15] Cunningham, B. A., Wang, J. L., Berggard, I., and Peterson, P. A., *Biochemistry*, **12**, 4811–4822 (1973).

Induction of lymphokine production by EAC and of blastogenesis by soluble mitogens during human B-cell activation

Two groups of human lymphocytes are distinguished on the basis of immune effector functions, membrane markers and *in vitro* mitogen reactivity. Thymus-derived (T) lymphocytes mediate cellular immunity either directly or by secreting pharmacologically active lymphokines[1], and are implicated in facilitating antibody responses[2]. In contrast, bone marrow-derived (B) lymphocytes are thought to produce only antibodies. Studies in the guinea pig, however, indicate that, appropriately stimulated, B cells can produce macrophage migration inhibitory factor (MIF)[3]. We have now found that the erythrocyte–antibody (19S)–complement (EAC) rosetting method, used to obtain pure human peripheral blood B cells, activates these B cells to produce mononuclear cell chemotactic (CTX) and mitogenic (MF) lymphokines. Furthermore, we have investigated the mechanism of B cell activation by EAC and the effects of mitogenic lymphokines on human T and B lymphocytes.

T and B lymphocytes were obtained as described in the legend to Table 1. Between 45 and 75% of peripheral blood lymphocytes were recovered as E-rosette-forming cells and 5–35% as EAC-rosette-forming cells. These high rates of recovery suggest that the subsequent biological studies are representative of T and B cell functions.

Purity of T and B cells was assessed by immunofluorescent staining of membrane-associated immunoglobulins, a characteristic of B cells. Different anti-human immunoglobulin sera were used: two conjugated with fluorescein (C20F-341, Meloy Laboratories), and 3031612 (Baltimore Biological Laboratories, Cockeysville, Maryland) and the other with rhodamine (6481, Cappel Laboratories, Dowingtown, Pennsylvania). All sera were titred to find their optimum staining concentrations. Rosetted cells (1.0×10^6) were treated with isotonic ammonium chloride lysing buffer, washed and kept at 3° C. A volume of 0.1 ml conjugated serum was added and the pelleted cells were incubated for 30 min at 4° C, washed three times in chilled azide-BSA buffer and wet mounts were made. In four experiments, the mean purity of T cells was 95% ± 1 and of B cells, 97% ± 2 with < 0.5% monocyte contamination in either population.

Table 1 Blastogeneic responses of human T and B lymphocytes

	T cells		B-EAC		Dissociated B cells	
	N	mean	*N*	mean	*N*	mean
Control	(19)	87±13	(15)	157±33	(4)	135±12
PHA (1 μg per well)	(19)	16,404±2,934	(17)	4,926±981	(4)	5,513±3,404
Con A (10 μg per well)	(14)	9,687±2,471	(12)	1,780±685	(3)	4,002±2,282

T and B cells (93–99% purity) were prepared from FH-separated (30–200ml) heparinised peripheral blood by a modification of a sequential rosetting technique unpublished results of W. West, J. McCoy, T. Doering and R. Herberman. Washed mononuclear cells were sequentially rosetted at 30° C with sheep erythrocytes (E) and foetal calf sera (heat-inactivated E absorbed). T cells were separated from non-rosetting cells on two consecutive Ficou-Hypaque (FH) gradients. NRFC were then rosetted with E coated with (19S) anti-E antibody-mouse complement (EAC) at 37° C and EAC-rosette-forming cells separated on two consecutive FH gradients. B-EAC-rosettes were dissociated with rabbit anti-C3 globulin (1 mg IgC per 1×10^7 cells; Cappel Laboratories) at 37° C for 30 min. Dissociated B cells were separated from EAC on FH gradients, washed and diluted to 2×10^6 viable cells per ml in RPMI-1640 (NIH) supplemented with a final concentration of 0.5% human AB sera and aliquoted in 0.1 ml volumes into microtitre trays. B and T cells with their adherent rosettes cells were diluted and aliquoted in a similar manner. The test mitogens PHA or con A were added in 0.1 ml volumes to triplicate wells and trays cultured for 72–96 h in 5% CO_2–95% air at 37° C; 18 h before collection 3.0 μCi of ^{3}H–TdR (6 Ci mol^{-1}, Schwarz/Mann) was added to each well. Cultures were processed automatically and total radioactivity incorporated into DNA was assessed. *N* represents the number of independent experiments used to calculate the results. Data are expressed as the mean c.p.m. of triplicate cultures ± s.e.

T and B cells with adherent E or EAC rosettes were placed in culture (as described in the Table legends) and stimulated with medium, concanavalin A (con A) or phytohaemagglutinin (PHA). Levels of incorporation of tritiated thymidine (^{3}H-TdR) in control unstimulated cultures in both populations were quite low (Table 1); with a mean of 87 c.p.m. ± 13 for T cells and 157 ± 33 for B cells. Mitogen-stimulated T cells incorporated considerable ^{3}H-TdR and to a lesser degree B cells were also stimulated by soluble PHA and con A.

We investigated whether B cell responses to mitogens were due to PHA and con A adsorbed on the EAC, since particulate mitogens stimulate B cells[4]. B cells were dissociated from erythrocytes with anti-C3 antibody[5] and separated by Ficoll-Hypaque (FH) gradient centrifugation. Dissociated B cells were viable as assessed by trypan blue exclusion and still gave PHA and con A-induced proliferative responses equivalent to B-EAC cell cultures. Even transient EAC binding to C3 receptors may have lowered the threshold for B cell stimulation so that the subsequent interaction with soluble mitogens induced blastogenesis.

We then investigated whether purified T and B cells can be activated by soluble mitogens to make mediators as well as to proliferate. Supernatants from medium, PHA and con A-stimulated T and B cell cultures were assessed for CTX activity as before[6]. Table 2 shows that supernatants from T cell cultures which had been activated with PHA or con A to synthesise DNA also had appreciable CTX activity (30–60 cells per 20 fields), while unstimulated control supernatants, reconstituted after culture with mitogen, had essentially none (8–9 cells per 20 fields). The mean values of the reconstituted controls were equivalent to those obtained with medium. In contrast, all B cell supernatants contained equal levels of CTX activity irrespective of whether they were from unstimulated, reconstituted or mitogen-stimulated cultures. The generation of CTX activity in B cell cultures did not correlate with ^{3}H-TdR incorporation (Table 1). The CTX activity in both control and mitogen stimulated B-EAC cell supernatants was two to five times greater than in supernatants from mitogen-stimulated T cell cultures, suggesting that CTX activity in B-EAC cell culture supernatants was not derived from the few contaminating nonimmunoglobulin-bearing cells. Furthermore, mitogen-stimulated B-EAC cultures did not contain more CTX than supernatants from control cultures. This indicates that the contaminating cells were not producing lymphokines, as would be expected if they were T cells; this is another indication of the purity of our B cell preparations.

To assess whether the attachment through the C3 receptor non specifically induced the production of lymphokines, B-EAC cell rosettes were dissociated with rabbit anti-mouse C3 globulin[5] (as described in Table legends) and dissociated B cells were cultured with or without mitogen. Supernatants from unstimulated B cells dissociated from EAC were devoid of high CTX activity compared with supernatants from cultures of undissociated B-EAC cell rosettes (Table 2). These data suggests that the attachment of EAC to B cells stimulates lymphokine production. But EAC binding was either insufficient to cause concomitant B cell blastogenesis or blastogenesis requires a different signal. In control experiments, E and EA (19S antibody) which do not bind to B cells failed to stimulate either lymphokine production or blastogenesis; however, EA (7S antibody) which binds to B cell membrane Fc receptors also induced B cell lymphokine production (B. F. M., L. C. A., and J. Jardszewski, unpublished data). Therefore, the induction of B cell lymphokine production seems to be facilitated by agents which bind to specific B cell membrane receptors. The biological activity of B cells dissociated from their adherent EAC was further assessed in terms of their ability to produce lymphokines after soluble mitogen stimulation (Table 2). Con A and PHA stimulation of dissociated B cell cultures induced CTX supernatant activity comparable with that of B-EAC cultures and twice as great as those of mitogen-stimulated T cells. The dissociated B cell culture supernatant levels of CTX were too high to be attributed to the low percentage of contaminating immunoglobulin-negative cells; however, we cannot ignore the possible contribution of helper cell effects. Table 2 suggests that with appropriate stimulation both human T and B lymphocytes produce lymphokines. Further, lymphokine was produced in B-EAC cultures in the absence of concomitant blastogenesis; an observation consistent with the reported dichotomy between lymphocyte proliferation and macrophage inhibitory factor synthesis[7,8].

CTX activity in B cell supernatants was not derived from the two chemotactic complement peptides, C3a and C5a. First, B cells purified using EAC prepared with C5 deficient mouse sera still generated CTX activity equivalent to B cells separated with EAC prepared with normal mouse sera and second, the treatment of B cell supernatants with antisera to C3 and C5 did not appreciably decrease their chemotactic activity; specificity of antisera was assayed by immunodiffusion. The CTX activity found in B cell culture supernatants was therefore presumed to be produced by B cells and seems to have physiochemical characteristics similar to those produced by T cells (L. C. A., B. F. M., and B. Chassy, in preparation).

Supernatants from T and B cell cultures were also assessed for MF. We asked two questions: first, could purified T and B cells produce MF and second, could B, as well as T cell MF stimulate pure T or B cells (Table 3).

Table 2 Chemotactic lymphokine activity in T and B lymphocyte culture supernatants

	T cell supernatants			B-EAC cell supernatants			Dissociated B cell supernatants		
	N	reconstituted	stimulated	E/C	*N*	reconstituted	stimulated	E/C	*N*	reconstituted	stimulated	E/C
Medium	(5)	—	9±2	—	(6)	—	93±7	—	(6)	—	22±6	—
PHA (μg ml^{-1})	(9)	9±2	66±3	7.3	(4)	82±2	99±14	1.2	(2)	18±3	104±11	5.7
Con A (10 μg ml^{-1})	(7)	8±1	30±2	3.8	(2)	134±12	142±10	1.1	(2)	18±3	114%11	6.3

T and B lymphocytes at 4 × 10^6 per 2 ml culture (RPMI-1640; 0.5% human AB sera supplemented) with or without stimulant were incubated at 37° C in 5% CO_2–95% air for 24 h. B-EAC rosettes immediately after purification were treated with rabbit anti-mouse C3 globulin (1 mg IgG per 1 × 10^7 cells; 37° C for 30 min with constant shaking). Dissociated B cells were separated from EAC and undissociated B cells by two FH gradient centrifugations. Total viable dissociated B cells were counted by trypan blue dye exclusion, then cells were diluted and cultured as described. Supernatants were collected and tested for CTX activity. The test population of mononuclear cells (MNC) used in the chemotactic assays was separated from human peripheral blood on FH gradients, washed twice in Gey's balanced salt solution (*p*H 7.0) and diluted to 5 × 10^6 cells ml^{-1}. Target MNC and culture supernatants were placed in modified Boyden chambers separated by a 5 μm polycarbonate filter (Nuclepore, Wallabs Inc.). The chambers were incubated for 90 min at 37° C in air[6]; the filters were removed, stained, and the cell migration was quantitated. Results are expressed as the mean number of migrating cells per 20 oil fields ± s.e. of triplicate samples. *N* represents the number of experiments used to calculate the mean. The E/C values represent the ratios of the CTX activity in supernatants from stimulated cultures divided by the activity in their reconstituted controls.

Supernatants from con A-stimulated and reconstituted T cell cultures were passed through Sephadex G-100 to remove most added con A (ref. 9); supernatants reconstituted with con A after incubation served as controls for the mitogenic activity of any residual con A. Since there was no difference in results for autologous and allogeneic secondary cultures the data were combined. Supernatants from con A-stimulated T cell cultures could activate both T and B cells. Reconstituted T cell supernatants had slightly more mitogenic activity than control supernatants, probably due to residual con A. Supernatants from control EAC-B cells CTX activity (144 ± 5 cells per 20 fields) irrespective of the presence of T cells; control unrosetted and unstimulated mononuclear cell supernatants had no activity. Second, the addition of T cells to purified B-EAC rosette cultures in ratios of 1:10 and 1:1 neither suppressed nor increased the generation of B cell CTX activity.

We conclude first that human peripheral blood T and B lymphocytes proliferate in response to soluble mitogens previously thought to stimulate only T cells. One explanation for this is a helper effect by the few contaminating immunoglobulin negative cells. Alternatively the interaction of activated EAC-3 with B cells through their C3 receptors, may have provided an initial signal so that subsequent binding of soluble PHA or con A became sufficient to stimulate B cell blastogenesis[10]. These findings are consistent with reports that human B cells purified on immunoabsorbent columns also respond to soluble mitogens[11,12]. These immunoabsorbents, like EAC rosettes, may lower the threshold for B cell activation by binding to a membrane receptor.

Second, the binding of EAC and EA (7S) to human B lymphocytes induced CTX and MF synthesis but not concomitant blastogenesis. In contrast, PHA and con A-stimulated dissociated B cells to both proliferate and synthesise lymphokines. These data indicate that lymphokine synthesis and blastogenesis are either functions of different lymphocyte populations or require different degrees of membrane stimulation for their initiation. Finally, the demonstration that B lymphocytes produce a mitogenic lymphokine which effects T cells suggests a previously unrecognised mechanism of immune regulation.

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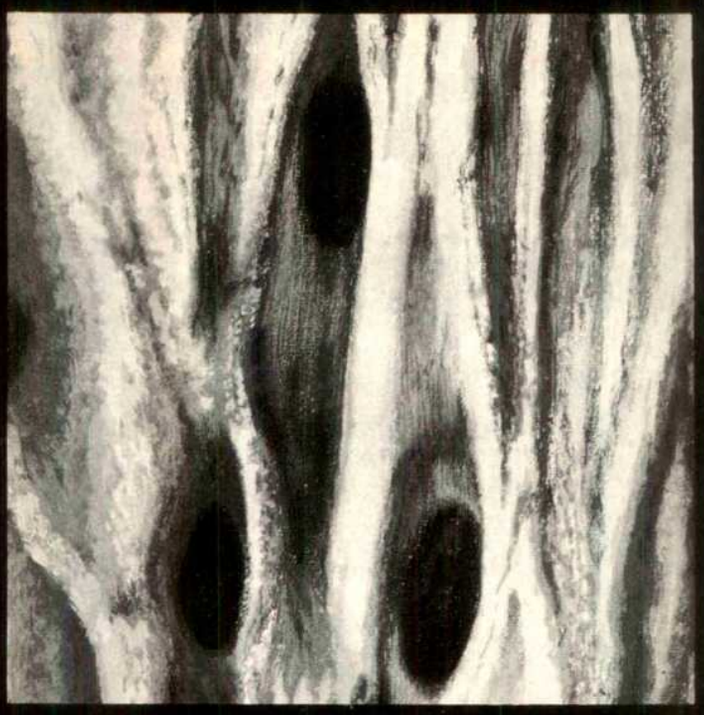
Table 3 Mitogenic lymphokine activity in supernatants of human T and B lymphocyte cultures

Primary cultures			Secondary target cell cultures					
				T lymphocytes			B lymphocytes	
cell type	stimulant	reconstituted	(*N*)	mean	E/C	*N*	mean	E/C
—	—	Medium	(3)	70±13	1.0	(4)	59±16	1.0
T	Medium	—	(3)	128±19	1.8	(4)	49±8	0.8
T	Medium	Con A	(5)	444±63	6.3	(3)	110±80	1.8
T	Con A	—	(5)	4,690±460	70.6	(3)	1,406±698	7.4
—	—	Medium	(15)	87±35	1.0	(18)	146±108	1.0
B	EAC Binding	—	(15)	429±97	4.9	(18)	579±107	4.0

T and B lymphocyte culture supernatants were prepared as described for Table 2. In experiments using con A as a stimulant, control supernatants were reconstituted after incubation with an equivalent concentration of con A (10 μg ml^{-1}) and reconstituted and stimulated (experimental) supernatants (4–6 ml) both passed through 10 ml Sephadex G-100 columns[9] (binding capacities of 300 μg con A per ml of wet G-100). Rosetted T and B target cells were prepared as previously described from allogeneic and autologous donors. A microtray system was used to assess MF activity; 0.1 ml containing 2 × 10^5 viable cells and 0.1 ml or 0.05 ml of each test supernatant or RPMI-1640 added in to each well; assays were performed in triplicate wells for each variable. Trays incubated at 37° C in 5% CO_2–95% air for 72 h; 18 h before collection 3 μCi ^{3}H-TdR was added to each well. Cultures were processed automatically and the total radioactivity incorporated into DNA assessed. *N* represents the number of supernatant pairs assessed with the results expressed as the mean (c.p.m.) ± s.e. The E/C values represent the stimulation index.

to which no mitogen was added and which did not incorporate ^{3}H-TdR also stimulated both T and B cell blastogenesis in autologous and allogeneic secondary cultures. This MF activity was not ascribed to conditioned medium effects since control T cell supernatants had no stimulatory effects. Significantly more blastogenesis occurred in EAC-B-cell-supernatant-stimulated secondary cultures than in secondary cultures to which only medium was added; however, blastogenesis did not approach that produced by con A-stimulated T cell supernatants. Thus, human B cells activated by EAC-rosetting produced MF similar to the MF in supernatants of con A-stimulated T cell cultures which activated both T and B cells. Furthermore, the incubation of purified T cells with EAC induced neither blastogenesis nor MF production indicating that EAC binding to B cells was the cause of MF synthesis.

Further control experiments were performed to assess whether lymphokine production by human B cells resulted from removal of suppressor T cells. First, B-EAC rosettes were formed but not separated from the total peripheral blood mononuclear cell population, and cultured for 24 h. Supernatants from these cultures contained high levels of



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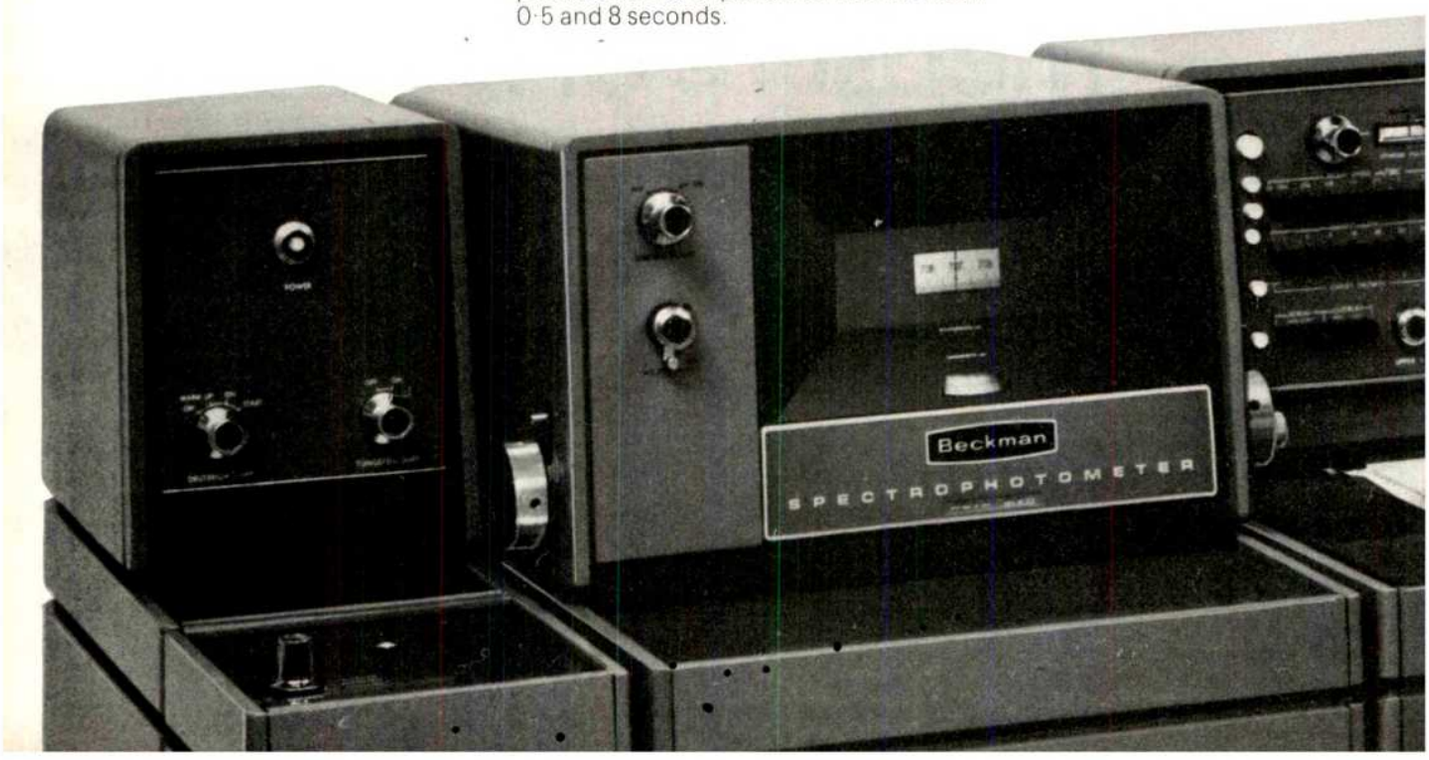
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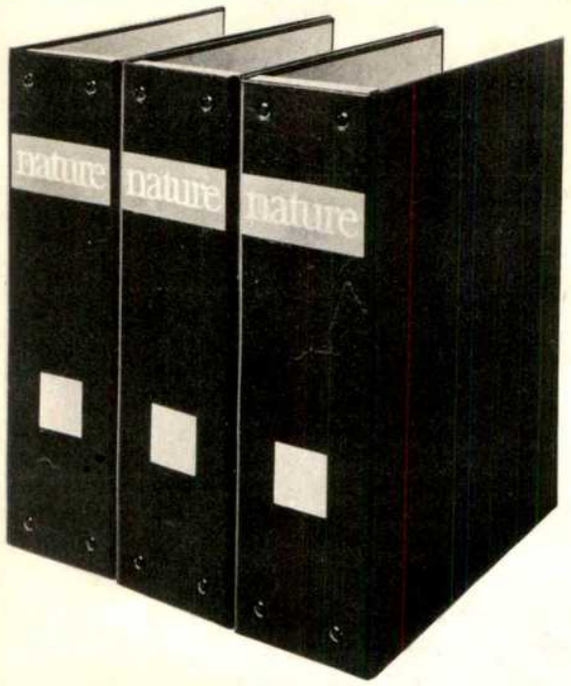
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Note added in proof: The report[14] that purified human B cells fail to proliferate in response to soluble T cell mitogens conflicts with our and other findings[11,12].

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1 Good, R. A., *Cell. Immun.*, **3**, 1 (1972).
2 Anderson, J., Möller, G., and Sjöberg, O., *Eur. J. Immun.*, **2**, 99 (1972).
3 Yoshida, T., Sonozaki, H., and Cohen, S., *J. exp. Med.*, **138**, 784 (1972).
4 Greaves, M. F., and Barminger, S., *Nature new Biol.*, **235**, 67 (1972).
5 Bianco, C., Patrick, R., and Nussenzweig, V. J., *J. exp. Med.*, **132**, 702 (1970).
6 Altman, L. C., Snyderman, R., Oppenheim, J. J., and Mergenhagen, S. E., *J. Immun.*, **110**, 801 (1973).
7 Chaparas, S. D., Thor, D. E., Godfrey, H. D., Baer, H., and Hedrick, S. T., *Science*, **170**, 637 (1970).
8 Rocklin, R. E., and Ratcliffe, H. E., *Fedn Proc.*, **31**, 753 (1972).
9 Mackler, B. F., Wolstencroft, R. A., and Dumonde, D. C., *Nature new Biol.*, **239**, 139 (1972).
10 Dukor, P., and Hartmann, K. U., *Cell Immun.*, **7**, 349 (1973).
11 Phillips, B., and Roitt, I. M., *Nature new Biol.*, **241**, 254 (1973).
12 Chess, L., MacDermott, R. P., and Schlossman, S. F., *Fedn Proc. Abstr.* (1974).
13 Mackler, B. F., Altman, L. C., Wahl, S., Rosenstreich, P. L., Oppenheim, J. J., and Mergenhagen, S. E., *J. dent. Res.*, **53**, 225 (1974).
14 Geha, R. S., Rosen, F. S., and Merler, E., *Nature*, **248**, 426 (1974).

Altered metabolism of carcinoembryonic antigen in hamsters bearing GW-39 tumours

THE liver is well known for its role in the catabolism of a variety of plasma glycoproteins[1]. Liver-mediated clearance of a tumour glycoprotein antigen, the carcinoembryonic antigen (CEA) associated with human gastrointestinal adenocarcinomas[2], has been demonstrated in dogs and rabbits[3]. Since circulating CEA is being extensively investigated for its diagnostic value in the detection of cancer patients, we were prompted to determine the metabolism of CEA in a xenogeneic species which supports the growth of a CEA-producing colonic cancer.

Female Syrian hamsters, 110–180 g, were heterografted with a CEA-producing colonic adenocarcinoma of human origin continuously propagated in hamsters[4]. CEA, purified from liver metastases of human colonic adenocarcinomas (E. S. Newman, H. J. Hager and H. J. H., manuscript in preparation), was radiolabelled with ^{125}I by the chloramine-T method[5]. Animals were injected intracardially with 7–9 μCi of aggregate-free ^{125}I-CEA (40-80 μCi μg^{-1}) diluted in phosphate buffered saline containing 10% autologous serum. At timed intervals, each animal was exsanguinated by cardiac puncture, its organs were removed and radioactivity was determined in each and in 1 ml of plasma, using a Model 3375 Packard gamma scintillation counter. The radioactive material in the plasma, urine, and liver was characterised by Sepharose 6B column chromatography and by immunoreactivity with goat anti-CEA antibody in the radioimmunoassay developed by Hansen *et al.*[6].

Figure 1 shows that in normal hamsters, 80–90% of the injected radioactivity was cleared by the liver within 5 min. This level of radioactivity was maintained in the liver until 30 min, at which time it began to decrease. Initially, 10–20% of the injected radioactivity was not cleared from the circulation; at 12 h, less than 5% was recovered from either the liver or blood. The accumulation of radioactivity in the urine coincided with its disappearance from the liver and by 24 h, 95% of the injected dose of radioactivity was found in the urine. At all times, the distribution of radioactivity was less than 1.0% in the spleen, kidneys, lungs, heart, and faeces, whereas 10–30% localised in the intestinal tract. The radioactivity reached a peak of 20–35% within the small bowel at 3 h and then declined, while a maximum of 10–15% was recovered in the large bowel 3 h later.

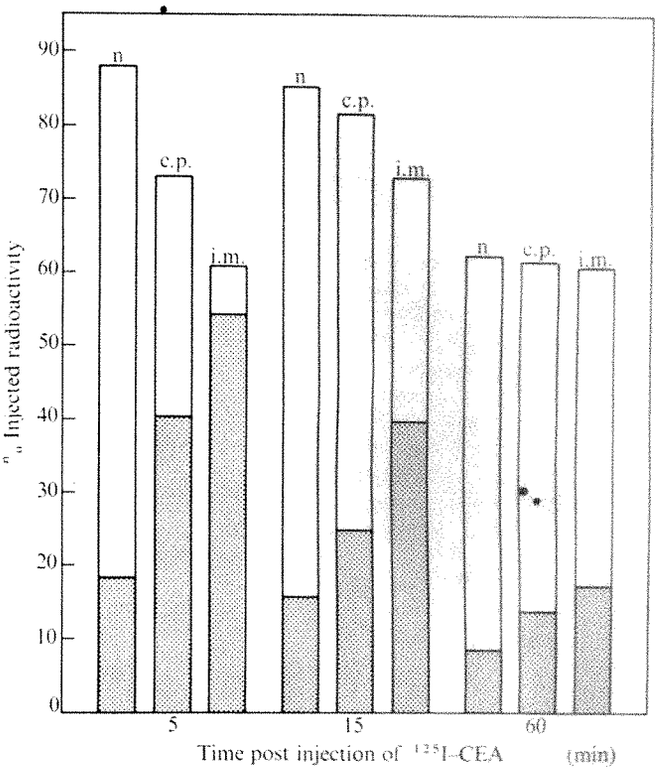


Fig. 1 Clearance of ^{125}I-CEA in normal and GW-39 tumour-bearing hamsters. Shaded area of each bar is the percentage of radioactivity remaining in the plasma of normal hamsters (n), and hamsters with cheek pouch (c.p.) or intramuscular (i.m.) tumours. Clear area of bar is the percent of radioactivity accumulated in the liver. The c.p. and i.m. tumours ranged in size from 2–4 and 20–35 g, respectively.

When radiolabelled CEA was injected into hamsters bearing cheek pouch and intramuscular GW-39 tumours, a decrease in the clearance of plasma radioactivity was observed in hamsters bearing tumours at either site, being more marked in animals with intramuscular tumours (Fig. 1). Initially, the increase in vascular radioactivity corresponded to a decrease in the quantity present in the liver. But as the plasma radioactivity decreased, its level began to rise in the liver, and by 60 min, the radioactivity in both the plasma and liver of tumour-bearing hamsters was similar to that of normal animals. The distribution of radioactivity among the other organs studied in tumour hamsters was identical to that found in normal hamsters.

The radioactivity which was present in a 30 min and 3 h liver sample of a normal hamster was characterised on Sepharose 6B columns calibrated with the preinjection radiolabelled CEA (Fig. 2). The first radioactive peak in a 30 min liver sample cochromatographed with the preinjection antigen, whereas at 3 h, this peak eluted at a slightly lower molecular weight. The second peak in both cases appeared after the total column volume was voided

and represented low molecular weight materials which lacked immunoreactivity with goat anti-CEA antibody. The percentage of this material in the liver was 10–15% from 30 to 360 min. Following perchloric acid (PCA) extractions, the radioactivity sequestered by the liver during the latter times was 80–90% immunoreactive.

The radioactivity not initially cleared by the liver and remaining in the plasma at 5 min was identical, according to Sepharose 6B chromatography, to the preinjection CEA. Subsequently, a low molecular weight, nonimmunoreactive radioactive peak, similar to that in the liver, gradually appeared and accompanied a decrease in the quantity of radioactive material which eluted as the preinjection CEA. By 3 h, the relative percentages of the former and latter were 70% and 25% of the plasma radioactivity, respectively. At 5 min, more than 70% of the plasma radioactivity was immunoreactive before PCA extraction but decreased to 10% by 3 h, owing to the raised levels of the low molecular weight radioactive material. Loss of the latter material during dialysis of 30 min to 3 h plasma samples extracted with PCA yielded material which was 70–75% immunoreactive. All of the radioactivity which accumulated in the urine at 30 min or 24 h eluted as the low molecular weight, nonimmunoreactive material.

Figure 3 shows that a major portion of the radioactivity in both 15 and 60 min plasma from hamsters bearing intramuscular tumours appeared in the void volume after Sepharose 6B chromatography. Although there was a 20% drop in the blood's radioactivity during this time, the relative percentage (45%) of the void volume material remained approximately the same.

Our results with normal hamsters are in general agreement with the report by Shuster *et al.*, in which the metabolism of CEA was investigated in dogs and rabbits[3]. Slight differences in the initial plasma clearance rates are most likely a result of variations in the sialic acid content of the CEA preparations used in the different studies, as previously reported for other serum glycoproteins[7]. We have demonstrated a delay in the blood plasma clearance of radiolabelled CEA in GW-39 tumour-bearing hamsters.

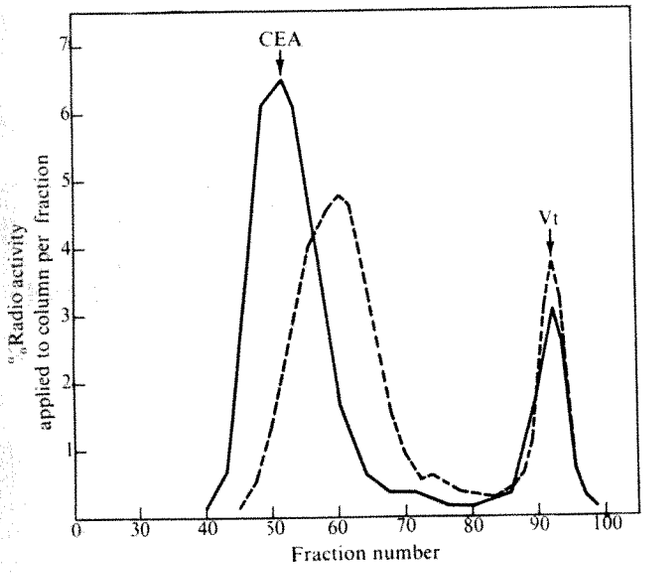


Fig. 2 Chromatography of 30 min (———) and 3 h (– – –) liver radioactivity on Sepharose 6B columns. Livers were homogenised in distilled water, 1:4 (w/v), and centrifuged at 12,000*g* for 30 min. After applying a sample from the supernatant to a 2.6 × 90 cm Sepharose 6B column, 5 ml fractions were collected and assayed for radioactivity. 85 to 98% of the radioactivity applied was recovered. Arrows, total column volume (Vt), and the elution volume of the preinjection radiolabelled CEA.

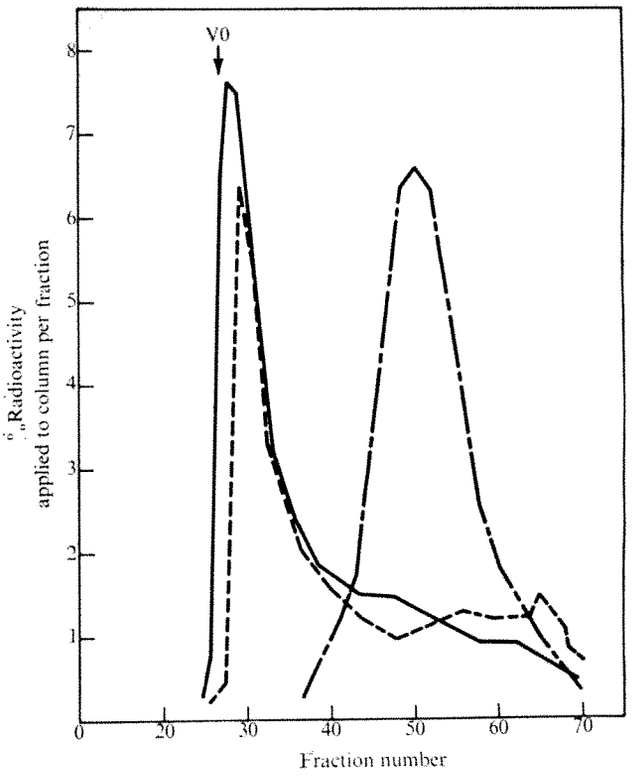


Fig. 3 Chromatography of normal and GW-39 tumour-bearing hamster plasma radioactivity on Sepharose 6B. 85 to 95% of the radioactivity applied was recovered. Arrow, void volume (Vo); (———), 15 min plasma sample from hamster with tumour; (– – –), 60 min plasma sample from hamster with tumour; (—— –), 15 min plasma sample from normal hamster.

Since the GW-39 tumour elicits the formation of a hamster anti-CEA IgM antibody[8], the plasma radioactivite macromolecule which appears in these animals is probably a CEA-antibody complex. Future studies will attempt to determine whether this antibody interferes with CEA clearance or is incidental to a more general state of tumour-induced suppression of liver function.

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1 Winzler, R. J., *Clin. Chem.*, **2**, 339 (1965).
2 Gold, P., and Freedman, S. O., *J. exp. Med.*, **122**, 467 (1965).
3 Shuster, J., Silverman, M., and Gold, P., *Cancer Res.*, **33**, 65 (1973).
4 Goldenberg, D. M., and Hansen, H. J., *Science*, **175**, 1117 (1972).
5 Greenwood, F. G., Hunter, W. M., and Glover, J. S., *Biochem. J.*, **89**, 114 (1963).
6 Hansen, H. J., Lance, K. P., and Krupey, J., *Clin. Res.*, **19**, 143 (1971).
7 Morell, A. G., Gregoriadis, G., Scheinberg, I. H., Hickman, J., and Ashwell, G., *J. biol. Chem.*, **246**, 1461 (1971).
8 Primus, F. J., Wang, R. H., Hansen, H. J., and Goldenberg, D. M., *Proc. Am. Ass. Cancer Res.*, **14**, 105 (1973).

Regulation of the corticosteroid inducibility of tyrosine aminotransferase in interspecific hybrid cells

TYROSINE aminotransferase (TAT) is not inducible by corticosteroid hormones in heterokaryocytes and hybrid cells resulting from the fusion of rat hepatoma cells with mouse fibroblasts[1,2]. Fusion of rat hepatoma cells with human fibroblasts also results in the loss of TAT inducibility, but because of the unidirectional loss of human chromosomes in the resulting hybrid cells[3], inducibility of TAT is re-expressed in some clones[4]. The suppression or modulation of the expression of TAT inducibility in such hybrid clones seems to be related to presence of the human X chromosome[4]. Since rat–human hybrid clones in which TAT inducibility is suppressed have the same corticosteroid receptor activity as the rat parental cells[4], the absence of inducibility cannot be ascribed to a decrease in corticosteroid receptor activity.

To identify the biochemical event involved in the suppression of the TAT inducibility in interspecific hybrid clones, we have analysed a series of rat–mouse and rat–human suppressed hybrid clones for corticosteroid receptor activity and for their ability to transfer the dexamethasone-receptor complex to the nucleus. The rat hepatoma cells (Fu5AH) used are resistant to 15 μg ml^{-1} of 8-azaguanine and deficient in hypoxanthine-phosphoribosyltransferase (HPRT) activity[5]. They were hybridised with mouse Cl-1D cells which are derived from L cells[6] and are resistant to 30 μg ml^{-1} of BUdR, and with human KOP fibroblasts which are derived from a subject with a translocation of the long arm of the human X chromosome to D-14 [t(14q, Xq)] (ref. 7). Fu5AH cells were fused with either Cl-1D cells or KOP cells in the presence of β-propiolactone-inactivated Sendai virus in Eagle's minimal essential medium (MEM) at *p*H 8.0 (ref. 8). Immediately after fusion, hypoxanthine-aminopterin-thymidine[9] (HAT) selective medium was added to the cultures. After 14 d, colonies of mouse–rat hybrid cells were isolated. These cells were then cloned in microtest plates.

After fusion of human and rat cells the cultures were maintained and passaged in HAT medium to eliminate human fibroblasts. Colonies of hybrid cells were then obtained 4–6 weeks after fusion, permitted to grow and subsequently cloned.

The chromosomes of the parental rat, mouse and human cells and of cells of each hybrid clone were stained by a modification of Seabright's Giemsa banding method[3,10], and a minimum of 20 karyotypes of each hybrid clone was analysed to identify the origin of the chromosomes of the hybrid cells. Chromosome preparations were treated with a solution of trypsin (0.05%) and EDTA (0.02%) (Gibco) for 5 min, rinsed with medium containing 5% foetal calf serum and then rinsed with Hanks solution. The slides were then stained in 2% Giemsa (*p*H 6.8) for 5 min, rinsed with *p*H 6.8 buffer (made by dissolving one tablet of G.T. Gurr *p*H 6.8 buffer in 100 ml water) and dried in humidified hot air.

Of the three parental cells studied, TAT was inducible by dexamethasone only in the rat Fu5AH cells (> four-fold induction) (Table 1).

In all the rat–mouse hybrid clones the TAT baseline activity was not expressed and the enzyme was not inducible by dexamethasone (specific activity = 0). In the rat–human (Fu5AH × KOP) hybrid clone 7, in which the only human chromosome present was the t (14q, Xq), the baseline TAT activity was similar to that of the rat parental cells (Table 1) whereas the inducibility of TAT was significantly reduced in comparison with that of the rat parental cells.

The rat–mouse hybrid clones contained between 83 and 103 chromosomes which could be identified by Giemsa banding (Fig. 1, Table 1). In these clones, most of the chromosomes of the rat and mouse parental cells were retained (Fig. 1), but occasionally rat chromosomes were preferentially lost.

Table 1 Induction of TAT in rat–mouse and rat–human hybrid cells

Cell line	Dexamethasone	TAT specific activity*	Average chromosome no.
Parental			
Fu5AH	−	0.5	52
	+	2.2	
Cl-1D	−	0	52
	+	0	
KOP	−	0	46
	+	0	
Hybrids			
Rat–mouse Cl 5	−	0	99
	+	0	
Rat–mouse Cl 7	−	0	95
	+	0	
Rat–mouse Cl 8	−	0	101
	+	0	
Rat–mouse Cl 10	−	0	89
	+	0	
Rat–mouse Cl 16	−	0	91
	+	0	
Rat–mouse Cl 17	−	0	98
	+	0	
Rat–mouse Cl 18	−	0	103
	+	0	
Rat–mouse Cl 20	−	0	83
	+	0	
Rat–human Cl 7	−	0.7	79
	+	1.1	

Almost confluent monolayers of parental and hybrid cells were kept in serum-free medium with or without 10 μM dexamethasone for 18 h. The cells were collected after centrifugation at 800*g* for 10 min at 4° C, washed, and resuspended in extraction medium (0.15 M KCl, 1 mM EDTA, 0.1 mM potassium phosphate buffer at *p*H 7.6). This suspension was sonicated by two 10 s bursts with a chilled probe of Branson sonifier at a setting of 5 A. The sonicated suspension was immediately centrifuged in a RC-2 Sorvall centrifuge at 20,000*g* for 15 min at 4° C. The supernatant solutions were tested for TAT by the method of Canellakis and Cohen[11] and for proteins by the method of Lowry *et al.*[12].

* TAT specific activity = μmol *p*-hydroxyphenyl pyruvate formed per 10 min (37° C) per mg of protein.

Such chromosomal loss seemed to be limited and usually involved less than 40% of the rat chromosomes (Fig. 1).

To survive in HAT medium[4], the rat–human hybrid cells had to retain the long arm of the human X chromosome which was translocated to D-14[7] (Fig. 2), since the human HPRT gene is located on the long arm of the X chromosome[7].

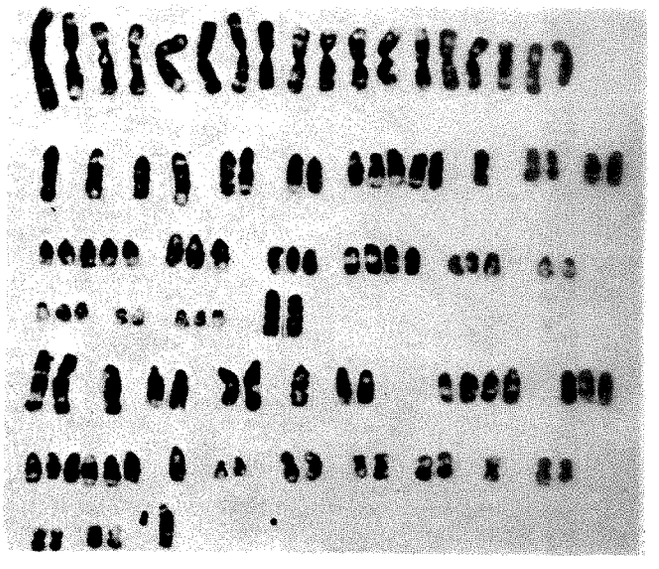


Fig. 1 Rat–mouse hybrid Cl 18. All the mouse Cl-1D chromosomes are present in this clone (upper four rows). Forty rat chromosomes are present in this hybrid (last three rows).

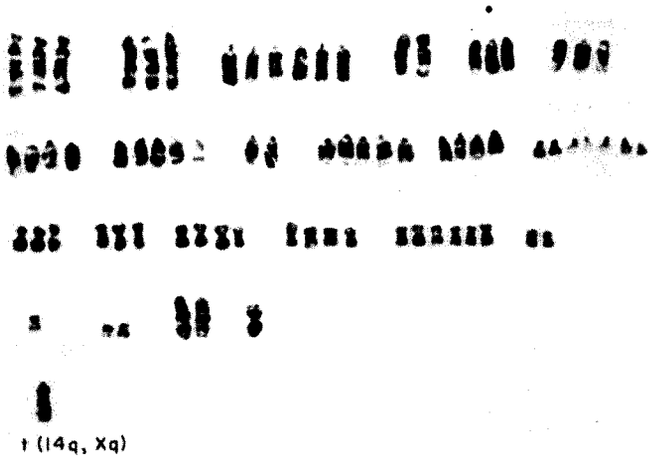


Fig. 2 Rat–human hybrid Cl 7. Seventy-five rat chromosomes are present in this hybrid. Only the human t (14q, Xq) chromosome is present in this clone.

As Table 2 shows, both rat and mouse parental cells displayed corticosteroid receptor activity. The amount of receptor activity was higher in the mouse parental cells (Table 2), in which TAT could not be induced by dexamethasone (Table 1). The KOP cells, as well as WI38 human fibroblasts[4], had low amounts of receptor activity (Table 2). Two noninducible mouse–rat hybrid clones (Table 1) displayed corticosteroid receptor activity which was higher than that of the parental mouse and rat cells (Table 2). The rat–human hybrid clone 7, in which TAT was only slightly inducible by corticosteroids, displayed higher dexamethasone-receptor activity (Table 2) than the rat hepatoma parental cells.

As Table 3 shows, transfer of radioactive dexamethasone to the nucleus proceeded to the same extent in both rat Fu5AH and mouse Cl-1D cells. In the three rat–mouse hybrid clones, in which TAT was not inducible (Table 1), the percentage of radioactive dexamethasone recovered in the nuclei at various times after exposure to the hormone was slightly higher than in the rat and mouse parental cells.

In the rat–human hybrid clone 7, the bound dexamethasone nuclear transfer proceeded to the same extent as in the Fu5AH parental cells (our unpublished results).

Since the rat–human hybrid cells that retained the human X (long arm)/D-14 chromosome had a much lower TAT inducibility than did the rat Fu5AH cells (Table 1), the gene(s) modulating TAT inducibility by corticosteroids seems to be located on the long arm of the human X chromosome. Previous studies excluded this role for the human D-14 chromosome[4].

Our results indicate that noninducibility of TAT in rat–mouse or rat–human hybrid cells cannot be attributed to an absence or decrease of dexamethasone-receptor activity. In fact the rat–human hybrid clone 7 in which TAT was only slightly inducible by dexamethasone had dexamethasone-receptor activity higher than that of the rat parental cells. In addition the mouse–rat hybrid cells have more dexamethasone-receptor activity than either of the parental cells. It is thus possible that in this case fusion of two cell types, both with dexamethasone-receptor activity, results in the formation of a hybrid cell with combined receptor activity of the two parental cells.

In all the clones tested in which TAT was not inducible but which had dexamethasone receptor activity, the transfer of radioactive dexamethasone to the nucleus proceeded to the same or greater extent than in the rat or mouse parental cells, indicating that the biochemical event which is missing in these noninducible hybrid clones is not the transfer to the nucleus of the dexamethasone-receptor complex. Therefore, the regulation of the inducibility of TAT in interspecific hybrid cells must take place either at the level of the binding of the dexamethasone receptor complex to the chromatin or at the transcriptional or post-transcriptional level. These problems are now being investigated.

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Table 2 Corticosteroid receptor activity of parental and hybrid cell lines

Cell line	Receptor activity (% hormone binding)	TAT inducibility
Fu5AH	21	++++
Cl-1D	45	−
KOP	4	−
Rat–mouse Cl 5	71	−
Rat–mouse Cl 18	67	−
Rat–human Cl 7	32	+

Cytosols of the cell lines were incubated for 90 min at 0° C with 2 nM 1,2,4-^{3}H-dexamethasone (New England Nuclear Corp.). A load sample of 0.5 ml was added on a 10 ml syringe column of Sephadex G-25 and eluted with 1 mM phosphate buffer (*p*H 7.6). Pre-bound, bound and unbound pools were collected and radioactivity of the pools was determined in a scintillation mixture containing Triton-X, toluene and methanol fluor[13]. These experiments were done essentially as described earlier[13]. Percentage binding was calculated by the following formula:

$$\frac{\text{c.p.m. bound pool}}{\text{c.p.m. load sample}} \times 100 = \% \text{ binding}$$

Using radioactive dexamethasone at this level in glucocorticoid responsive cells in culture we observed that only one major dexamethasone-bound macromolecule appears on ion-exchange chromatography which accounts for about 95% of the binding[14]. This fraction is identical to the binder II dexamethasone binding protein of rat liver cytosol which has been shown to be the major hormone receptor protein[15]. In addition, when this level of radioactive dexamethasone is used in *in vitro* binding experiments with adrenalectomised liver cytosol, there is only one major peak on ion-exchange chromatography which is characterised as binder II, the hormone receptor (unpublished experiments). Thus, the values reported for binding of ^{3}H-dexamethasone in cytosol represent about 95% hormone receptor.

Table 3 Nuclear transfer of ^{3}H-dexamethasone in rat–mouse hybrids

Cell line	% Nuclear transfer 2 h	4 h	6 h	18 h
Fu5AH	17.8	25.9	34.1	20.5
Cl-1D	26.9	26.5	23.4	28.1
Rat–mouse Cl 5	33.6	28.1	30.6	41.4
Rat–mouse Cl 8	ND**	56.3	42.1	48.6
Rat–mouse Cl 10	ND**	43.1	54.5	49.3

Almost confluent monolayers of parental and hybrid cells were exposed to 3.3×10^{-8} M 1, 2, 4-^{3}H-(N)-dexamethasone (specific activity 21.2 Ci mmol^{-1}) in serum-free medium. At different intervals the cells were trypsinised, centrifuged and washed three times in ice cold phosphate-buffered saline (PBS). The cells were resuspended in hypotonic reticulocyte standard buffer (RSB), composed of 10 mM NaCl, 10 mM Tris-HCl, *p*H 7.4, and 1.5 mM $MgCl_2$, and homogenised. The cytoplasmic fraction was centrifuged in a RC-2 Sorvall centrifuge at 20,000*g* for 15 min at 4° C. The nuclei were stripped of the outer nuclear membrane by double detergent procedure according to the method described by Penman[16]. The percentage of nuclear transfer of the dexamethasone-receptor complex was determined by the following formula:

$$\% \text{ Nuclear transfer} = \frac{\dfrac{\text{c.p.m.}}{\text{mg of nuclear proteins}}}{\dfrac{\text{c.p.m.}}{\text{mg of nuclear proteins}} + \dfrac{\text{c.p.m.}}{\text{mg of cytosol proteins}}} \times 100$$

We conclude that the activity of the hormone receptor has indeed been measured by our techniques for the reasons set forth in Table 2. Furthermore, nuclear translocation experiments ascertain the presence and biological activity of the receptor as shown by the results described in this table.

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[1] Thompson, E. B., and Gelehrter, T. D., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2589–2593 (1971).
[2] Schneider, J. A., and Weiss, M. C., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 127–131 (1971).
[3] Croce, C. M., Kieba, I., and Koprowski, H., *Expl Cell Res.*, **79**, 461–463 (1973).
[4] Croce, C. M., Litwack, G., and Koprowski, H., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1268–1272 (1973).
[5] Croce, C. M., Bakay, B., Nyhan, W. L., and Koprowski, H., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2590–2594 (1973).
[6] Dubbs, D. R., and Kit, S., *Expl Cell Res.*, **33**, 19–28 (1964).
[7] Ricciuti, F., and Ruddle, F. H., *Nature new Biol.*, **241**, 180-182 (1973).
[8] Croce, C. M., Koprowski, H., and Eagle, H., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1453-1456 (1972).
[9] Littlefield, J. W., *Expl Cell Res.*, **41**, 190–196 (1966).
[10] Seabright, M., *Lancet*, **ii**, 971–972 (1971).
[11] Canellakis, Z. N., and Cohen, P. P., *J. biol. Chem.*, **222**, 53–62 (1956).
[12] Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **143**, 265–275 (1951).
[13] Litwack, G., and Singer, S., in *Biochemical actions of hormones* (edit. by Litwack, G.), **2**, 113–163 (Academic Press, New York, 1972).
[14] Singer, S., Becker, J. E., and Litwack, G., *Biochem. Biophys. Res. Commun.*, **52**, 943–950 (1973).
[15] Litwack, G., Filler, R., Rosenfield, S. A., Lichtash, N., Wishman, C. A., and Singer, S., *J. Biol. Chem.*, **248**, 7481–7486 (1973).
[16] Penman, S., in *Fundamental techniques in virology* (edit. by Habel, K., and Salzman, N. P.), 35–48 (Academic Press New York, 1969).

New biological activity following intravascular activation of the complement cascade

ACTIVATION of the complement cascade (C_1–C_9) by the classic or alternate pathways is associated with the development of several biological activities[1]. These include the elaboration of chemotactic factors (C_{3a}, C_{5a}, and a trimolecular complex $C_{\overline{5,6,7}}$), opsonic activity associated with the formation of C_{3b} from the third component, anaphylatoxin activity associated with the formation of C_{3a} and C_{5a} from the third (C_3) and fifth (C_5) components, and lysis of certain cells after the formation of lesions at the cell surface associated with fixation of the eighth (C_8) and ninth (C_9) components. We have detected another activity which follows intravascular activation of the complement cascade with the elaboration of the low molecular weight factor that produces an immediate neutropenia followed by marked neutrophilia.

Activation of the complement cascade intravascularly by the alternate pathway was achieved in New Zealand White rabbits or Hyline Sandy Lop rabbits, weighing approximately 2.5 kg, by intravenous injection 0.6–1.0 ml of purified cobra venom factor or 50 mg of a suspension of inulin (Sigma). The CVF was purified from the crude venom of *Naja naja* (Sigma) on DEAE cellulose and Sephadex G-200 columns after the method of Ballow and Cochrane[2]. The complement activators were injected directly into the marginal ear vein of the rabbit and sequential heparinised venous blood samples were obtained. Leukocyte counts and Wright-stained differential counts were performed by standard techniques.

Figure 1 shows the effects of intravenous administration of the two activators of complement on neutrophils. A profound neutropenia occurred within 60–120 s and this was followed by neutrophilia within 0–3 h. Figure 2 shows the time course of depletion of the opsonin activity and antigenic levels of C_3 in rabbit serum after challenge with CVF. Opsonic activity was determined by counting the total number of zymosan particles ingested in 100 neutrophils (PMN). Values are expressed as percentages of a parallel control. The alterations in PMN counts in Fig. 2 coincided more closely with depletion of opsonic activity than with reduction in antigenic C_3 levels.

To determine whether the biological activity was associated with complement activation, rabbits were first depleted of complement by sequential injections of purified CVF as described by Brown[4]. When the antigenic C_3 level was less than 10% the animal was challenged with 1.0 ml of CVF. In the complement-depleted animal there was little effect on PMN counts after rechallenge with 1.0 ml of CVF.

Further substantiation that the biological effect was associated with complement was obtained by infusing 40 ml of fresh rabbit plasma into an animal that was complement-depleted but still had circulating CVF. It has been demonstrated that, after sequential intravascular administration of CVF, the CVF continues to circulate until antibodies against it are formed[5]. Ths infusion of the 40 ml of fresh heparinised plasma into a complement-depleted rabbit (opsonic index <10%) caused acute neutropenia followed soon by neutrophilia (Fig. 3).

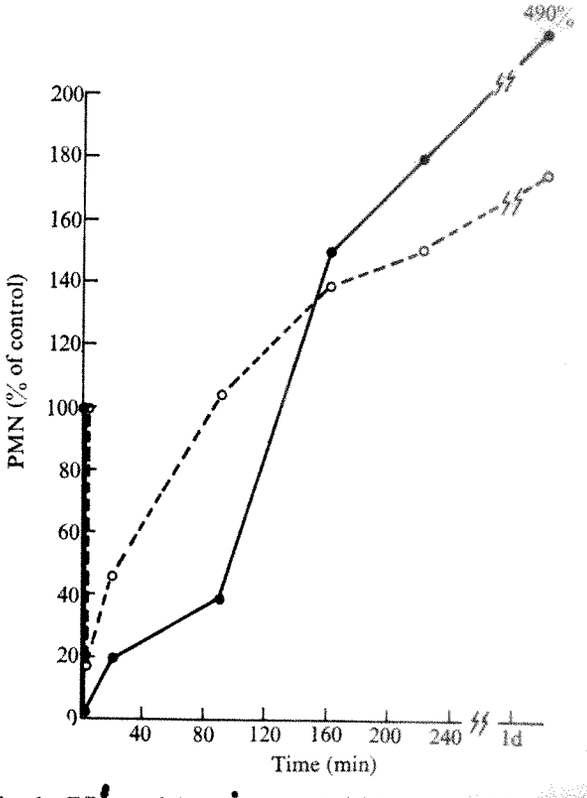


Fig. 1 Effects of intravenous administration of 1.0 ml of CVF (●) or 50 mg of inulin (○) on rabbit peripheral blood PMN counts. Values are expressed as percentages of control value, which was obtained 1–5 min before infection. The values are representative experiments, selected from more than five individual observations, all done in duplicate.

Both CVF and inulin activate complement by the alternate pathway which results in formation of a C_3-convertase which then activates C_3–C_9 (ref. 1). To identify further the components upon which biological activity might depend, CVF was injected into C_6-deficient rabbits. Acute neutropenia followed by neutrophilia occurred, suggesting that the biological activity was dependent on either C_3 and/or C_5 (data not shown).

Further studies were then designed to further characterise the active factor. Fresh plasma (15 ml) was treated with either CVF (1:10 v/v) or 6 mg ml^{-1} of inulin suspension. The mixtures were reacted for 15 min at 37° C and immediately filtered at 4° C through a UME 20,000 Amicon Diaflo filter. To enhance the filtration the plasma was first diluted 1:1 with sterile, isotonic saline. The filtrate was collected and then filtered through a 0.4 μm Millipore filter before infusing it into the rabbit ear vein. Appropriate saline controls were performed.

Figure 4 shows that the biological activity could be recovered in the filtrate. The small pore size would exclude CVF (molecular weight >180,000) and the C_{3b} immune adherence molecule (molecular weight ~150,000). The low molecular weight fraction recovered after treatment of plasma with CVF or inulin caused a biphasic curve following infusion. There is no explanation for this difference from *in vivo* adminstration of CVF or inulin. Low molecular weight filtrate produced very little neutropenia when the plasma treated *in vitro* with CVF was obtained from an animal first depleted of C_3 by sequential doses of CVF. There was, however, pronounced neutrophilia, suggesting that neutrophilia follows activation of less substrate or that it is determined by a different mechanism.

Preliminary studies suggest that the low molecular weight factor acts directly on PMN and that the cells are removed by sequestration rather than lysis (our unpublished observations).

Alterations in neutrophil kinetics during acute inflammatory states have interested investigators for some time. Some of the most intriguing early observations were made by Andrewes[6]. This investigator demonstrated in rabbits that acute neutropenia followed intravenous administration of the live Gram-negative bacteria, and antigen–antibody complexes. Neutrophilia occurred later. Reactions to Gram-positive bacteria were mild unless animals were previously

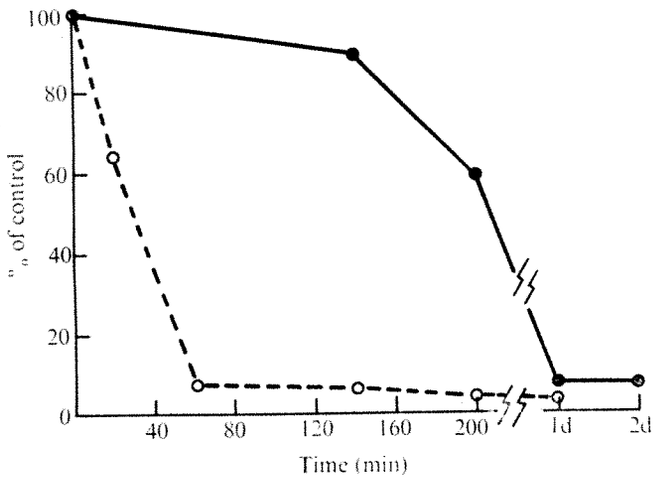


Fig. 2 Effect of two sequential doses of 1.0 ml CVF on antigenic C_3 levels of serum (●) and zymosan opsonic index (○). The first dose of CVF was given at zero time and the second at 160 min. Antigenic C_3 levels were measured by radial immudiffusion using goat anti-rabbit C_3 (ref. 3). The opsonic index for zymosan was obtained by counting the number of intracellular zymosan particles in 100 PMN (100 particles: 1 PMN) after incubating in a shaker bath at 37° C for 15 min.

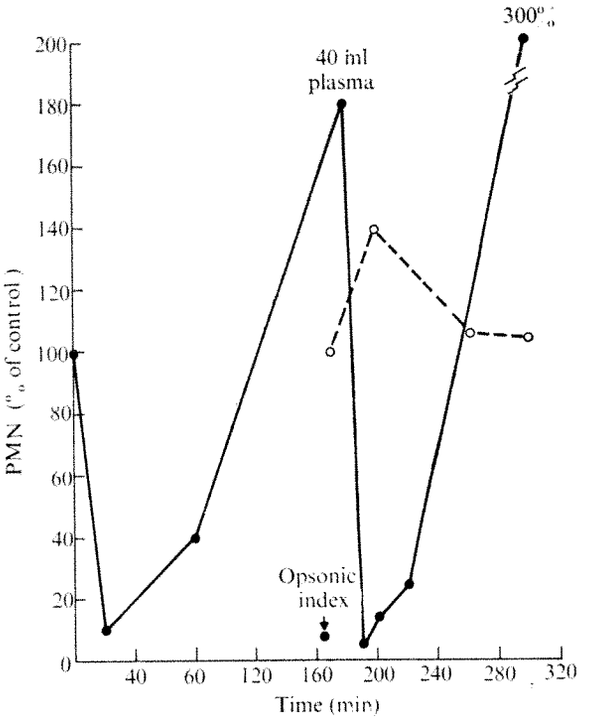


Fig. 3 Effect of infusion of 40 ml of fresh plasma in a rabbit which received 1.0 ml of CVF at zero time (●). The opsonic index for zymosan at the time of plasma infusion was 8% of control. A saline control in a normal rabbit is included (○).

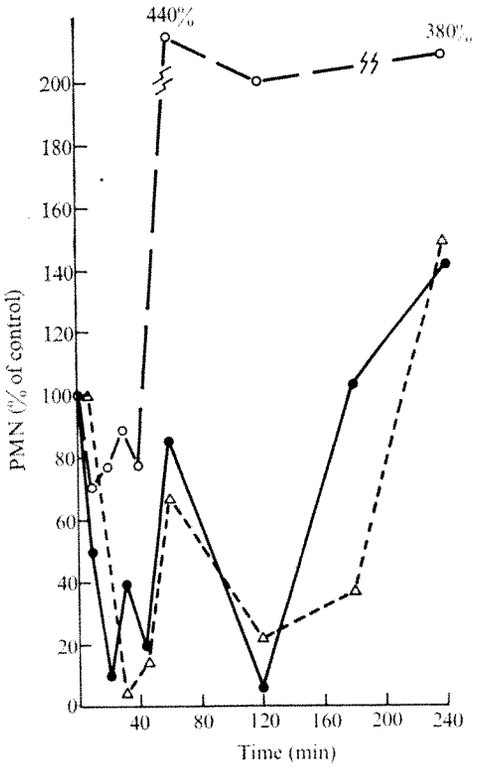


Fig. 4 Effects of infusion of 15 ml of low molecular weight filtrate (< 20,000) on rabbit PMN counts. Before filtration for approximately 2 h through an Amicon Diaflow UME 20,000 filter, 15 ml of fresh plasma was incubated with either CVF (1:10 v/v) or inulin 6 mg ml^{-1} plasma. The plasma was diluted 1:1 with saline before filtration in order to increase the rate of filtration. ●, Filtrate from CVF treated plasma; △, filtrate from inulin-treated plasma; ○, filtrate from CVF treated plasma obtained from a rabbit that had received for sequential doses of CVF (0.7 ml) which reduced the antigenic C_3 level to 20% of control before the time plasma was collected.

immunised. Most PMN were sequestered in the lung and there was no evidence that their destruction was the cause of the profound neutropenia. Interpreted in the light of contemporary knowledge, these early studies suggest that the acute neutropenia may have been complement dependent. With Gram-negative bacteria, the complement cascade would be activated by endotoxin[1]. The reaction to Gram-positive bacteria would be mild unless it was enhanced by the presence of specific antibody, after which the complement cascade would be activated by the antigen–antibody complex[1]. Moreover, both neutropenia and neutrophilia may occur in humans during acute bacterial infection, and the shock syndrome of infection is associated with activation of the complement cascade[7]. Neutropenia follows the injection of endotoxin into both humans and experimental animals and during this time the cells are primarily sequestered in the pulmonary capillaries[8]. Recent studies have indicated that the neutropenia induced after infusion of viable Gram-negative bacteria into the blood of subhuman primates is diminished if the animals are first depleted of the complement by CVF (ref. 9). In our investigation the possibility of contamination of the CVF, inulin or low molecular weight fraction by endotoxin was considered. This was excluded by constant temperature measurements for up to 3 h in rabbits receiving each of these. No significant alteration in rectal temperature was observed in any animal, thus making contamination with endotoxin highly unlikely.

A neutrophilia has been observed several hours after the infusion of CVF, but careful studies of time points earlier than 1 h were not done[5,10]. The magnitude of the neutrophilia was somewhat less than we observed and the reasons for this are not evident at present. A possible association of leukocytosis with complement activation has been recorded by Rother who observed that factors released during complement activation induce release of leukocytes from bone marrow[11].

This investigation indicates that the alternate pathway activation of the fluid phase by CVF or by inulin is associated with alterations in PMN kinetics. The changes depend on elaboration of a factor of low molecular weight (< 20,000) which may be derived from either the C_3 or C_5 molecule. This factor may be responsible for alterations in PMN during acute inflammation.

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1 Ruddy, S., Gigli, I., and Austin, K. F., *New Engl. J. Med.*, **248**, 489, 545, 592, 624 (1972).
2 Ballow, M., and Cochrane, C. G., *J. Immun.*, **103**, 944 (1969).
3 Lachmann, P. J., Hobart, N. J., and Aston, W. P., in *Experimental immunology*, second ed. (edit. by Weir, P. M.), (Blackwell, Oxford, in the press).
4 Brown, D. L., dissertation, Univ. Cambridge (1972).
5 Cochrane, C. G., Müeller-Eberhard, H. J., and Akin, B. S., *J. Immun.*, **105**, 55 (1970).
6 Andrewes, F. W., *Lancet*, **i**, 8 (1910).
7 McCabe, W. R., *New Engl. J. Med.*, **288**, 21 (1973).
8 McKay, D. G., *Thrombos. Diathes. Haemorrah.*, **22**, 11 (1973).
9 Gilbert, D. N., Barnett, J. A., and Sanford, J. P., *J. clin. Invest.*, **52**, 406 (1973).
10 Dodds, W. J., and Pickering, R. J., *Blood*, **40**, 400 (1972).
11 Rother, K., *Eur. J. Immun.*, **2**, 550 (1972).

Evidence that most noradrenaline is stored without ATP in sympathetic large dense core nerve vesicles

THE concept that catecholamine (CA) in sympathetic nerve vesicles is stored together with ATP, in a fixed molar ratio of 4/1, is so widely accepted that it is even quoted in general textbooks on physiology and pharmacology for medical students[1-3]. This fixed ratio of CA to ATP has been convincingly demonstrated in purified preparations of adrenomedullary vesicles[4,5], whereas the evidence concerning the ratio of noradrenaline (NA) to ATP in sympathetic nerve vesicles has been based on preparations from bovine splenic nerves[6-9], rat heart[10] and rat vas deferens[11] contaminated to an unspecified extent with other organelles such as mitochondria. The aim of the present study was to determine the molar NA/ATP ratio in a highly purified preparation of bovine splenic nerve vesicles.

Large dense core vesicles were isolated from bovine splenic nerves using differential and density gradient centrifugation[12,13]. The supernatant obtained after centrifugation of the nerve homogenate at $10{,}500g_{max}$ for 10 min was layered on sucrose-D_2O gradients (linear from 0.25 M to 0.6 M with a step of 1.2 M in the bottom). The gradients were spun at 280,000 g_{max} for 90 min in a SW 40 rotor (Beckman). The band just above the bottom step (fraction III) in the gradients was taken out, diluted three-fold with isotonic sucrose and centrifuged at $10{,}500g_{max}$ for 10 min. The supernatant obtained was centrifuged at $20{,}000g_{max}$ for 10 min and the supernatant from this centrifugation was spun at $230{,}000g_{max}$ for 20 min.

Each pellet was resuspended in 1.5 ml 20 mM K-phosphate buffer (*p*H 7.3); 0.5 ml was used for determination of cytochrome oxidase as a mitochondrial marker enzyme[14]. The rest (1 ml) was extracted with 0.4 M perchloric acid and then diluted with distilled water to 2 ml. After removal of the protein precipitate, 1 ml was used for fluorimetric determination of NA (ref. 12) and the remaining 1 ml was titrated with Tris-buffer and KOH to *p*H 7.0–7.4. The perchlorate precipitate was carefully removed by centrifugation. The supernatant was used for ATP assay by the firefly method[15]. The recovery of ATP added as internal standard ranged from 59–73%.

The ATP values were corrected for the loss actually measured in each individual experiment. The protein precipitate was solubilised in NaOH and protein was determined by the Folin method.

NA storage particles and mitochondria from splenic nerve sedimented to about the same level in the density gradients[12]. But a separation of NA storage particles and mitochondria could be achieved by differential centrifugation of the diluted gradient fraction as shown in Fig. 1. Cytochrome oxidase activity was highest in the first two pellets obtained after low speed centrifugations, while most of the NA was found in the last pellet which was obtained after high speed centrifugation. In this way about 80% of the cytochrome oxidase activity could be removed while still more than 60% of the NA was retained in the particles.

The high speed sediment with the lowest cytochrome oxidase activity was used for determination of the NA/ATP ratio. In the first few experiments there was a great variability in NA/protein ratio due to variable *post mortem* delay at the slaughter house before the nerves could be chilled with ice. When special precautions were taken to shorten the delay to 15–20 min, the average NA/protein ratio was 5.30 ± 0.74 μg mg^{-1} (s.e.m., $n = 11$) and the molar NA/ATP ratio 7.32 ± 0.30.

The original density gradient fraction (fraction III) consisted mainly of large dense core vesicles (750 Å), contaminated almost exclusively with mitochondria according to biochemical[12] and morphological analyses[16]. As seen in Fig. 1 most of the mitochondria likely to contain appreciable amounts of ATP, could be effectively separated from the NA storage particles, as has also been confirmed by morphological studies[16].

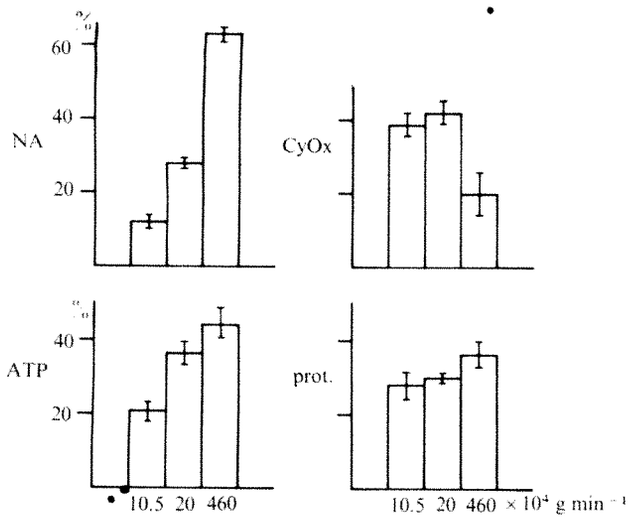


Fig. 1 Distributions of NA, ATP, cytochrome oxidase (CyOx) and protein (prot.) in the three sediments. These were prepared from a 'nerve vesicle fraction' (obtained from a density gradient after ultracentrifugation) by fractional centrifugations at 10,500 g_{max} for 10 min; 20,000g_{max} for 10 min and 230,000g_{max} for 20 min. The values are expressed in percentages ± s.e.m. Each bar represents the mean of at least four experiments.

The much more complete removal of cytochrome oxidase than of ATP indicates that the ATP sedimenting together with NA truly belongs to the NA storage particles. The possibility of contamination with mitochondrial fragments containing ATP but not cytochrome oxidase (outer membrane fractions) cannot be ruled out. But these particles would probably not sediment to the same level in the density gradients[14].

It was not possible to get rid of all the cytochrome oxidase in the final sediment. According to the morphological studies[16] the mitochondria constituted a few per cent of all the particles. Therefore the NA/ATP ratio was certainly slightly higher than 7.3 in the NA storage particles in the final sediment.

We have not excluded the possibility that there were nerve vesicles with relatively higher ATP content which were lost during the first centrifugations. The overall NA/ATP ratio was however found to be 5.1/1 in the original preparation containing at least 10% mitochondria according to morphological analysis[16], so it is quite possible that the NA/ATP ratio also was higher than 4/1 in the lost vesicles.

The 'true' *in vivo* ratio of NA to ATP may be even higher than the value 7.3 obtained in the present study. Since there is evidence that thermal activation leads to a selective loss of NA without concomitant loss of ATP[8], it seems probable that *post mortem* delay at body temperature reduces the NA/ATP ratio of the vesicles. Thus, when the delay was 30–40 min the NA/protein ratio was about 2 μg mg^{-1} and the molar NA/ATP ratio was about 3.5. When the delay was reduced to about 10 min, vesicle preparations with a NA/protein ratio of 10 μg mg^{-1} could be obtained. The NA/ATP ratio in these preparations was 7–12.

Extrapolation to zero time[13] suggests that the NA/ATP ratio *in vivo* may be as high as 20/1 which would indicate that only 20% of the NA in these sympathetic nerve vesicles could be stored in the postulated NA/ATP complex. High values for the NA/ATP ratio have been observed previously; a level of 7.5–12 has been calculated to occur in sympathetic large dense core vesicles[17]. Moreover, in a series of studies on phaeochromocytoma tumours a wide range of CA/ATP ratios were found, from 8 to as much as 35 (refs 18, 19). It may be of further interest that cholinergic nerve vesicles seem to store acetylcholine in a molar ratio to ATP of 10/1; and 5/1 in the two discernable pools[20].

The occurrence of other nucleotides in sympathetic nerve vesicles cannot be ruled out, but only trace amounts of such material, including ADP, could be detected in partially purified splenic nerve vesicles (DaPrada, personal communication).

This study concerns the NA storage particles in nerve trunk. It is possible that the vesicles in the nerve terminals have a relatively higher content of ATP, corresponding to the proximo-distal increase in NA/protein ratio[21]. But preliminary studies do not support this possibility, since no significant rise was found in the ATP/protein ratio in the vesicles isolated from the intrasplenic distal part of the nerve trunk, compared with vesicles from the proximal part, while the NA/protein ratio increased markedly.

The present results provide very strong evidence that a major proportion of the NA in the sympathetic nerve vesicles is stored differently from the bulk of the CA in the adrenomedullary vesicles. This is further supported by the findings of striking differences in kinetic properties[8,12]; between nerve and adrenomedullary vesicles.

The nerve vesicles are, for example, very resistant to osmotic shock, but they lose NA rapidly during thermal activation, while the adrenomedullary vesicles behave in the opposite way[8]. Furthermore, the nerve vesicles have a very low content of soluble proteins such as chromogranin A compared with the chromaffin vesicles[9]. The nerve vesicles may contain a small NA/ATP/soluble protein complex of the same kind as that which predominates in adrenomedullary vesicles; it seems possible that this complex in nerve vesicles is functionally insignificant and may represent an evolutionary dead end. If ionically bound, most of the NA in nerve vesicles has to be bound by negatively charged constituents other than ATP such as phospholipids.

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1 Ganong, W., *Medical Physiology*, Lange Medical Publications, Los Altos, (1973).
2 Keele, C. A., and Neil, E., *Samson Wright's Appplied Physiology* (Oxford University Press, London, 1971).
3 Goodman, L. S., and Gilman, A., *The Pharmacological Basis of Therapeutics* (Macmillan, New York, 1970).
4 Blashko, H., Born, G. V. R., D'Iorio, A., and Eade, N. R., *J. Physiol. Lond.*, **133**, 548 (1956).
5 Falck, B., Hillarp, N.-A., and Högberb, B., *Acta physiol. scand.*, **36**, 360 (1956).
6 Schümann, H. J., *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **233**, 296 (1958).
7 Euler, U.S.v., Lishajko, F., and Stjärne, L., *Acta physiol. scand.*, **59**, 495 (1963).
8 Stjärne, L., *Acta physiol. scand.*, **62**, suppl. 228 (1964).
9 Helle, K. B., Lagercrantz, H., and Stjärne, L., *Acta physiol. scand.*, **81**, 565 (1971).
10 Potter, L. T., and Axelrod, J., *J. Pharmac. exp. Ther.*, **142**, 291 (1963).
11 Geffen, L. B., and Livett, B. G., *Physiol. Rev.*, **51**, 98 (1971).
12 Lagercrantz, H., *Acta physiol. scand.*, suppl. 366 (1971).
13 Yen, S. S., Klein, R. L., and Chen-Yen, S. H., *J. Neurocytol.*, **2**, 1 (1973).
14 Sottocasa, G. L., Kuylenstierna, B., Ernester, L., and Bergstrand, A., *J. Cell Biol.*, **32**, 415 (1967).
15 Strehler, B. L., and Totter, J. R., *Methods of Biochemical Analysis*, **1**, 341 (1954).
16 Thureson-Klein, A., Klein, R. L., and Lagercrantz, H., *J. Neurocytol.*, **2**, 13 (1973).
17 De Potter, W. P., Smith, A. D., and De Schaepdryver, A. F., *Tissue Cell*, **2**, 547 (1970).
18 Schumann, H. J., *Klin. Wschr.*, **38**, 11 (1960).
19 Stjarne, L., Euler, U.S.v., and Lishajko, F., *Biochem. Pharmac.*, **13**, 809 (1964).
20 Dowall, M. J., and Zimmerman, H., *Brain Res.* (in the press).

[21] Klein, R. L., in *Frontiers in Catecholamine Research* (Pergamon Press, New York, in the press).

(+) and (−)-bicuculline methochloride as optical isomers of a GABA antagonist

THE discovery by Curtis *et al.*[1], that bicuculline would antagonise both the depressant effects of microiontophoretically applied γ-aminobutyric acid (GABA) and certain strychnine resistant central inhibitions has provided further support for the view that GABA might be a central transmitter. Their studies have also stimulated much thinking and research into the structural relationship of GABA and its receptor. On the basis of examination of Dreiding stereomodels of GABA and bicuculline, the Canberra group suggested that the nitrogen atoms and the three atoms associated with the carboxylate in GABA (O = C − O) could be isosteric with the nitrogen atom and the O = C − C grouping in bicuculline[1]. Indeed, recent physiochemical work[2] on the two substances indicates that the distance from the onium group to a carboxylate oxygen in GABA is fixed at between 5.2 and 5.8 Å and this agrees well with a distance of 5.6 Å between the equivalent groups in bicuculline. It would therefore seem reasonable to suppose that the GABA antagonist properties of bicuculline are due to the occupation of the GABA receptor by a part of the bicuculline molecule that is isosteric with the biologically active conformation of GABA[1].

Closer inspection of the portion of the bicuculline molecule that is isosteric with GABA reveals that it contains two asymmetric centres and thus four optical isomers are possible. Such variation in the optical properties would be unlikely to change interatomic distances along the skeleton of the molecule and so may not affect the potency as a GABA antagonist. If, on the other hand, the stereochemical requirements for combination with the GABA receptor are more subtle, then different optical isomers would be expected to have widely differing potencies. All pharmacological experiments so far performed with bicuculline[3] have used the commercially available (+) form (K & K Chemical, Pierce Chemical) and, so we thought it important to examine the properties of the (−) isomer. Although the (−) form occurs in nature[4] it is rare and we have therefore prepared it by synthesis from (−) β-hydrastine[5].

In comparing the biological activities of the two stereoisomers of bicuculline certain problems arise, chiefly associated with their very low solubility at biological *p*H, and this has already led to dispute over the efficacy of the (+) isomer[6,7]. This would obviously lead to uncertainty over the correct interpretation of a negative effect with the (−) form. More recently (+) bicuculline methochloride has been shown to be an effective GABA antagonist and as this substance is about 100 times more soluble in water than bicuculline base, its use, particularly in microiontophoresis experiments, is easier and gives more reproducible results[8]. As the asymmetric centres are not affected by methylation we have chosen to prepare the methochlorides from our samples of (+) and (−)-bicuculline and to compare the relative biological activities of these derivatives.

All experiments have been performed in piebald Lister rats anaesthetised with urethane, and respiring spontaneously. Microiontophoretic techniques were as previously described[9] and all recordings of unit activity were extracellular. Neurones were located in the cerebral cortex and cuneate nucleus, and (+)-bicuculline methochloride (10–50 nA, from 5 mM solution in 150 mM saline) consistently antagonised the depressant action of GABA on these neurones and produced concurrent excitation. (−)-Bicuculline methochloride (prepared in a similar solution, 10–100 nA) applied to the same neurones, from the same pipette, was ineffective as a GABA antagonist but produced neuronal excitation in a similar manner to the (+) isomer (Fig. 1).

Presynaptic inhibition was measured in the cuneate nucleus by analysis of the evoked potential recorded there in response to stimulation of a forepaw ipsilateral to the recording electrode. The amplitude of the positive-going potential (P wave) was taken as an index of presynaptic terminal depolarisation[10]. (+)-Bicuculline methochloride (0.5 mM solution in normal saline), applied topically to the medulla, consistently and reversibly antagonised presynaptic inhibition in the nucleus, whereas (−)-bicuculline methochloride (4 mM solution in normal saline) was without effect on the P wave (Fig. 2).

The relative convulsant potencies of the two isomers were tested by making microinjections into cerebral cortex and recording the resultant convulsive discharges in the EEG, the injection cannula serving as recording electrode. Very small quantities of the (+)-bicuculline methochloride produced sustained convulsive activity ('spiking') (2 μl of 0.5 mM solution in normal saline) whereas injection of 400 times this amount of the (−) form to the same animals (10 μl of 4 mM solution in normal saline) produced only transient convulsive activity.

More recently, experiments in the cat have shown that (+)-bicuculline methochloride will readily antagonise both GABA and synaptic inhibition in the cuneate nucleus, but that (−)-bicuculline methochloride does neither. Both isomers seemed to have little effect on depression of these neurones by glycine[11]. In comparison with other GABA antagonists that we have studied, (+)-bicuculline methochloride stands out for both it potency and its lack of effect on glycine (R. G. H. and M. A. Simmonds, in preparation).

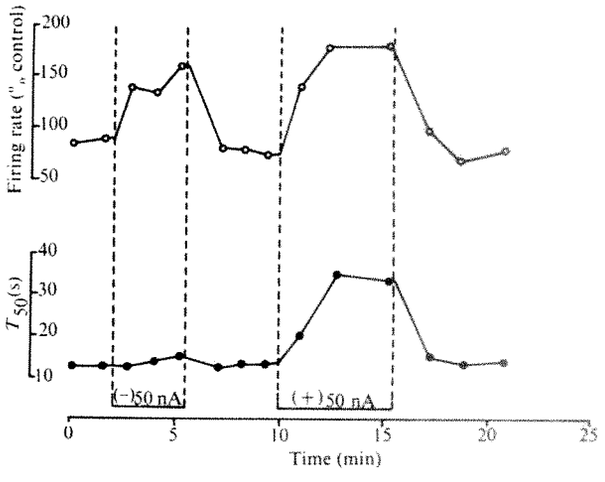


Fig. 1 Production of an increase in firing rate of a single cortical neurone by both (+) and (−)-bicuculline methochloride and GABA antagonism by the (+) isomer. The firing of this neurone was assisted with a continuous application of *d,l*-homocysteate 5 nA. Firing rate (○) immediately preceding each GABA application is plotted against elapsed time. The time taken to achieve 50% depression of firing (T_{50}) (●), by successive application of GABA 40 nA, measures the effectiveness of the GABA[9]. Each point represents one GABA application. Where shown by the first horizantal bar (−)-bicuculline methochloride 50 nA was applied and produced an increase in the firing rate, although it did not affect the response to GABA. The second horizontal bar represents an application of (+)-bicuculline methochloride 50 nA and this produced a similar increase in firing rate. Additionally the GABA T_{50} was much prolonged, indicating GABA antagonism had occurred. Both effects were readily reversible.

Thus, from these experiments, it would appear that only the (+) form of bicuculline methochloride is capable of antagonising both the depressant effects of GABA and presynaptic inhibition. Additionally, (+)-bicuculline methochloride is at least 400 times as potent a convulsant as (−)-bicuculline methochloride. It would, therefore, seem that the biological activity of these compounds is dependent on the absolute configuration around the asymmetric centres.

The GABA molecule is flexible and can exist in many different conformations. Attempts have been made[1,12,13], using molecular models, to explain the GABA antagonist action of bicuculline in terms of a stereochemical match with GABA. Blount and Gilardi have recently, independently determined the absolute configuration of bicuculline using X-ray analysis and Gilardi has pointed out that the previous model studies[1,12,13] on the preferred conformations for GABA and bicuculline were probably based on an incorrect configuration for bicuculline. Some workers[2,14–16] have suggested using molecular orbital and NMR calculations for protonated bicuculline and bicuculline methochloride in solution, as a way of determining the correct preferred conformations. Our results suggest that whatever the preferred conformations of bicuculline and bicuculline methochloride are found to be, the conformation of the (+) isomer will be more correctly isosteric with the preferred biologically active conformation of GABA than will the (−) enantiomer. This stereospecificity parallels that seen elsewhere in the nervous system, for example with muscarinic agonists of acetylcholine, narcotic analgesics and their antagonists and β-receptor blocking agents.

(+)-Bicuculline methochloride (Fig. 3) possesses the 1S, 9R configuration[17], whereas (−)-bicuculline methochloride (Fig. 3) has the 1R, 9S configuration[17], these configurations of course, being shared by the parent compounds (+) and (−)-bicuculline. These observations are compatible with the work of Johnston and his co-workers who revealed that corlumine (Fig. 3) is an effective GABA antagonist[8] for on examination of the stereochemistry we found that this substance also had the 1S, 9R configuration.

We are now examining the biological activity of bicuculline epimers and related compounds to determine whether the particular stereochemical arrangement involving a 1S, 9R configuration is the unique criterion for GABA antagonism in this series of alkaloids.

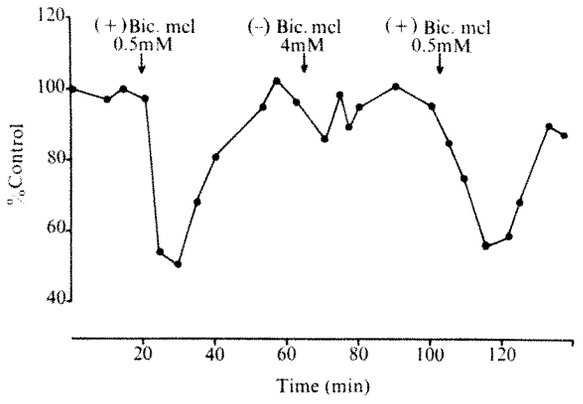


Fig. 2 Reduction of presynaptic inhibition in the rat cunate nucleus by (+)-bicuculline methochloride and the lack of effect of the (−) isomer. The inhibition was measured as the amplitude of the P wave (expressed as percentage of control) recorded in the cuneate in response to peripheral stimulation. At the first arrow (+)-bicuculline methochloride 0.5 mM solution in saline was applied to the surface of the medulla and rapidly reduced the P wave amplitude. After 40 min the P wave amplitude had returned to control values and application of 4 mM (−)-bicuculline methochloride solution at the second arrow had a negligible effect. A subsequent application of the (+) isomer (third arrow) again readily and reversibly depressed the P wave.

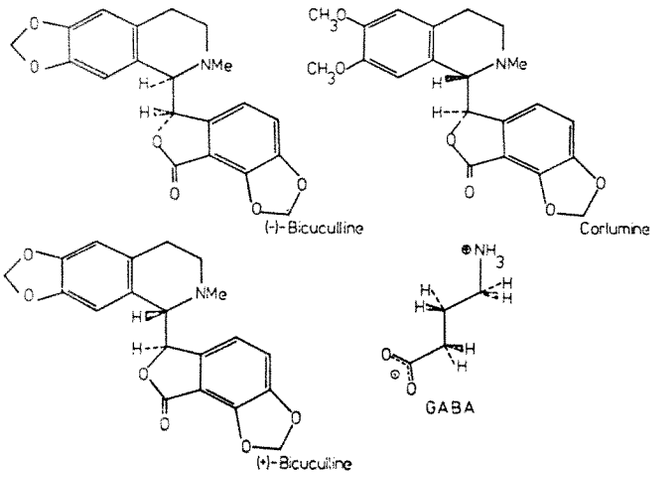


Fig. 3 Structural formulae showing the configurations of (+) and (−)-bicuculline and corlumine about their asymmetric centres. GABA is shown for comparison in one of its possible conformations. Although precise conformational information is not yet available, the preferred biologically active conformation of GABA is more likely to resemble the configurations of the isosteric groupings in (+)-bicuculline. Although the conformation of (+) and (−)-bicuculline methochloride will not be precisely that of (+) and (−) bicuculline due to steric effects of the extra methyl group[14,15], the configuration about the asymmetric centres will be identical.

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1 Curtis, D. R., Duggan, A. W., Felix, D., and Johnston, G. A. R., *Nature*, **226**, 1222 (1970).
2 Kier, L. B., and George, J. M., *Experientia*, **29**, 501 (1973).
3 Welch, A. D., and Henderson, V. E., *J. Pharmac.*, **51**, 482 (1934).
4 Yunusov, S. M., and Yunusov, S. Yu., *Khim. Prirodn. Soedin.*, **4**, 61 (1968).
5 Teitel, S., O'Brien, J., and Brossi, A., *J. org. Chem.*, **37**, 1879 (1972).
6 Godfraind, J. M., Krnjević, K., and Pumain, R., *Nature*, **228**, 675 (1970).
7 Straughan, D. W., Neal, M. J., Simmonds, M. A., Collins, G. G. S., and Hill, R. G., *Nature*, **233**, 352 (1971).
8 Johnston, G. A. R., Beart, P. M., Curtis, D. R., Game, C. J. A., McCulloch, R. M., and McLachlan, R., *Nature new Biol.*, **240**, 219 (1972).
9 Hill, R. G., and Simmonds, M. A., *Br. J. Pharmac.*, **48**, 1 (1973).
10 Banna, N. R., and Jabbur, S. J., *Int. J. Neuropharmac.*, **8**, 299 (1969).

[11] Hill, R. G., Simmonds, M. A., and Straughan, D. W., *J. Physiol., Lond.* (in the press).
[12] Beart, P. M., Curtis, D. R., and Johnston, G. A. R., *Nature new Biol.*, **234**, 80 (1971).
[13] Steward, E. G., Player, R., Quilliam, J. P., Brown, D. A., and Pringle, M. J., *Nature new Biol.*, **233**, 87 (1971).
[14] Blount, J. F., cited in ref. 5.
[15] Gilardi, R. D., *Nature new Biol.*, **245**, 86 (1973).
[16] Andrews, P. R., and Johnston, G. A. R., *Nature new Biol.*, **243**, 29 (1973).
[17] Santavy, F., in *The Alkaloids* (edit. by Manske, R. H. F.) **12**, 396 (Academic Press, New York, 1970).

Spin-lattice relaxation times of imidazole protons and their relevance to NMR studies of proteins

THE spin lattice relaxation time (T_1) is the exponential time constant for re-equilibration of nuclear spins with their surroundings (the lattice) following resonance excitation, and is consequently related to the correlation time of the nuclei in solution. Thus, the determination of T_1 values would be of interest in many biological systems. Measurement of the relative motility of different portions of a protein molecule, for example, could shed light on conformational and functional properties.

Recent technical innovations, notably the introduction of the Fourier transform (FT) technique in nuclear magnetic resonance (NMR) (ref. 1) have made determinations of T_1 almost routine. There are several reports of the measurement of T_1 values of imidazole C_2 protons of histidine residues in peptides and proteins[2-5], as well as applications of ^{13}C NMR (refs 6–11). Previous work has indicated the value of measuring the chemical shift of the imidazole proton resonances as a function of *p*H (ref. 12 and ref. 13 and refs therein). We now report the T_1 values of imidazole protons (Figs 1 and 2) as a function of *p*H.

The T_1 values of both the C_2 and $C_{4,5}$ protons of fresh solutions of imidazole (0.073 M) in 0.1 M sodium chloride in deuterium oxide, showed a minimum at about neutral *p*H (Fig. 3). Measurements made in the absence of (paramagnetic) oxygen by freeze-thawing five times under high vacuum then sealing the tubes, showed an even more pronounced minimum in the data (Fig. 3). Similar results were also observed for histidine, including degassed samples (Table 1). Increasing the salt concentration by a factor of ten in non-degassed samples of imidazole resulted in no significant change. The imidazole concentration was also decreased by a factor of ten and the absence of any change eliminated the possibility of intermolecular association as the origin of the *p*H dependence (Fig. 3).

Values measured in the presence of 10^{-4} M ethylenediamine tetra-acetate (EDTA) increased at neutral *p*H (Table 1 and Fig. 4). By contrast, the addition of 10^{-5}–10^{-6} M Cu^{2+} resulted in an expected lowering of the T_1 values of the ring protons at neutral *p*H, but had less effect in acidic or basic solution (Table 1 and Fig. 4). These results indicate that the phenomenon of the minimum in the *p*H dependence of the T_1 values of the imidazole protons arises from the binding of paramagnetic metal ion impurities in the samples at concentrations of 10^{-7} M or below. At low *p*H these ions would be displaced by deuterons and at high *p*H would bind more strongly to deuteroxide ions. The T_1 value of H_2O is reported to show no *p*H dependency[14,15]. (No significant *p*H dependence was observed for the T_1 value of HDO in the present work, even in the presence of 7×10^{-5} M Cu^{2+} ion, as expected[16].)

If the *p*H dependence of T_1 values were to be explained by a change in the correlation time (τ_c) of the imidazole ring at different *p*H values, this would require, for example, a fifteen-fold change in τ_c of the imidazole C_2 proton between *p*H 1.4 and 7.1 in the degassed samples. The viscosity of water is relatively insensitive to *p*H, and such a large change in τ_c is considered unlikely.

Table 1 Spin-lattice relaxation times (T_1) of imidazole protons

Sample	*p*H	Conditions*	T_1 s C2H	T_1 s C4H
His	0.0	Degassed	18 ± 1.0	9.9 ± 0.5
	4.6	Degassed	8.1 ± 0.5	7.0 ± 0.5
	11.5	Degassed	24 ± 2.0	15.6 ± 2.0
Im A	6.77	—	5.04 ± 0.03	6.57 ± 0.03
		EDTA		
	6.77	(10^{-4} M)	12.4 ± 0.1	13.2 ± 0.1
Im B	7.1	—	6.01 ± 0.14	8.19 ± 0.03
		EDTA		
	7.1	(10^{-4} M)	12.6 ± 0.2	15.5 ± 0.2
		EDTA		
	7.1	Degassed	14.4 ± 0.1	18.8 ± 0.1
Im C	9.46	—	2.21 ± 0.07	3.14 ± 0.05
		EDTA		
	9.46	(10^{-4} M)	15.79 ± 0.21	14.62 ± 0.16
Im D	0.7†	Cu^{2+}		
		(7×10^{-5} M)	21.9 ± 1.8	16.2 ± 0.2
Im E	3.43	—	21.3 ± 0.3	18.1 ± 0.2
		Cu^{2+}		
	3.43	(7×10^{-6} M)	10.6 ± 0.2	11.6 ± 0.2
Im F	7.1	Cu^{2+}		
		1.5×10^{-5} M)	0.35 ± 0.02	0.53 ± 0.02
Im G	7.1	Cu^{2+}		
		(3×10^{-5} M)	0.20 ± 0.08	0.37 ± 0.05
Im H	0.7	Im Dil × 10	33.1 ± 0.7	31.1 ± 0.8
		EDTA		
		(10^{-3} M)		
		Degassed		
Im I	3.3	Degassed	31.8 ± 1.2	26.6 ± 0.6
Im J	6.9	Degassed	0.83 ± 0.04	1.51 ± 0.02
Im K	7.7	Degassed	1.96 ± 1.1	3.14 ± 0.18

* Unless otherwise stated imidazole is 0.073 M in 0.1 M NaCl-D_2O. Most values for imidazole shown in this table are not given in Figs 3 and 4.
† At *p*H 7.1 the C2 and C4 proton lines are extremely broad in the presence of 7×10^{-5} M Cu^{2+}.

Estimates of the T_1 values for a nucleus can be made on the assumptions that only intramolecular dipole–dipole interactions are effective, and that rapid isotropic motion is occurring[1,2,5]. Since the C_4 and C_5 protons effectively relax each other, the T_1 values for these protons in acid *p*H are about half those of the C_2 proton. At neutrality, however, the ratio of the T_1 values is reversed (Table 1). This implies that the relaxation times are dominated by the paramagnetic ions present, and that these spend more time on average closer to the C_2 proton than the $C_{4,5}$ protons.

Calculations were made of the effect of protonation of the imidazole ring on the T_1 values[17] using the geometry determined by X-ray crystallography[18]. Results indicated only a small

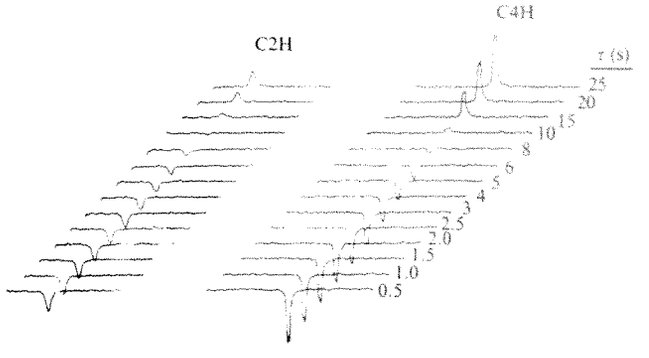


Fig. 1 Spectra of imidazole (0.073 M in 1.0 M NaCl, *p*H 2.0) at 220 MHz recorded on a Varian 220 FT spectrometer at 20 ± 1° C. A single (d, 180°, t, 90°) pulse sequence was used, where d is a delay time ($d > 5T_1$) and t is a variable time. At short values of t the spins are recorded as essentially inverted by the 180° pulse, while at long values of t they approach their maximum intensity value (I_∞). The T_1 values determined in this case were C_2H, 20.6 ± 0.30 s; $C_{4,5}H$, 17.6 ± 0.30 s. Values of *p*H are direct meter readings in D_2O (ref. 12).

change in the T_1 value of both the C_2 and $C_{4,5}$ protons would be expected on protonation of the ring assuming τ_c the same in both cases. This effect would probably be comparable with errors in the T_1 measurements. The increase observed in the T_1 values at low *p*H for the imidazole protons in histidine seems to have been mistakenly interpreted to indicate the titration of the ring[3], rather than the *p*H dependence of the binding of paramagnetic impurities.

Sodium chloride was used in these experiments to act as a reservoir of ionic strength to avoid change in this parameter when *p*H changes. Similar experiments with proteins would require a salt or buffer for reasons of solubility. Thus, the levels of impurity observed in these experiments are not above a reasonable value for practical applications of this technique. Analyses of highly purified imidazole grade III (Sigma) by atomic absorption gave 0.17×10^{-7} mg Cu^{2+} mg^{-1} imidazole or 0.12×10^{-8} M Cu^{2+}. The sodium chloride used in these experiments was Baker Analyzed reagent, with a specified heavy metal analysis including iron of 0.0002%. Analysis of the copper content in the standard 0.1 M NaCl–D_2O solution used gave a value of 2×10^{-5} mg Cu^{2+} ml^{-1}, corresponding to 8×10^{-8} M. Low temperature electron spin resonance (ESR) spectra of the solid NaCl and imidazole showed negligible amounts of paramagnetic material present. Further, the use of 1.0 M NaCl gave only a slight decrease in the T_1 values at neutral *p*H (Fig. 3) indicating that the extra NaCl did not provide a substantial increase in the level of paramagnetic impurity above the background range of approximately 10^{-7} M.

In view of the effects of degassing (Fig. 3) and EDTA (Fig. 4) separately on the T_1 values of the imidazole protons, experiments were performed by degassing in the presence of 10^{-4} M EDTA. As expected, T_1 values of the imidazole protons were increased, but there was still a minimum in the *p*H dependence (Fig. 4). This implies that the EDTA does not completely scavenge metal ions, allowing partial interaction with imidazole at neutral *p*H. Indeed there is evidence for the formation of a ternary copper-EDTA-imidazole complex[19], as for many other such combinations[20,21]. To test whether such a complex was being formed in our experiments, we reduced the ratio of concentrations of imidazole:EDTA by two orders of magnitude. If EDTA was

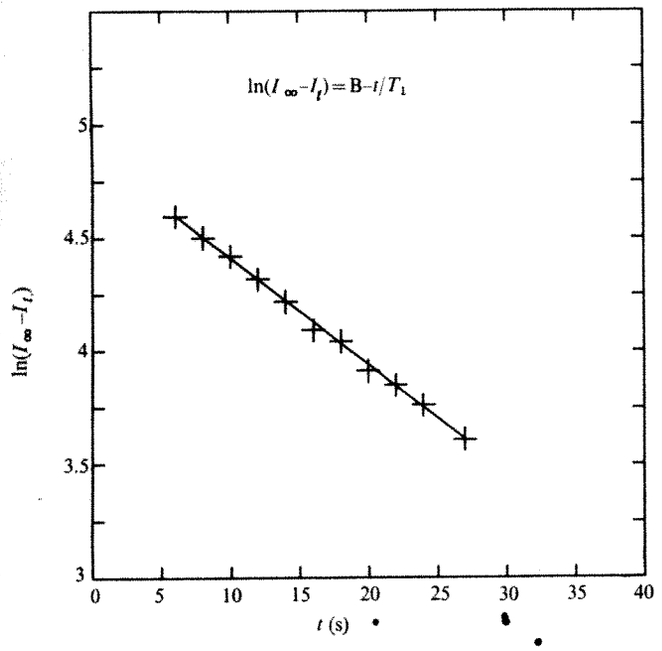


Fig. 2 Plot of the log of the intensity difference at time t ($I_\infty - I_t$) against t derived as in Fig. 1. The equation provides the best straight-line fit to the data, the slope of the line then gives the value of T_1. The value of the intercept B theoretically equals $\ln(2I_\infty)$. Imidazole *p*H = 3.43; $T_1 = 21.29 \pm 0.32$.

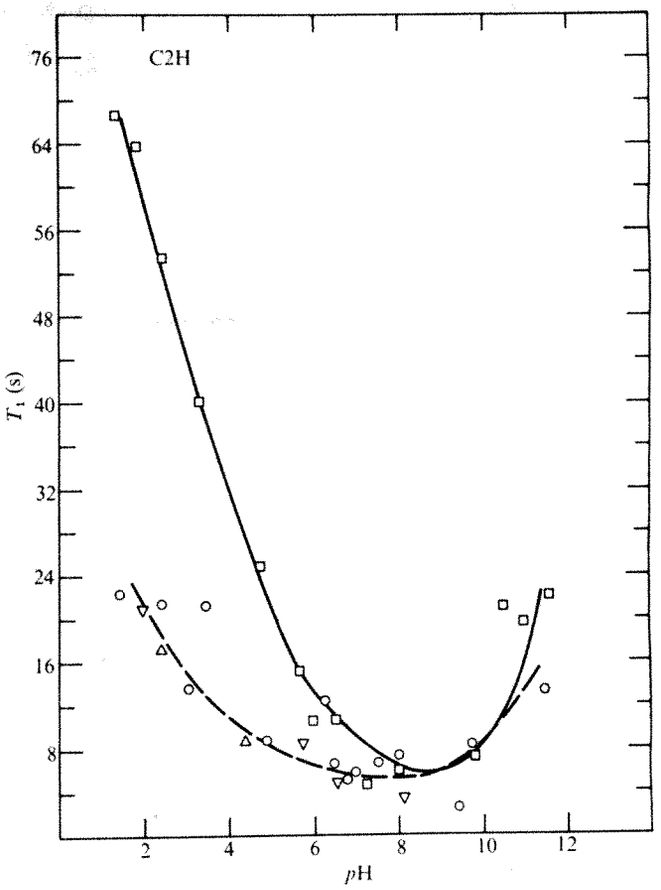


Fig. 3 Plot of the spin-lattice relaxation time (T_1) against *p*H for the C_2 proton of imidazole. Unless otherwise shown, all measurements were carried out in 0.1 M NaCl–D_2O in undegassed samples. The curves are hand-drawn indications of the *p*H dependence. The standard error in the T_1 determinations (Fig. 2) varied with the value obtained, for example, for the degassed samples for C_2H, *p*H 1.40, T_1 66.5 ± 0.7 s; 6.07, 10.6 ± 0.4 s; 10.50, 20.8 ± 0.6 s. □, Degassed; ○, Undegassed; △, diluted ten times; ▽, 1.0 M NaCl.

effectively scavenging metal ions the T_1 values of the imidazole protons would be expected to increase. But if a ternary complex was being formed, the T_1 values would be expected to decrease. In fact, a significant decrease in the T_1 values was observed for 7×10^{-3} M imidazole with 10^{-3} M EDTA (Table 1) in degassed samples, supporting the formation of a ternary complex.

The sensitivity of spin–spin relaxation times (T_2) of purine proton resonances to impurities of copper ion at concentrations of 10^{-6} to 10^{-5} M has recently been emphasised[22-24]. In the light of such considerations, T_2 evaluations from line width studies, particularly as a function of *p*H, must be treated with caution, except where the binding of paramagnetic ion well beyond the impurity level is being studied[25]. Calculations of T_2 from the line widths of the imidazole proton resonances in the presence of Cu^{2+} in this work show that $T_1 \approx 10\ T_2$. Thus, calculations based on the common assumption that $T_1 \approx T_2$ for such systems are probably in error. An increase in the line widths of imidazole proton resonances at intermediate *p*H values commonly observed may result at least in part from the *p*H dependence of the ion binding reported here, rather than the *p*H dependence of the protonation rate[26,27]. Thus, the interpretation of line widths of resonance in proteins in terms of structural effects[28] may be difficult.

T_1 values reported for the C_2 proton of imidazole residues in proteins have been interpreted to be due to the close approach of protons on other residues in the folded tertiary conformation of the native structure[2,3]. Due to the reciprocal relationship

$$(1/T_1)_{obs} = (1/T_1)_{intrinsic} + (1/T_1)_{para}$$

the effect of paramagnetic species would be expected to be greater on protons with intrinsically longer, rather than shorter,

Table 2 Spin-lattice relaxation times (T_1) of imidazole protons of histidine residues in several proteins

Protein	Histidine residues	Salt	*p*H	T_1 (s)
Hen egg white lysozyme	15	0.1 M NaCl	1.4	0.69 ± 0.02
		0.1 M NaCl	3.6	0.93 ± 0.03
		0.1 M NaCl	4.3	0.90 ± 0.03
		0.1 M NaCl	6.0	0.82 ± 0.06
Human lysozyme	78	0.1 M NaCl	3.0	2.65 ± 0.07
		0.1 M NaCl	4.5	2.41 ± 0.12
		0.1 M NaCl	6.0	1.33 ± 0.04
RNase A	105 (H-1)	0.1 M NaCl	1.7	0.88 ± 0.05
		0.1 M NaCl	3.4	1.02 ± 0.04
		0.1 M NaCl	6.0	1.07 ± 0.06
		0.2 M CD_3COONa	5.7	1.09 ± 0.02
		0.2 M CD_3COONa	5.5*	0.77 ± 0.03
	12 (H-2)	0.1 M NaCl	1.7	0.76 ± 0.02
		0.1 M NaCl	3.4	0.42 ± 0.02
		0.1 M NaCl	6.0	0.39 ± 0.04
		0.2 M CD_3COONa	5.7	0.92 ± 0.04
		0.2 M CD_3COONa	5.5*	0.28 ± 0.02
	119 (H-3)	0.1 M NaCl	1.7	0.72 ± 0.05
		0.1 M NaCl	3.4	0.41 ± 0.02
		0.1 M NaCl	6.0	0.82 ± 0.04
		0.2 M CD_3COONa	5.7	1.07 ± 0.03
		0.2 M CD_3COONa	5.5*	0.40 ± 0.02
	48 (H-4)	0.1 M NaCl	1.7	1.03 ± 0.09
		0.1 M NaCl	3.4	1.07 ± 0.04
		0.1 M NaCl	6.0	Not observed
		0.2 M CD_3COONa	5.7	1.14 ± 0.07
		0.2 M CD_3COONa	5.5*	0.51 ± 0.03

Values were calculated as shown in Fig 2. It is not easy to measure T_1 values of C_2 protons in proteins at *p*H > 6 due to the poor baseline from adjacent resonances. The four C_2 proton resonances in RNase A are resolved at only a few *p*H values. Assignments of the resonances (numbered H-1 to H-4 at *p*H 5.5) are taken from ref. 34.

* From ref. 2, determined at 100 MHz at 33° C.

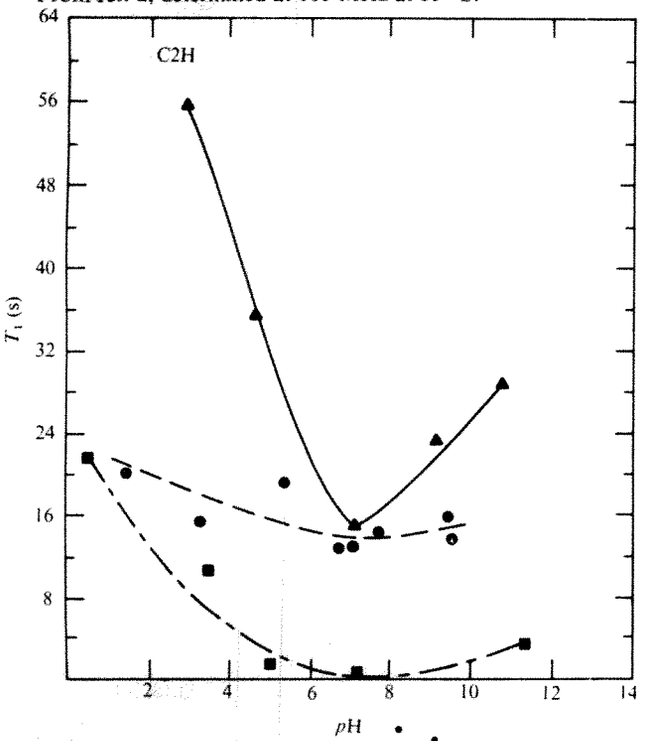


Fig. 4 Plot of the spin-lattice relaxation time (T_1) against p*H* for the C_2 proton of imidazole in the presence of EDTA and Cu^{2+} ion. ▲, EDTA 10^{-4}M degassed; ●, EDTA 10^{-4}M undegassed; ■, Cu^{2+} 7×10^{-6}M.

T_1 values. We have measured T_1 values of imidazole resonances in several proteins (Table 2), and have observed values as long as two seconds for the single histidine residue present in human lysozyme. Indeed, the *p*H dependence in this case showed an increase in acid *p*H similar to the phenomenon seen in imidazole itself. The values obtained for bovine pancreatic RNase A under essentially the same conditions were longer than those previously reported[3], as expected from the frequency dependence of T_1 values[1,5]. The T_1 values observed may reflect the environments of the histidine residues in the structure of RNase A (ref. 29). In view of the known binding of Cu^{2+} ion to these residues[30], however, and the phenomenon reported here for imidazole, such interpretations may not be justified.

The above considerations may not be as important for groups which do not bind metal ions. Nor would such a large effect be expected for the T_1 values of carbon nuclei observed by ^{13}C NMR. Other studies, however, have indicated the sensitivity of ^{13}C resonances to paramagnetic metal ions[31]. In view of the above, interpretations of regional unfolding on denaturation of proteins from relative values of T_1 (refs 3, 7, 8) must be viewed with some caution, and may also reflect local changes of ion binding on changing solution conditions and conformation. Addition of EDTA to protein solutions may not simply solve the problem of such measurements since EDTA binds to some proteins[32]. For many other proteins, there is a requirement for a metal ion[3,33], which although not itself paramagnetic may be accompanied by sufficient paramagnetic metal ion impurity to affect the T_1 values significantly.

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1 Farrar T. C., and Becker, E. D. *Pulse and Fourier Transform NMR* (Academic Press New York, 1971).
2 Benz, F. W., Roberts, G. C. K., Feeney, J., and Ison, R. R., *Biochim. biophys. Acta*, **278**, 233 (1972).
3 Arata, Y., Khalifah, R., and Jardetzkey, O., *Ann. N.Y. Acad. Sci.*, **222**, 230 (1973).
4 Pesando, J., *Fedn Proc.*, **31**, 452 (1972).
5 Coates, H. B., McLauchlan, K. A., Campbell, I. D., and McColl, C. E., *Biochim. Biophys. Acta*, **310**, 1 (1973).
6 Sternlicht, H., Kenyon, G. L., Packer, E. L., and Sinclair, J., *J. Am. chem. Soc.*, **93**, 199 (1971).
7 Allerhand, A., Doddrell, D., Glushko, V., Cochran, D. W., Wenkert, E., Lawson, P. J., and Gurd, F. R. N., *J. Am. chem. Soc.*, **93**, 544 (1971).
8 Glushko, V., Lawson, P. J., and Gurd, F. R. N., *J. biol. Chem.*, **247**, 3176 (1972).
9 Smith, I. C. P., DesLauriers, R., Saito, H., Walters, R., Garrigou-Lagrande, C., McGregor, H., and Sarantakis, D., *Ann. N.Y. Acad. Sci.*, **222**, 597 (1973).
10 Browne D. T., Kenyon, G. L., Packer, E. L., Wilson, D. M., and Sternlicht, H., *Biochem. biophys. Res. Commun.*, **50**, 42 (1973).
11 Chaiken, I., Freedman, M. H., Lyerla, J. R., jun. and Cohen, J. S., *J. biol. Chem.*, **248**, 844 (1973).
12 Sachs, D. H., Schechter, A. N., and Cohen, J. S., *J. biol. Chem.*, **246**, 6576 (1971).
13 Cohen, J. S. Griffin, J. H. and Schechter A. N. *J. biol. Chem.* **248**, 4305 (1973).
14 Meiboom, S., Luz, Z., and Gill, D., *J. chem. Phys.*, **27**, 1411 (1957).
15 Glick, R. E., and Tewari, K. C., *J. chem. Phys.*, **44**, 546 (1965); *Nature* **211**, 739 (1966).
16 Morgan, L. O., and Nolle, A. W., *J. chem. Phys.* **31**, 365 (1959).
17 Jardetzky, O., *Adv. Chem. Phys.* **7**, 499 (1964).

[18] Martinez-Carrera, S., *Acta Cryst.*, **20**, 783 (1966).
[19] Israeli, J., and Saulnier, H., *Inorg. Chem. Acta.* **2**, 482 (1968).
[20] Sunberg, R. G., and Martin, R. B., *Chem. Res.* (in the press).
[21] Walter, W. R., Shaw Y. L., and Li, N. C., *J. Coord. Chem.* **3**, 77 (1973).
[22] Wong, Y. P., *J. Am. chem. Soc.*, **95**, 3511 (1973).
[23] Pieber, M., Kroon, P. A., Prestegard, J. H., and Chan, S. I., *J. Am. chem. Soc.*, **95**, 3408 (1973).
[24] Gorenstein, D. G., and Wyrwicz, A., *Biochem., biophys. Res. Commun.*, **54**, 976 (1973).
[25] Sigel, H., and McCormick, D. B., *J. Am. chem. Soc.*, **93**, 2041 (1971).
[26] Patel, D. J., Woodward, C. K., and Bovey, F. A., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 599 (1972).
[27] McDonald, C. C., and Phillips, W. D., *Biochemistry*, **12**, 3170 (1973).
[28] Bradbury, J. H., Chapman, B. E., and King, N. L. R., *Int. J. protein Res.*, **3**, 351 (1971).
[29] Richards, F. M., and Wyckoff, M. W., in *The Enzymes* (edit. by Boyer, P.), **4**, 647 (Academic Press, New York, 1971).
[30] Ihnat, M., *Biochemistry*, **11**, 3483 (1972).
[31] Kotowycz, G., and Suzuki, O., *Biochemistry*, **12**, 5325 (1973).
[32] Putter, J., Barretto, A., Markley, J. L., and Jardetzky, O., *Proc. natn. Acad. Sci. U.S.A.*, **64**, 1396 (1969).
[33] Cuatrecasas, P., Fuchs, S., and Anfinsen, C. B., *J. biol. Chem.*, **242**, 3063 (1967).
[34] Meadows, D. H., Jardetzky, O., Epand, R. M., Ruterjans, H. H., and Scheraga, H. A., *Proc. natn. Acad. Sci. U.S.A.*, **60**, 766 (1968).

Detection of metabolic carcinogen intermediates in urine of carcinogen-fed rats by means of bacterial mutagenesis

SEVERAL developments suggest the possibility of a new means of assessing the exposure of human populations to chemical carcinogens. Carcinogens such as 2-acetylaminofluorene (AAF) and *p*–dimethylaminoazobenzene (DAB) have been shown[1-4] not to be carcinogenically active agents themselves, but to be converted into such agents by metabolic processes in the target organs (in these instances, liver). These studies also show that actively carcinogenic metabolites (as well as other metabolic products which are not carcinogenic) may be excreted in the urine, apparently as glucuronides[4,5]. Similar results have been obtained with human subjects ingesting aflatoxin B_1 from contaminated peanut butter[6].

Miller *et al.* showed that active AAF-intermediates are mutagenic in a test system involving *in vitro* interactions with transforming DNA followed by inoculation of the latter into a suitable strain of *B. subtilis*[1,7]. More recently Ames *et al.* have found that 18 of 20 chemical carcinogens that were studied are highly mutagenic toward *Salmonella typhimurium* test strains if they are incubated during exposure of the bacteria in a partially purified liver homogenate (a 9,000*g* supernatant)[8]. These observations considerably reduce the disparity between carcinogenicity and mutagenicity of organic compounds, which has until now discouraged the use of mutagenesis as a test for carcinogenicity. The technique developed by Ames *et al.* is simple, rapid, and sensitive (capable of detecting less than 1 μg of AAF) and hence well adapted to screening of environmental samples. We have investigated whether, in rats exposed to a carcinogen, metabolic intermediates of the latter can be detected in the urine by means of their mutagenic activity in the *Salmonella* test system.

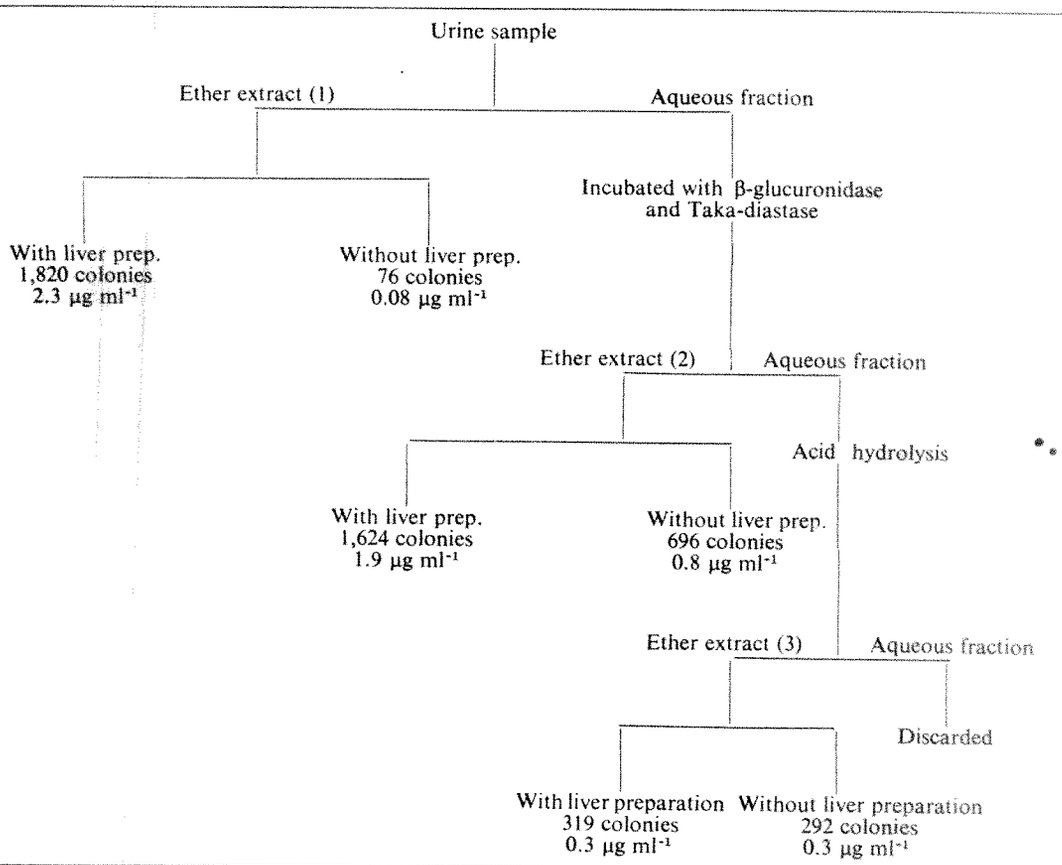
Control male Holtzman rats were maintained on a balanced semisynthetic diet (see ref. 9 for details); experimental groups were maintained on the same diet supplemented with 0.67 mg g^{-1} of AAF or 0.6 mg g^{-1} of DAB. The animals were supplied with distilled drinking water *ad libitum*. At one week intervals after the onset of feeding, two rats from each group were placed in separate collecting

Table 1 Mutagenic substances in urine of rats fed control and carcinogen-containing diets

Diet (no. of weeks)	Sample: Type	Sample: Equivalent vol. of urine (ml)	Treatment: β-glucuronidase and Taka-diastase	Treatment: Liver preparation	No. of colonies*: Total no. per plate	No. of colonies*: No. per plate per ml urine	Equivalent conc. of AAF in urine μg ml^{-1}†
Control (1)	3×conc.‡	0.9	−	−	16	—	—
Control (1)	Direct	0.3	−	+	27	—	—
Control (2)	3×conc.	0.9	−	−	14	—	—
Control (2)	3×conc.	0.9	−	+	33	—	—
Control (3)	Direct	0.6	−	−	18	—	—
Control (3)	Direct	0.6	−	+	25	—	—
Control (3)	Ether extract§	0.9	+	−	12	—	—
Control (3)	Ether extract	0.9	+	+	22	—	—
Control (4)	3×conc.	0.9	−	−	18	—	—
Control (4)	3×conc.	0.9	−	+	35	—	—
AAF (1)	3×conc.	0.9	−	−	63	70	0.25
AAF (1)	Direct	0.3	−	+	341	1,137	5.2
AAF (2)	Direct	0.6	−	−	56	93	0.35
AAF (2)	Direct	0.6	−	+	1,947	3,245	16.0
AAF (2)	Ether extract	0.9	+	−	175	194	1.0
AAF (2)	Ether extract	0.9	+	+	3,672	4,060	20.2
AAF (3)	Direct	0.6	−	−	153	255	1.1
AAF (3)	Direct	0.6	−	+	2,292	3,820	18.9
AAF (3)	Ether extract	0.9	+	−	207	230	1.1
AAF (3)	Ether extract	0.9	+	+	6,348	7,053	35.1
AAF (4)	3×conc.	0.9	−	−	46	51	0.16
AAF (4)	3×conc.	0.9	−	+	1,842	2,047	10.1
DAB (4)	Direct	0.3	−	−	15	—	—
DAB (4)	Direct	0.3	−	+	28	—	—
DAB (4)	Ether extract	0.9	+	−	26	—	—
DAB (4)	Ether extract	0.9	+	+	479	532	—

* Number of revertant colonies produced per plate by *Salmonella typhimurium* test strain TA 1538.
† These values are approximated from the calibration experiment, on the basis that 1 μg of AAF produces about 200 colonies. Corrections have been made for the number of colonies produced by urine samples from rats on the control diet.
‡ Urine concentrates were prepared by evaporating the urine to one-third the original volume.
§ In these instances the urine samples, after incubation with β-glucuronidase and Taka-diastase at *p*H 7, were extracted with ether. The ether extracts were evaporated to dryness and taken up in one-third the original volume (of urine) of DMSO and aliquots were tested against the bacteria.

Table 2 Analysis of mutagenic constituents of urine



21 ml of urine, adjusted to *p*H 7 and diluted to 75 ml with water was mixed with 60 ml of 0.1 M *p*H 7 phosphate buffer and extracted four times with 50 ml aliquots of ether (1). The aqueous phase, freed of ether, was incubated for 16 h at 37° C with 3,200 units each of bovine β-glucuronidase and Taka-diastase (Sigma). After cooling it was extracted four times with 50 ml aliquots of ether (2). The aqueous phase was adjusted to *p*H 1, refluxed 15 min, neutralised, and extracted four times with 50 ml aliquots of ether (3). The ether extracts (1), (2), and (3) were evaporated and residues dissolved in 2.6, 2.5 and 2.0 ml of DMSO respectively. 0.5 ml aliquots were used for each assay, which represented 4, 4.2 and 5.25 ml of urine for ether extracts 1, 2 and 3 respectively. The procedure is similar to that described in ref. 4. Actual colony counts per plate (test strain TA 1538) are given. Concentrations of mutagens are approximated as described in Table 1.

cages and urine samples were collected for 24 h. The samples were frozen until analysed, and sterilised by Millipore filtration before use.

Tests for mutagenicity were carried out according to the procedures developed by Ames *et al.*[8], using a strain of histidine-negative *S. typhimurium* (TA 1538) kindly supplied by Dr Ames. In this procedure, the sample to be tested is mixed with aliquots of a 9,000*g* supernatant prepared from a homogenate of normal rat liver, and with the bacterial inoculum incubated on an agar nutrient medium lacking histidine. Colonies that are formed in this medium represent reverse mutations of the original histidine-negative genome to histidine-positive. Counts of revertant colonies were made after 48 h of incubation at 37° C. All tests were made in duplicate; colony counts are reported as averages of the two values.

First we carried out a calibration experiment to determine the number of mutant colonies of *S. typhimurium* TA 1538 produced when a series of known amounts of AAF were incubated with the 9,000*g* supernatant. The results showed a linear response in the test range (0–50 μg of AAF, that is 0–0.2 mM concentrations of AAF in the final test solution) yielding about 200 colonies per μg of AAF with a blank value of 20 colonies in the absence of added AAF.

Table 1 shows that when rats are maintained on a normal diet for a 4 week period, weekly tests of urine samples show no significant variation in the number of revertant colonies produced either in the presence or absence of the liver preparation. AAF-fed rats excrete in their urine considerable concentrations of mutagenic substances. In the absence of liver preparation, the urine samples obtained in successive weeks, yielded respectively, 70, 93, 255 and 51 colonies per ml urine. These values are sufficiently above the control value (16 colonies per plate) to suggest the presence of intrinsically mutagenic substances in these urine samples. The evidence relative to the presence of activatable mutagens (that is those activated by exposure to the liver preparation) is unequivocal: in the presence of the liver preparation between 1,137 and 3,820 colonies per ml of urine are observed.

Also shown in Table 1 are the results of tests in which the urine samples were incubated with a mixture of β-glucuronidase and Taka-diastase. The incubation mixture was then extracted with ether, the extract evaporated to dryness, taken up in DMSO (dimethylsulphoxide) and tested in the usual way (see Table 2 for details of this procedure). As shown in Table 1, this procedure considerably increases the mutagenic activity of the samples, both in the presence and absence of the liver preparation. These data indicate that part of both the inherently active intermediates (that is, those which do not require activation by the liver preparation) and of carcinogen intermediates that become active only on treatment with the liver preparation occur in the urine as glucuronides.

DAB was one of the two carcinogens among the 20 tested by Ames *et al.*, which failed to yield mutagenic activity on incubation with the liver preparation. We also found (Table 1) that urine samples from DAB-fed rats tested directly by the Ames method show no mutagenic activity. But when tested according to the procedure (described above) for releasing intermediates from their conjugated forms, significant mutagenic activity is observed. Tested in this way

urine from DAB-fed rats produced 532 revertant colonies per ml of urine, in the presence of the 9,000*g* supernatant. Thus, DAB-fed rats excrete in their urine glucuronides of intermediates of the carcinogen that are mutagenically active in the presence of the liver preparation, following removal of the sugar residue.

By means of suitable extraction and incubation procedures, similar to those used by Weisburger *et al.*[4], it is possible to estimate the contribution of the several constituents to the overall mutagenicity of urine samples. The results of such an experiment, using urine from rats maintained on the AAF diet for 1 week, are shown in Table 2. In this experiment the following constituents were found in the urine sample, expressed in amounts equivalent to AAF: (1) activatable, unconjugated mutagen (probably AAF of faecal origin), 2.3 μg ml^{-1}; (2) intrinsically active, unconjugated intermediates, 0.08 μg ml^{-1} (an amount of marginal significance in this assay); (3) glucuronides of intrinsically active intermediates, 0.8 μg ml^{-1}; (4) glucuronides of activatable intermediates, 1.1 μg ml^{-1} (1.9–0.8); (5) acid-hydrolysable esters of intrinsically active intermediates, 0.3 μg ml^{-1}. Thus of a total about 4.5 μg ml^{-1} of carcinogen-derived material, 2.2 μg ml^{-1} is certainly of urinary origin and represents both inherently active and activatable intermediates of AAF. Since the mutagenic activity of the urine of DAB-fed rats becomes evident only after treatment with β-glucuronidase and Taka-diastase all of this activity must be due to glucuronides of urinary origin.

Thus metabolic intermediates of AAF and DAB can be detected in the urine of rats fed on diets that contain these carcinogens by means of tests based on mutagenicity towards a strain of *Salmonella typhimurium*, while the urine of rats on normal diets yield no significant response in these tests. This procedure should be applicable to the detection of exposure of human populations to carcinogens. Preliminary tests using human urine are in progress.

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Note added in proof: After the completion of this work we learned that similar results have been obtained by Ames and Durston (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 737; 1974).

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1 Miller, E. C., and Miller, J. A., *in Chemical Mutagens, Principles and Methods for their Detection* (edit. by Hollander, A.), **1**, 83–119 (Plenum Press, New York, 1971).
2 Miller, J. A., *Cancer Res.*, **30**, 559 (1970).
3 Weisburger, E. K., and Weisburger, J. H., *Adv. Cancer Res.*, **5**, 331 (1958).
4 Weisburger, J. H., Grantham, P. H., Vanhorn, E., Steigbigel, N. H., Rall, D. P., and Weisburger, E. K., *Cancer Res.*, **24**, 475 (1964).
5 Miller, J. A., Cramer, J. W., and Miller, E. C., *Cancer Res.*, **20**, 950 (1960).
6 Campbell, T. C., Caedo, jun., J. P., Bulatoa-Jane, J., Salamat, L., and Engel, R. W., *Nature*, **227**, 403 (1970).
7 Maher, V. M., Miller, E. C., Miller, J. A., and Szybalski, W., *Molec. Pharmac.*, **4**, 411 (1968).
8 Ames, B. N., Durston, W. E., Yamasaki, E., and Lee, F. D., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2281 (1973).
9 Commoner, B., Woolum, J. C., Senturia, jun., B. H., and Ternberg, J. L., *Cancer Res.*, **30**, 2091 (1970).

Primary defect in copper transport underlies mottled mutants in the mouse

THE results presented here show that a primary defect in copper transport underlies the mottled syndrome in the mouse. The X-linked mottled mutants thus offer an excellent system for the study of mammalian copper metabolism. They also provide an animal model of the inherited human copper deficiency, Menkes kinky hair disease[1,2], which is also X-linked.

A number of different mutant alleles at the mottled locus are known and these are listed, together with their phenotypic effects, in Table 1. In all cases where the male hemizygote survives beyond birth, the resulting mouse shows a severe dilution in hair pigment (Fig. 1), although the individual hairs are pigmented at their tips, together with an altered hair structure[3] and curly whiskers. A persistent neurological disturbance is also apparent, consisting of a mild sustained tremor and general inactivity[4]. The more severe alleles *Mo* and *Mo^{dp}* die respectively *in utero* and at birth, and extensive skeletal defects have been reported in *Mo^{dp}* hemizygotes and heterozygotes[5].

Recent work[4] has demonstrated a severe deficiency in central noradrenaline (NA) levels in *Mo^{br}* and *Mo^{vbr}* males and this has been traced to a reduced *in vivo* conversion of dopamine to NA by the enzyme dopamine-beta-hydroxylase (DBH)[10]. A deficiency in NA was also found in mice hemizygous for the viable allele *Mo^{blo}* (Table 2). But direct *in vitro* estimates of DBH activity in *Mo^{br}* and *Mo^{blo}* brain tissue (Table 2) showed an elevated activity, in contrast to the *in vivo* situation. The DBH assay used[11] contains exogenous copper to inactivate endogenous inhibitors of the enzyme. Since DBH is a copper-containing enzyme with a cofactor requirement for cupric ion[12], the difference between the *in vivo* and *in vitro* results may reside in a copper deficiency in mutant brain tissue.

Estimates of liver and brain copper levels (Table 3) confirm the presence of a copper deficiency in *Mo^{br}*. The accumulation of copper in the intestinal wall indicates that uptake from the gut lumen is normal but that the general body deficiency arises from a defective transport within the intestinal epithelial or mucosal cells or across the membrane on the serosal aspect of these cells. To test this interpretation, the *in vitro* uptake of isotopic copper (^{64}Cu) into intestinal cells from *Mo^{br}* males and normal littermates was examined. Segments of intestinal wall were incubated in Krebs–phosphate buffer containing 1 mg ml^{-1} bovine serum albumin and 1 μg ml^{-1} $^{64}CuCl_2$ The results (expressed in ng $^{64}CuCl_2$ per 5 min per g $\pm$ s.e.m.) at 0°C (normal: 65.9 $\pm$ 11.9; *Mo^{br}*: 68.5 $\pm$ 11.5) and at 37°C (normal: 131.7 $\pm$ 27.4; *Mo^{br}*: 138.9 $\pm$ 27.7) confirm that the initial uptake into the intestinal epithelium is not defective.

Serum copper is largely contained in ceruloplasmin[15]. Ceruloplasmin levels were estimated by the method of

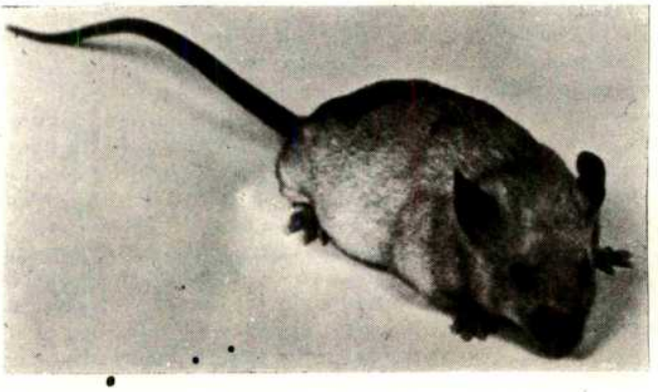


Fig. 1 A rare surviving *Mo^{br}* male.

Table 1 Phenotype characteristics of alleles at the X-linked mottled locus in the mouse

Allele	Male hemizygote	Female heterozygote	Ref
Blotchy (*Mo^{blo}*)	Pale fur, curly whiskers. Viable and fertile.	Variegated coat colour, curly whiskers. Viable and fertile.	6
Brindled (*Mo^{br}*)	Pale fur, curly whiskers. Dies at about 14 d *post partum*.	Variegated coat colour, curly whiskers. Viable and fertile.	7
Dappled (*Mo^{dp}*)	Pale fur, curly whiskers, skeletal defects. Dies at birth.	Variegated coat colour, curly whiskers, skeletal defects. Viable and fertile.	5
Mottled (*Mo*)	Lethal *in utero*.	Variegated coat colour, curly whiskers. Viable and fertile.	7, 9
Viable-brindled (*Mo^{vbr}*)	Pale fur, curly whiskers. Reduced viability and sterile.	Variegated coat colour, curly whiskers. Viable and fertile.	8

Ravin[13]. The results (normal: 6.52 ± 0.60; *Mo^{br}*: 4.33 ± 0.50 expressed in A_{530} units per h per ml serum $\pm$ s.e.m. show a significant reduction ($P = < 0.01$) in ceruloplasmin concentration in mutant mice. The precise function of ceruloplasmin is still obscure but it is unlikely that it represents a copper transport protein since the copper content of ceruloplasmin is not exchangeable *in vivo*[14]. Ceruloplasmin may function in the maintenance of a copper balance[15] and its reduced level in *Mo^{br}* supports this interpretation.

The experimental findings are consistent with a deficiency in copper transport and the relationship of a copper deficiency to the different pleiotropic effects of these mutants indicates that it is probably primary. The initial effect of a copper deficiency will be on the copper-requiring enzymes and the reduction in the *in vivo* activity of DBH has already been described. The elevated *in vitro* activity of this enzyme may be a reflection of an increased synthesis of the enzyme protein in response to the deficiency in NA biosynthesis. The neurological symptoms are consistent with a deficiency of NA[10], although a reduction in cytochrome oxidase, another copper-dependent enzyme, may contribute. The virtual absence of hair pigmentation can also be explained by a direct effect of the copper deficiency on the activity of tyrosinase, the first enzyme in the melanin biosynthetic pathway. The pigmented tip of each hair may arise from an initial but rapidly depleted copper store in the hair follicle.

Table 2 Brain noradrenaline and *in vitro* activity in *Mo^{br}* and *Mo^{blo}*♂♂ and normal (+) littermates

	Age (d)	NA	% difference from normal	DBH	% difference from normal
+	7	1.35 ± 0.19(6)†		33.14 ± 2.39(13)	
Mo^{br}	7	0.45 ± 0.10(6)†	−66.7*	48.81 ± 2.92(12)	+47.3*
+	28	1.96 ± 0.09(6)		33.02 ± 3.20(6)	
Mo^{blo}	28	1.34 ± 0.06(6)	−31.6*	77.63 ± 5.26(6)	+135.1*

DBH activity was assayed by the method of Molinoff *et al.*[11] with the following modifications. Brains were homogenised in 10 volumes of 0.005 M Tris-HCl buffer *p*H 6.0 containing 0.2% Triton-X100 and assays carried out using tyramine as substrate and a final concentration of 3.25×10^{-4} M $CuSO_4$. NA was assayed by the method of Anton and Sayre[22] as previously modified[10]. DBH activity is expressed as nmol octopamine g^{-1} h^{-1} $\pm$ s.e.m. and NA concentration is expressed in nmol g^{-1} $\pm$ s.e.m. Numbers in parentheses refer to number of mice used.

*Significance at the 1% probability level.

†Data previously published[4].

Table 3 Copper levels in brain, liver and intestinal wall from 7–10 d *Mo^{br}*♂♂ and normal (+) littermates

	+	*Mo^{br}*	% difference from normal
Brain	2.43 ± 0.27(7)	1.43 ± 0.24(7)	−41.1*
Liver	24.23 ± 1.98(6)	9.37 ± 1.14(6)	−61.3*
Intestinal wall	4.39 ± 0.87(12)	10.13 ± 1.84(12)	+131.3*

Tissue copper levels were estimated by the spectrophotometric method of Carter[23]. Tissue homogenates were hydrolysed with 2N HCl and precipitated with 22.6% trichloroacetic acid. After centrifugation, 0.5 ml of glacial acetic acid containing 40 µg dicyclopentamethylene thiuram disulphide was added to 1.0 ml of tissue supernatant and absorption determined after 10 min at 420 nm. Appropriate standards and blanks were carried through the procedure. Copper levels are expressed in µg g^{-1} $\pm$ s.e.m.

*Significance at the 1% probability level.

Numbers in parentheses refer to numbers of mice used.

Aortic aneurism arising from the fragmentation of the internal elastic lamina of the arterial wall has been reported in a number of mottled alleles[16] and it is significant that the formation of cross linkages between lysine residues in the synthesis of elastin and collagen is a copper-dependent process[17]. Recent results[19] indicate a reduced elastin cross linking in mottled mutants and the lysyl oxidase identified in chick bone collagen[18] seems a likely candidate for the action of the copper deficiency. The skeletal defects may similarly arise from a defective synthesis of bone collagen. The formation of disulphide bonds in keratin is also copper dependent, suggesting defective keratin synthesis as the basis of the curly whiskers and altered hair structure.

Copper deficiency has been shown[2] to underlie the human X-linked progressive brain disease, Menkes kinky hair disease[1]. The phenotypic effects of this mutant include abnormal white hair, altered hair structure[1], and arterial disease[2]. Copper levels are depressed in liver and serum, and the accumulation of copper in the intestinal epithelium[21] directly parallels that in the mottled syndrome in mice. Catecholamine biosynthesis has not been examined although the reported hypothermia[20] indicates that a deficiency in NA may be present.

Phenotypic variegation arising from random X inactivation[24] in a female heterozygote for an X-linked coat colour mutant depends on the cell autonomy of the mutant defect. The variegated appearance of the mottled heterozygote would therefore argue for the presence of the copper transport defect in the follicle and pigment cells of the skin, and possibly elsewhere. Copper-deficient cells would be expected to be at a selective disadvantage in competition with their normal counterparts, leading to a progressive dilution of mutant cells during development. The progressive darkening of *Mo^{br}* heterozygotes with age[3] supports this conclusion.

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1 Menkes, J. H., Alter, M., Steigleder, G. K., Weakley, D. R., and Sung, J. H., *Pediatrics*, **29**, 764–779 (1962).
2 Danks, D. M., Stevens, B. J., Campbell, P. E., Gillespie, J. M., Walker-Smith, J., Blomfield, J., and Turner, B., *Lancet*, **i**, 1100–1103 (1972).
3 Grüneberg, H., *J. Embryol. exp. Morph.*, **22**, 145–179 (1969).
4 Hunt, D. M., and Johnson, D. R., *Biochem. Genet.*, **6**, 31–40 (1972).

[5] Phillips, R. J. S., *Genet. Res.*, **2**, 290–295 (1961).
[6] Russell, L. B., and Saylors, C. L., *Genetics*, **47**, 7–10 (1962).
[7] Fraser, A. S., Sobey, S., and Spicer, C. C., *J. Genet.*, **51**, 217–221 (1953).
[8] Cattanach, B. M., Pollard, C. E., and Perez, J. N., *Genet. Res.*, **14**, 223–235 (1969).
[9] Falconer, D. S., *Z. indukt. Abstamm. VererbLehre*, **85**, 210–219 (1953).
[10] Hunt, D. M., and Johnson, D. R., *J. Neurochem.*, **19**, 2811–2819 (1972).
[11] Molinoff, P. B., Weinshilboum, R., and Axelrod, J., *J. Pharmac. exp. Ther.*, **178**, 425–431 (1971).
[12] Kaufman, S., and Friedman, S., *Pharmac. Rev.*, **17**, 71–99 (1965).
[13] Ravin, H. A., *J. Lab. clin. Med.*, **58**, 161–168 (1961).
[14] Steinlieb, J., Morell, A. G., Tucker, W. D., Green, M. W., and Scheinberg, I. H., *J. Lab. Invest.*, **40**, 1834–1840 (1961).
[15] Dowdy, R. P., *Am. J. clin. Nur.*, **22**, 887–892 (1969).
[16] Grahn, D., Fry, R. J. M., and Hamilton, K. T., *Genetics*, **61**, 522 (abst.) (1969).
[17] Carnes, W. H., *Fedn Proc.*, **30**, 995–1000 (1971).
[18] Siegel, R. C., and Martin, G. R., *J. biol. Chem.*, **245**, 1653–1658 (1970).
[19] Grahn, D., *Mouse News Letter*, **48**, 22 (1973).
[20] Danks, D. M., Cartwright, E., Campbell, P. E., and Mayne, V., *Lancet*, **ii**, 1089 (1971).
[21] Danks, D. M., Cartwright, E., Stevens, B. J., and Townley, R. R. W., *Science*, **179**, 1140–1142 (1973).
[22] Anton, A. H., and Sayre, D. F., *J. Pharmac exp. Ther.*, **138**, 360-375 (1962).
[23] Carter, P., *Clin. chim. Acta*, **39**, 497–505 (1972).
[24] Lyon, M. F., *Nature*, **190**, 372–373 (1961).

Uptake of radioactive DOPA by *M. leprae*

THE inability to culture *Mycobacterium leprae in vitro* has been a great obstacle to the progress of research in leprosy. This obstacle has been in part overcome by the introduction of the morphological index for the assessment of the viability of *M. leprae*, although the index is relatively crude. Biochemical studies[1,2] have revealed the specificity of the enzyme *o*-diphenoloxidase in relation to *M. leprae* as opposed to other bacteria included in the genus of mycobacteria. *o*-Diphenoloxidase oxidises 3,4-dihydroxyphenylalanine (DOPA) to quinone.

It seemed possible that DOPA might be a metabolite of the living bacilli and that radioactive atoms within the molecule would become incorporated into macromolecules within the living organism. We have shown this to be the case by high resolution autoradiography. Bacterial suspensions were prepared by the homogenisation of biopsies from untreated patients without disruption of bacilli and from patients in early stages of treatment, with a relatively high morphological index. Drops of suspension were added to a smear of warm agar on coverslips and incubated for various periods up to 48 h in the presence of 5 μCi ml^{-1} of ^{3}H-DOPA. The cultures were carefully washed and fixed in formol saline and then examined by autoradiography with Ilford K5 Emulsion as a thin film.

The high resolution obtained made it possible to observe the grains on individual bacilli (Fig. 1). A number of bacilli were clearly labelled after 48 h and some were intensely labelled; granular bacilli were unlabelled. The heavily labelled bacilli were often seen as a small centre of compact organisms within a large group. These clearly labelled bacilli might originate from centres of active growth within the population. In a parallel test with ^{3}H-thymidine a few of the bacilli in the aggregates showed grains. Labelled bacilli were also observed after uptake by mouse peritoneal macrophages in culture. Drutz and Cline[3] have observed labelling of some globi in human peripheral blood monocytes using thymidine. The high resolution autoradiography combined with the use of DOPA shown here enables the metabolic activity of individual bacilli to be assessed.

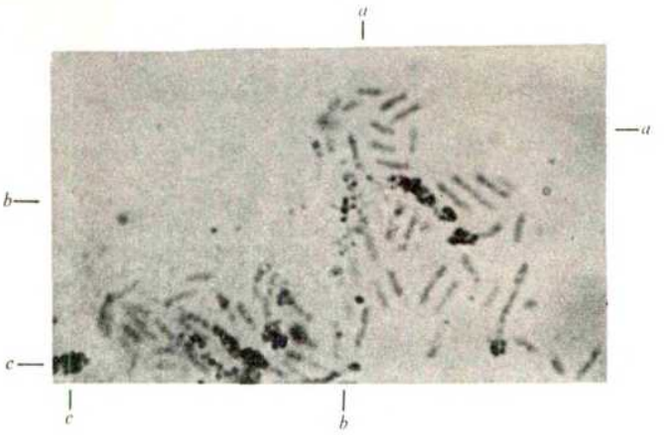


Fig. 1 Autoradiograph of *M. leprae* labelled with radioactive DOPA (5 μCi ml^{-1} for 48 h). Patient in initial stages of treatment. Light staining for acid-fast bacilli to show contrast with dense silver grains. *a*, Unlabelled bacilli; *b*, separate bacilli showing one to three grains; *c*, small groups of densely labelled bacilli.

By combining the use of labelled DOPA and labelled thymidine it therefore becomes possible to give, in addition to the morphological index, an assessment of metabolic activity (from DOPA labelling) and of rate of multiplication (from thymidine labelling) for individual bacilli of *M. leprae* in a given population. Such measurements can be made on biopsy specimens from new patients and also during treatment of patients as described above. It should also be possible to use the test for short term bacteriological investigations in the laboratory (somewhat similar to those used in the study of effects on metabolism and growth with organisms which grow well *in vitro*) for example to test the effects on metabolism and growth of potential chemotherapeutic agents, of immune serum and of lymphocytes. A more rapid method than autoradiography for assessing uptake of labelled metabolites is to use scintillation counting for bulk suspensions. Preliminary experiments measuring uptake of ^{3}H-thymidine by *M. leprae* by this method have also led to promising results.

These methods do not obviate the need for the use of mouse footpad experiments to assess the growth potential of *M. leprae in vivo*[4], particularly the use of immunologically deprived animals[5]. The most useful experimental approach in cell studies is generally to carry out parallel *in vivo* and *in vitro* investigations. The rapidity with which results can be obtained by the method described makes it attractive for a number of laboratory investigations.

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[1] Braganca, B. M., and Prabhakaran, K., thesis, Bombay University (1964).
[2] Prabhakaran, K., *Int. J. Lep.*, **35**, 42–51 (1967).
[3] Drutz, D. J., and Cline, M. J., *J. infect. Dis.*, **125**, 416–419 (1972).
[4] Shepherd, C. C., *Am. J. Hyg.*, **71**, 147–157 (1960).
[5] Rees, R. J. W., Walters, M. F. R., Weddell, A. G. M., and Palmer, E., *Nature*, **215**, 599–602 (1967).

Reduced susceptibility to scrapie in mice after steroid administration

ANIMALS deficient in lymphoreticular function either by age, genotype or immunosuppression are more susceptible to infection by most known pathogens mainly on account of their depressed immunological responses. An intact lymphoreticular system (LRS), however, can itself contribute to disease by various mechanisms including; providing sites for multiplication[1], producing infectious complexes[2] and precipitating autoaggressive accompaniments and sequalae[3]. It is possible that a special class of this paradoxical relationship is exemplified by the slow encephalopathies of which the best understood is scrapie, others being kuru, Creutzfeldt-Jakob disease and mink encaphalopathy. In these diseases of the central nervous system, infection normally occurs by some peripheral route and there always seems to be an initial, probably obligatory, replicative phase in the LRS before gaining access to the target organ[4,5]. In spite of this involvement of the LRS, and indicating some very special relationship as its basis, there is a complete absence of conventional immunological responses. No scrapie-specific antibodies[6] or interferons[7] have been found, and this is not due to any generalised depression of immune reactions[8]. Immunosuppressive techniques, which severely impair the functions of the B and T-cell elements of the LRS, do not affect the pathogenesis of peripherally injected scrapie[9,10]. Also congruent with an absence of immune responses is the non-inflammatory nature of the brain lesions even in the later stages of the disease[11,12].

A number of findings, however, can be construed as indicating some positive relation between LRS function and the pathogenesis of scrapie agents. Apart from the early high titres of agent in these tissues[13], there is an increased incubation period in splenectomised[14] and spleenless mutant mice[15]; the unexpected finding of decreased susceptibility to extraneurally injected scrapie in newborn mice[16]; the demonstration that lymphocytes can become sensitised to scrapie-damaged tissue[17]; and the decreased levels of circulating polymorphonuclear neutrophils soon after scrapie infection[18,19].

In the light of these findings it is interesting to report that the pathogenesis of scrapie in young mice can be profoundly modified by early injections of the anti-inflammatory steroid prednisone acetate (PA). Injections of PA in physiologically high doses on the days immediately before and after an intraperitoneal (i.p.) injection of scrapie-infected brain homogenate results, unexpectedly, in prolonged incubation periods and a proportion of long term survivors using an agent dose which is 100% lethal in untreated controls (Figs 1 and 2). As the doses required for the effect rise rapidly with the age of the host, it is possible that the drug operates on the same tissues as those responsible for the reduced susceptibility of neonatal mice to scrapie[16].

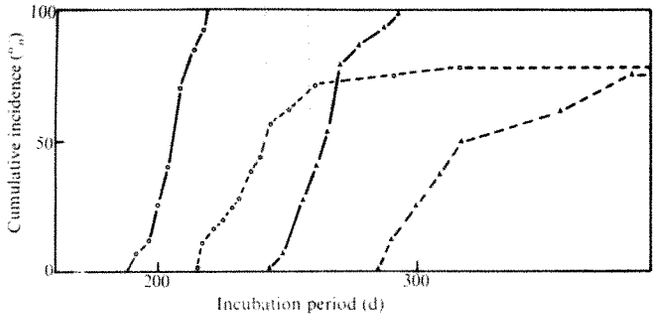


Fig. 1 Cumulative incubation periods for VL mice injected intraperitoneally (i.p.) as adults (21–42 d old) with 10% brain homogenate (500*g*, 10 min supernate) of 79A-strain scrapie agent (○) or 22C agent (▲); with (— — —) or without (———) the following high-dose prednisone acetate regime. Three days before the scrapie injection, 0.1 ml of a suspension of PA (250 mg kg^{-1}) in 2% Tween 20 in saline was injected subcutaneously (s.c.) over the shoulder, and then, beginning the day after the scrapie injection, 21 daily injections of PA suspension (15 mg kg^{-1}) were given s.c. as a maintenance dose. The controls received Tween 20 in saline only. The 0.02 ml injections of scrapie in saline contained approximately 1,000 i.p. LD_{50} infectious units.

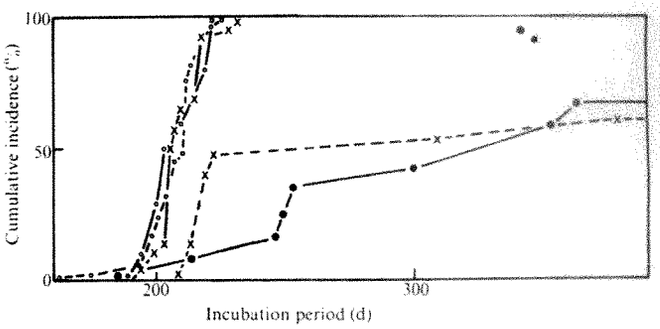


Fig. 2 Cumulative incubation periods for VL mice of different ages injected with 79A scrapie agent, together with (— — —) or without (———) a single moderate dose of PA. A similar 79A brain homogenate to that used in Fig. 1 was injected (i.p.) into mice aged 22–46 d (○), 14–20 d (×) or on the day of birth (●) (using due precautions against leakage). Half of the 22–46 and 14–20 d-old animals received a single s.c. injection of PA (50 mg kg^{-1}) in 2% Tween 20 in saline 3 d before the scrapie injection. No neonates were given PA treatment.

Figure 1 shows the effect on incubation periods of injecting adult VL mice with 79A or 22C scrapie, i.p., with or without a short, intensive course of PA injections at high dose levels during the first 3 weeks of infection. These steroid injections caused prolonged incubation periods, as judged by the onset of clinical disease seven months later, and a proportion (20%) of long term survivors, that is, individuals still alive and healthy more than 200 d after the death of all mice without steroid treatment. (In earlier pilot experiments some of these animals lived more than 700 d without contracting the disease although there were also some very late cases).

Figure 2 shows the effect of age and of a much lower dose of PA on the incubation period of 79A agent in VL mice of different ages between birth and 6 weeks. The single, previous injection of PA had no effect on the pathogenesis of the older animals (22–46 d at the time of scrapie injection), but it prolonged the incubation period of the younger age group (14–20 d) and caused 40% long term survivors. The cumulative incidence curve for this latter group is very similar to that for the neonatal mice which were injected at the same time and with the same dose of agent, but without a PA injection.

In spite of the fact that PA shares a number of immunosuppressive effects with thymectomy[9], and cyclophosphamide[10] and other drugs[20] it seems unlikely that these shared effects, particularly on B and T cells, are the basis of its influence

on scrapie incubation. It is not yet possible, however, to identify the relevant tissues or functions, which may indeed involve some that are not yet understood biologically[21]. Interim results from experiments to identify the relevant effects of PA show that: the kind of PA regime described seemed to have no effect on agent injected intracerebrally; there was no agent in the spleen of PA-treated mice surviving without signs to 340 d after scrapie injection; neonatal injections of PA did not affect scrapie susceptibility permanently (that is, by a developmental effect); and a variety of other PA regimes have been shown to be ineffective in modifying scrapie incubation periods (initial high dose without the maintenance course in adult mice); or high doses of PA either 6 weeks after i.p. injection of agent, or after the onset of clinical signs.

Before attempting to formulate detailed models based on these observations it will be necessary to be more specific about which tissues and cell types are involved. This will entail the use of other steroids and procedures, with different and more selective actions on various cell populations, together with a study of the dynamics of titre rise in those populations. We anticipate that macrophages, reticular cells, capillary sheath cells, polymorphonuclear neutrophils, mast cells, relatively steroid-resistant lymphocytes, various stem cells and sympathetic nerve terminals will yield the cell types involved. Sympathetic nerve terminals are not an obvious choice but they have several attractive features and have been discussed in connection with mink encephalopathy[22]. As a nervous, non-lymphoidal site of replication they would provide an explanation for the moderately high titres of agent found in organs such as the lungs, salivary glands and adrenals, and would permit the hypothesis that replication occurs in only one cell type instead of at least two. This hypothesis is more satisfactory in view of the fact that the onset of replication in both the periphery and the brain is controlled by the same gene[23], furthermore, the metabolism of sympathetic neurones can be modified by high doses of glucocorticosteroids[24]. This possibility would not, of course, exclude a role for lymphoidal elements in the uptake, accumulation and transportation of agent.

These findings carry the important experimental implication that attempts to encourage intraspecific passage of these 'slow viruses' by immunosuppressing the prospective hosts may be self-defeating[25]. As scrapie and other slow encephalopathies must infect by a peripheral route in the natural disease, a knowledge of the details of the early steps in their pathogensis—especially as these include some natural mode of inactivation—could be relevant for devising prophylactic treatments. Further investigation of this steroid effect could help to identify some of these early steps in infection and agent inactivation, and help to explain why the lymphoid system appears to act as a 'Trojan horse' instead of a defence mechanism in these diseases.

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1 Wheelock, E. F., and Toy, S. T., *Adv. Immun.*, **16**, 123–184 (1973).
2 Notkins, A. L., *J. exp. Med.*, **134**, 41s–51s (1971).
3 Choppin, P. W., in *Textbook of Immunopathology*, (edit. by Miescher, P. A., and Muller-Eberhard, H. J.), **1**, 337–349 (Grune and Statton, New York, 1968).
4 Eklund, C. M., Kennedy, R. C., and Hadlow, W. J., *J. infect. Dis.*, **117**, 15–22 (1967).
5 Kimberlin, R. H., *Scrapie in the mouse: a model slow disease*, (Merrow Monograph, 1974).
6 Porter, D. D., Porter, H. G., and Cox, N. A., *J. Immun.*, **111**, 1407–1410 (1973).
7 Worthington, M., *Infect. Immun.*, **6**, 643–645 (1972).
8 Gardiner, A. C., and Marucci, A. A., *J. comp. Path.*, **79**, 233–235 (1969).
9 McFarlin, D. E., Raff, M. C., Simpson, E., and Nehlsen, S. H., *Nature*, **233**, 336 (1971).
10 Worthington, M., and Clark, R., *J. gen. Virol.*, **13**, 349–351 (1971).
11 Zlotnik, I., *Acta Neuropath.*, supplement 1, 61–70 (1962).
12 Fraser, H., thesis, Univ. of Edinburgh, (1971).
13 Dickinson, A. G., and Fraser, H., *J. comp. Path.*, **79**, 363–366 (1969).
14 Fraser, H., and Dickinson, A. G., *Nature*, **226**, 462–463 (1970).
15 Dickinson, A. G., and Fraser, H., *Heredity*, **29**, 91–93 (1972).
16 Outram, G. W., Dickinson, A. G., and Fraser, H., *Nature*, **241**, 536–537 (1973).
17 Field, E. J., and Shenton, B. K., *Nature*, **240**, 104–106 (1972).
18 Licursi, P. C., Merz, P. A., Merz, G. S., and Carp, R. I., *Infect. Immun.*, **6**, 370–376 (1972).
19 Dickinson, A. G., Taylor, D. M., and Fraser, H., *Nature*, **248**, 510 (1974).
20 Nettesheim, P., and Hammons, A. S., *Proc. Soc. exp. Biol. Med.*, **133**, 696–701 (1970).
21 Pierpaoli, W., and Sorkin, E., *Experientia*, **28**, 1385–1389 (1972).
22 Marsh, R. F., Miller, J. M., and Hanson, R. P., *Infect. Immun.*, **7**, 352–355 (1973).
23 Dickinson, A. G., *Genetics*, (in the press).
24 Poherecky, L. A., and Wurtman, R. J., *Pharm. Rev.*, **23**, 1–35 (1971).
25 Gibbs, C. J., and Gajdusek, D. C., *Science*, **182**, 67–68 (1973).

Absence of a pilus receptor for filamentous phage IKe

Plasmid-specific filamentous bacteriophages have been known to attach to the tips of certain pili present on the surface of their *Escherichia coli* or *Salmonella typhimurium* hosts; the synthesis of these appendages is determined by specific plasmids which reside in the host cell[1–4]. As well as providing receptor sites for such phages, these pili have been found to be essential for the conjugal transfer of the plasmids[5–7] and some hypotheses of

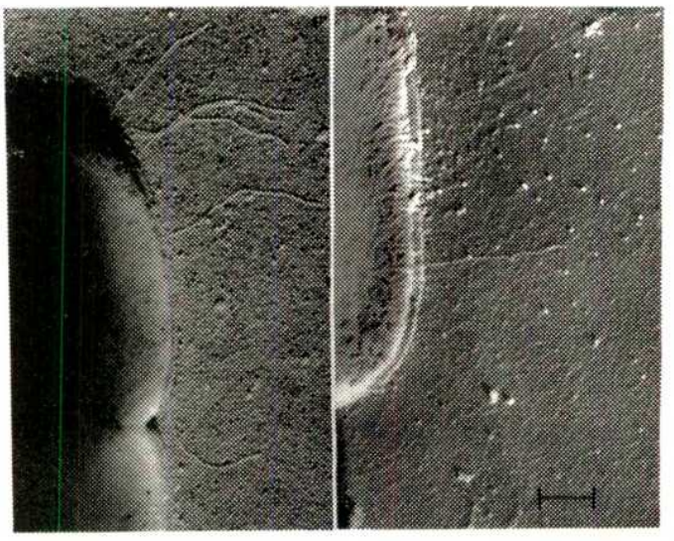


Fig. 1 The attachment of IKe particles to the surface of *E. coli* RM98$^+$ cells (× 81,600). The scale represents 100 nm.

Table 1 Adsorption of M13 and IKe to formalin killed F42$^+$ and RM98$^+$ cells

Experiment	Plasmid	Phage	MOI	Treatment of cells	Phage input (c.p.m. ml^{-1} phage/cell mixture)	C.p.m. ml^{-1} recovered in phage/cell complexes†	Unadsorbed phage (% of input)
A*	F42	M13	20	Unblended	13,500	393	
	F42	M13	20	Blended		255	
B*	F42	M13	20	Unblended	7,000	123	
	F42	M13	20	Blended		95	
C*	RM98	IKe	20	Unblended	19,700	226	
	RM98	IKe	20	Blended		801	
D*	RM98	IKe	20	Unblended	16,900	234	
	RM98	IKe	20	Blended		676	
E‡	F42	M13	0.0007	Unblended			77
	F42	M13	0.0007	Blended			99
F‡	RM98	IKe	0.0007	Unblended			83
	RM98	IKe	0.0007	Blended			68

* Phages were labelled with ^{3}H-thymidine. Phage/cell mixtures were layered on L broth containing 10% sucrose and centrifuged at 2,800 r.p.m. at 4° C for 25 min (ref. 12). The pellets were resuspended in L broth and 1 ml aliquots counted after mixing with 10 ml aquasol (New England Nuclear) in a Beckman LS-250 liquid scintillation system.

† The figures in this column have been corrected for background counts (30–40 c.p.m. ml^{-1}) obtained from counting 1 ml of the uninfected plasmid-bearing culture in 10 ml aquasol.

‡ Formalin-killed cells were washed free of formalin on sterile Millipore filters before resuspending in L broth and infecting with phage. Phage/cell mixtures were centrifuged following incubation for 10 min at 37° C and the supernatant assayed for unadsorbed phage on Hfr or RM98$^+$ indicator strains.

conjugal transfer consider that they are directly involved in the transfer process[6,8]. Previously, we have described a filamentous phage IKe, specific for the R factor RM98 and other R factors of the N compatibility group[9]. Here we describe evidence which indicates the direct attachment of IKe to the surface of its host which lacks an observable pilus-like appendage.

An *E. coli* F$^-$ auxotroph, strain JE2571, unable to synthesise common pili or flagella was used as a host for RM98. This host was capable of synthesising F- and I-type pili following the introduction into it of the plasmids F42 and a derepressed mutant of Col I transferred from *S. typhimurium* LT2 ColI drd. Strains bearing F42 or RM98 were grown to logarithmic phase in L broth[10]; the ColI$^+$ strain was cultivated in trypticase soy broth. These conditions yielded cells which always showed maximum adsorption of the respective phages. Drops of these cultures were placed on carbon-coated, formvar-supported 400 mesh copper grids. Dried grids were washed by touching the formvar surface of the grid to four drops of sterile distilled water. Shadowing was done at an angle of 11° with platinum–palladium or platinum–carbon in an Edwards High Vacuum Evaporator. The grids were examined in a Philips 300 electron microscope used at an accelerating voltage of 60 kV, at a main screen magnification of 14,000–35,000. The F42$^+$ and ColI$^+$ cultures showed their respective pili which were either surface attached or free. The RM98$^+$ strain showed neither surface attached nor free pili when we examined 10 grids prepared on three different occasions. We examined 1,300 isolated cells whose surfaces were clearly visible. At the magnification used, linear structures having a diameter of 5 nm or more should have been detected. Neither were any protrusions suggestive of very short pili observed.

In a second and independent approach, we assumed that if pilus-type appendages attached to the surface were present, they would be mechanically removable by agitating the cells. This treatment is known to detach F pili from the surface of F$^+$ cells[7,11].

L broth cultures of F42$^+$ or RM98$^+$ cells grown at 37° C to the logarithmic phase were chilled and treated with formalin (3.7% v/v) for 5 min. No viable cells could be recovered following this treatment which has been used to prevent the outgrowth of F pili following blending[11]. An aliquot of the formalin treated culture (40 ml) was blended at 0° C for 2 min at 10,000 r.p.m. in a 250 ml polypropylene bottle of a Sorvall Omnimixer to yield the 'blended' preparation (Table 1). Each cell preparation was infected with purified phage M13 or IKe; the phages were either labelled with ^{3}H-thymidine or unlabelled. Phage/cell mixtures were incubated at 37° C rather than at 0° C because IKe does not adsorb at the lower temperature.

The results in Table 1 indicate a reduction in the ability of blended F42$^+$ cells to adsorb M13. At high multiplicities of infection this reduction was 23–35% (Table 1, Experiments A and B) and 96% at low multiplicities of infection (Table 1, Experiment E) relative to unblended controls. Blended RM98$^+$ cells showed a two to three-fold increase in IKe adsorption; this was observed at high as well as low multiplicities of infection (Table 1, Experiments C, D and F). High speed blending of hosts bearing R factors of the N compatibility group has been reported to be ineffective in removing IKe receptors[13].

Table 2 Complex formation between IKe and RM98$^+$ cells at 37° C in L broth

Incubation (min)	% cells with attached IKe*	Numbers of IKe particles attached per cell 1	2	3	4 or more
0	0.4	0.2	0.2		
1	5.24	4.5	0.5	0.24	
2	16.9	15.3	1.6		
4	42.2	28.4	10.2	1.6	2.0
7	42.6	26.2	10.4	4.9	1.1
10	43.4	24.1	10.4	6.0	2.9

Phage/cell complexes were permitted to form at 37° C for 0–10 min in L broth and chilled immediately to prevent further adsorption. The complexes were separated from unadsorbed phage as described in the legend to Table 1. Pellets from the sucrose centrifugation were washed with chilled water and used for making grids which were shadowed.

* Three grids were prepared at each incubation time and at least 150 isolated cells examined per grid.

This evidence indicates the absence of a pilus-type receptor for IKe. It was therefore reasonable to expect that the phage would be seen to attach directly to the cell surface on which the receptor was located. This was in fact observed (Fig. 1) when complexes formed after mixing phage and cells were examined either immediately or following incubation of the mixtures at 37° C. The only difference noted was in the numbers of cells which showed attached phage particles (Table 2) but no difference could be seen in the manner of this attachment.

We conclude that a pilus-type receptor is not involved in the attachment of IKe to RM98$^+$ cells when the latter are grown in conditions which give a maximum specific susceptibility to

infection by IKe. We are now attempting to identify the location and nature of this receptor.

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1 Caro, L. G., and Schnos, M., *Proc. natn. Acad. Sci., U.S.A.*, **56**, 126-132 (1966).
2 Meynell, G. G., and Lawn, A. M., *Nature*, **217**, 1184-1186 (1968).
3 Crawford, E. M., and Gesteland, R. F., *Virology*, **22**, 165-167 (1964).
4 Meynell, G. G., and Lawn, A. M., *Gen. Res.*, **9**, 359-367 (1967).
5 Brinton, C. C., jun., Gemski, P., jun., and Carnahan, J., *Proc. natn. Acad. Sci., U.S.A.*, **52**, 776-783 (1964).
6 Brinton, C. C., jun., *Trans. N. Y. Acad. Sci.*, **27**, 1003-1054 (1955).
7 Novotny, C., Raizen, E., Knight, W. S., and Brinton, C. C., jun., *J. Bact.*, **98**, 1307-1319 (1969).
8 Brinton., C. C., jun., *C. R. C. Crit. Rev. Microbiol.*, **1**, 105-160 (1971).
9 Khatoon, H., Iyer, R. V., and Iyer, V. N., *Virology*, **48**, 145-155 (1972).
10 Lennox, E. S., *Virology*, **1**, 190-206 (1955).
11 Novotny, C., Carnahan, J., and Brinton, C. C., jun., *J. Bact.*, **98**, 1294-1306 (1969).
12 Jacobson, A., *J. Virol.*, **10**, 835-843 (1972).
13 Dennison, S., and Baumberg, S., *Proc. Soc. gen. Microbiol.*, September 14-15 (1973).

Photophosphorylation of an ADP analogue by spinach chloroplasts

THE mechanism of ATP synthesis during photosynthetic phosphorylation is not yet known. It has been shown, however, that a coupling factor protein is necessary for coupling ATP synthesis to electron transport[1]. An interesting mechanism for photophosphorylation has been proposed[2,3] whereby photosynthetic formation of ATP is brought about by transphosphorylation of two coupling factor-bound ADP molecules to form AMP and ATP. The AMP would then be rephosphorylated in a step dependent on electron transport to form coupling factor-bound ADP which would act as the high energy intermediate (which has been thought to exist) to phosphorylate another ADP molecule to ATP.

We have used α,β-methhylene adenosine 5′-diphosphate (AOPCP), an analogue of ADP, to determine whether ADP undergoes transphosphorylation to AMP and ATP during photosynthetic ATP formation. Since the two phosphates in AOPCP are linked by a non-hydrolysable-methylene group[4], this ADP analogue cannot transfer its β phosphate in a myokinase type of reaction. Any photophosphorylation of AOPCP to α,β-methylene adenosine 5′-triphosphate (AOPCPOP) would therefore mean that photosynthetic formation of ATP is due to direct phosphorylation of ADP. Our results indicate direct phosphorylation of ADP to ATP.

Experiments in which AOPCP was used as substrate for the photophosphorylation reaction are shown in Table 1. Incubation mixtures containing AOPCP incorporated P_i into 'organic phosphate'. This reaction was light-dependent since no incorporation was obtained in the dark (experiment 1). The incorporation was not due to the presence of endogenous nucleotides since very little activity was found when AOPCP was omitted (experiment 2). Photosynthetic phosphorylation of AOPCP to AOPCPOP was catalysed at rates of 30 to 50 μmol AOPCPOP formed per h per mg chlorophyll. This reaction was six to ten times slower than phosphorylation of ADP. One of the reasons for the slower phosphorylation of AOPCP might be the differences in bond angles and distances of the P–CH_2–P and P–O–P groups[8], and so AOPCP may not fit into the active site for photophosphorylation as well as does ADP. The time course of photophosphorylation of AOPCP showed that the reaction was almost linear for 10 min, at which time more than 0.8 μmol of the total of 6 μmol of AOPCP in the incubation mixture was esterified with $^{32}P_i$.

To show conclusively that AOPCP was phosphorylated to AOPCPOP during photophosphorylation, the nucleotides from denatured incubation mixtures were separated by thin layer chromatography (Fig. 1). The reaction mixture incubated in the dark contained only AOPCP (spot 1), while the light incubated sample contained both AOPCP (spot 3) and AOPCPOP (spot 4). Radioactivity was found only in the AOPCPOP spot indicating that $^{32}P_i$ reacted with AOPCP to form AOPCPOP during photophosphorylation.

Table 1 Photophosphorylation of AOPCP by a spinach chloroplast preparation.

Nucleotide added	Illumination	P_i esterfied (μmol per h per mg chlorophyll)
Experiment 1		
AOPCP	No	0
AOPCP	Yes	47
Experiment 2		
None	Yes	0.4
ADP	Yes	299
AOPCP	Yes	31

One hundred grams of deveined spinach leaves were homogenised in 300 ml of 0.4 M sucrose containing 40 mM TES-NaOH and 0.2% fatty acid poor bovine serum albumin (Calbiochem) at *p*H 8 in a Waring blender. The homogenate was filtered through eight layers of cheesecloth and centrifuged for 5 min at 4,000*g*. The chloroplast pellet was resuspended in a solution containing 45 ml of the homogenisation medium and 15 ml of glycerol. Aliquots (1 ml) of the resulting suspension were stored in sealed vials in liquid nitrogen[5] and were thawed at room temperature immediately before use. Photophosphorylation was measured as described previously[6] using 2 mM ADP (Sigma) or AOPCP (Miles Laboratories) and 0.1 ml of chloroplast suspension (117 μg of chlorophyll). Incubation time was 5 min at 40,000 lux of incandescent light, or in the dark, as indicated. Chlorophyll was measured in 80% acetone extracts using the equation of MacKinney[7].

These results indicate that the ADP which serves as substrate in the photophosphorylation reaction is phosphorylated directly to ATP and does not undergo a transphosphorylation to AMP and ATP. Roy and Moudrianakis[2,3], Forti *et al.*[10] and McPhee and Brody[11], however, have obtained evidence for a transphosphorylation reaction. Moreover, both chloroplast[2] and mitochondrial[12,13] coupling factors have been found to bind adenosine nucleotides strongly. It is possible therefore, that the isolated chloroplasts contained coupling factor-bound ADP which underwent transphosphorylation to AMP and ATP and that the resultant coupling factor-bound ATP served as the 'high energy intermediate' for the phosphorylation of AOPCP. If this

were the case, our results would point to the existence of two separate ADP pools involved in photophosphorylation. One would be the substrate ADP (represented by AOPCP in our experiments) which would undergo direct phosphorylation to ATP. The other would be coupling factor-bound ADP which would act as a coenzyme for the coupling factor and which would undergo the transphosphorylation reaction proposed by Roy and Moudrianakis[2,3]. Figure 2 shows the reactions which might occur on the coupling factor; the proposed scheme is in agreement with our results as well as those of Roy and Moudrianakis[2,3]. It assumes that coenzyme ADP which undergoes the myokinase type of reaction stays bound to the coupling factor while the substrate ADP associates with the coupling factor only transitorily to accept the phosphate from coupling factor bound ATP produced by the myokinase-like reaction.

It has been observed[12] that the mitochondrial coupling factor contains two binding sites for nucleotides, one with high and one with low affinity for ADP. The low affinity site was identical with the active site for the ATPase reaction catalysed by the coupling factor. The function of the high affinity site is not known. Since the mitochondrial and chloroplast coupling factors are very similar, it is possible that the high affinity binding site is the site of the myokinase-like reaction involving the coupling factor-bound coenzyme ADP, while the low affinity site is the site of phosphorylation of substrate ADP. Reactions at the high affinity site should therefore have the properties ascribed to the formation of the 'high energy intermediate', while reactions at the low affinity site should be identical to those involving the last step in photosynthetic ATP formation.

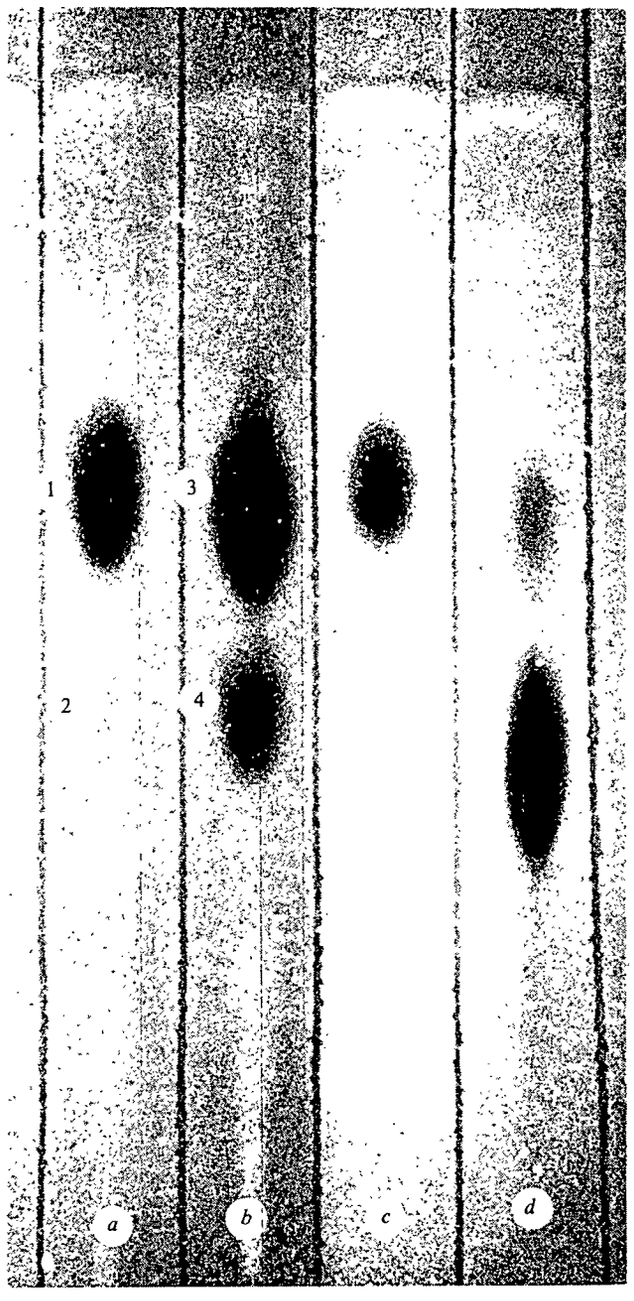


Fig. 1 Thin layer chromatography of nucleotide analogues. Chromatography was on cellulose coated Eastman Chromagram sheets using the solvent system isobutyric acid: concentrated ammonia: water = 66:1:33 (ref. 4). To remove salts which interfered with the separation, nucleotides from deproteinated incubation mixtures (3.2 ml) were adsorbed on 0.2 g of acid washed Norit charcoal. The charcoal was washed three times with 5 ml H_2O and nucleotides were eluted with 5 ml of 1% NH_3 in 60% ethanol[9]. The eluates were concentrated and aliquots used for chromatography. The resulting chromatograms were photographed under ultraviolet light with a Wratten 2A filter. Top and bottom correspond to front and start respectively. Chromatographed samples were: *a*, 10 min dark incubation; *b*, 10 min incubation at 40,000 lux; *c*, AOPCP standard; *d*, AOPCPOP standard. Analogue standards were purchased from Miles Laboratories. Numbered areas were scraped out and radioactivity of the material was counted in the liquid scintillation counter. The activities were: spot 1, 20 c.p.m.; 2, 17 c.p.m.; 3, 28 c.p.m.; 4, 1,278 c.p.m.

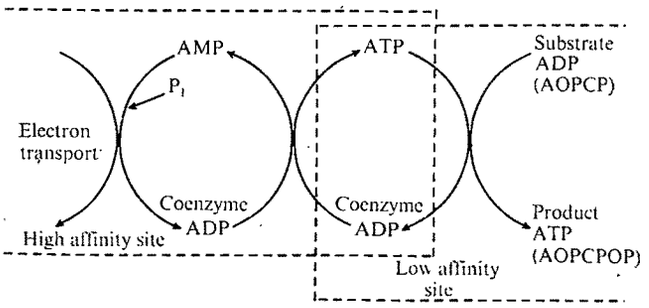


Fig. 2 Hypothetical scheme of reactions occurring on the coupling factor during photophosphorylation. The high affinity site is assumed to contain coupling factor bound nucleotides while the low affinity site is thought to be the site of phosphorylation of substrate ADP.

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1 Vambutas, V. K., and Racker, E., *J. biol. Chem.*, **240**, 2660 (1965).
2 Roy, H., and Moudrianakis, E. N., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 464 (1971).

[3] Roy, H., and Moudrianakis, E. N., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2720 (1971).
[4] Myers, T. C., Nakamura, K., and Flesher, J. W., *J. Am. chem. Soc.*, **85**, 3292, (1963).
[5] Bekina, R. M., and Krasnovskii, A. A., *Biokhimiya*, **33**, 178 (1968).
[6] Horak, A., and Hill, R. D., *Plant Physiol.*, **49**, 365 (1972).
[7] MacKinney, G., *J. biol. Chem.*, **140**, 315 (1941).
[8] Larsen, M., Willett, R., and Yount, R. G., *Science*, **166** (1969).
[9] Avron, M., *Anal. Biochem.*, **2**, 535 (1961).
[10] Forti, G., Rosa, L., and Garlaschi, F., *FEBS Lett.*, **27**, 23 (1972).
[11] McPhee, J., and Brody, S. S., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 50 (1973).
[12] Hilborn, D. A., and Hammes, G. G., *Biochemistry*, **12**, 983 (1973).
[13] Harris, D. A., Rosing, J., Van De Stadt, R. J., and Slater, E. C., *Biochim. biophys. Acta*, **314**, 149 (1973).

Sap stain in *Antiaris africana*, an economically important tropical white wood

MANY countries lose thousands of pounds annually through natural staining of valuable wood. Usually the organism which causes staining is the fungus *Botryodiplodia theobromae* Pat[1]. Field and laboratory experiments have shown that *Antiaris africana* becomes stained blue within 4 d of felling[2,3]. The fungus enters the cut surface of the wood through the vessels and within 2 d heavily colonises the surrounding vasicentric parenchyma cells. Infection of ray cells occurs through the vasicentric parenchyma cells[1]. The fungus has two types of hyphae: some are stout and brown in colour, others are thin and hyaline. The brown hyphae are predominantly found in vessels, tracheids and fibres (Fig. 1), whereas the parenchyma cells are heavily infected by the hyaline hyphae.

It is significant that not all fungi to which cut logs of *A. africana* are exposed gain entrance to the wood through vessels. *Phialophora fastigiata*, a sap stainer with a tendency to behave like a soft-rot fungus enters the wood through the ray parenchyma cells from which it ramifies into the vasicentric parenchyma cells. The vessels are infected through parenchyma cells. Within 14 d of infection, the fungus successfully digests the secondary wall of the fibres, creating cavities in oblique, transverse and longitudinal directions. *Rhinocladiella atrovirens*, a sap stainer, and *Chaetomium globosum* infect the wood through the rays and the vasicentric parenchyma cells.



Fig. 1 Brown hyphae in vessels of *Antiaris africana*. × 480.

Shoots of *A. africana* were exposed to $^{14}CO_2$ for 45 min three times a week for 2 weeks and then cut into small logs. The cut surface of each log was sprayed with spores of *B. theobromae* and the fungus was isolated from the logs after 7 d. The fungus was extracted with boiling ethanol and the ethanol-insoluble residue was wet-combusted[4]. Radioactivity was detected especially in the enthanol extract of the isolated fungus, providing direct evidence that *B. theobromae* uses food reserves of the xylem. There is also experimental evidence that there is no correlation between the severity of the blue-stain infection and the concentration of carbohydrate in the xylem[3]. It is, however, essential for carbohydrate to be present in the xylem for successful establishment of the fungus. Experiments on the food reserves of the xylem of *A. africana*[5] show that it is rich in various carbohydrates. Kozlowski and Keller[6] have reported that carbohydrates are stored in the living parenchyma cells of the xylem of trees. It is, therefore, most probable that the hyaline hyphae predominantly found in the living parenchyma cells of the wood of *A. africana* are nutritional in function.

Several methods have been suggested for the control of sap blue stain fungi[2,7,8]. Eight antitranspirants have recently been tried in the laboratory on germination of spores of *B. theobromae*, and in the field on the control of blue stain in *A. africana*, and four have been very effective. Spores were mounted in various concentrations of antitranspirants and incubated at 100% relative humidity. Percentage germination was counted over a period of 24 h. In the field experiments conducted during the rainy season when the incidence of blue-stain in *A. africana* is known to be heaviest[2], cut surfaces of logs were dipped in antitranspirants. Needle Fast, Spruce Seal and Vapor Gard (1–5%) Wilt Pruf (5–25%) prevented spore germination and significantly reduced blue stain infection of wood left in the forest for 4 weeks. Each of the four antitranspirants significantly reduced water loss from the cut surfaces of the logs. There was no infection when the water content of the wood was more than 50%.

These observations indicate that successful control of blue stain of wood should be aimed at either depleting the food reserves in the wood or using chemicals that will prevent rapid water loss from cut surfaces of logs.

I am grateful to Dr J. F. Levy of Imperial College of Science and Technology, London who supplied me with *R. atrovirens*, *C. globosum* and *P. fastigiata*.

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[1] Olofinboba, M. O., and Lawton, J. R. S., *Inst. W. Sci.*, **21**, 6 (1968).
[2] Momoh, Z. O., *Technical note 36* (Dept. of Forest Research, Ibadan, 1966).
[3] Olofinboba, M. O., *Nig. J. For.*, **2**, 54 (1972).
[4] van Slyke, D. D., and Folch, J. J., *J. Biol. Chem.*, **136**, 509 (1940).
[5] Olofinboba, M. O., *Ann. Bot.*, **33**, 339 (1969).
[6] Kozlowski, T. T., and Keller, T., *Bot. Rev.*, **32**, 294 (1966).
[7] Scheffer, T. C., and Lindgreen, R. M., *Tech. Bull. (US Dept of Agric., Washington, D.C.*, 714, 1940).
[8] Cartwright, K. S. C., and Findlay, W. P. K., *Decay of Timber and its Prevention* (HMSO, London, 1958).

matters arising

Spotted centromeres in human chromosomes

SIR,—The demonstration by Eiberg[1] of equal sized Giemsa-stained paired dots in the centromeric regions of human chromosomes, raises the question of whether these dots are equivalent to, or associated with, the paired spheres, or dots, observed in these regions in chromosomes preparations handled in different ways.

Sharply delineated pairs of unstained spheres associated with the centromere are often to be seen in human, mouse and other mammalian chromosome preparations of cells pretreated with colcemid and stained with orcein, or Giemsa, in the conventional way. These spheres stand out more clearly in cells with cytoplasmic staining and they have the appearance of unstained vacuoles (Fig. 1*a*). Dots, perhaps more closely similar to those described by Eiberg[1], are also to be seen in optically shadowed, but unstained, human chromosomes[2] that have been exposed to the alkali and saline pretreatments necessary before Giemsa staining in a technique[3] to give C-banded chromosomes, or to a trypsin schedule[4] to give G banding. These dots appear as raised prominences in the centromere regions of the collapsed chromosomes and are similar in size and position in all members of the chromosome complement (Fig. 1*b* and c). They cannot be simply equated to the densely stained Giemsa C bands, as the size of the centromerically located C bands differs between chromosomes and indeed some C bands are very large (such as those of chromosomes 1, 9 and 16) and are not confined in their location to the centromere regions as, for example, in the Y chromosome. Differences between C bands and a more localised centromeric staining have also been noted by Voiculescu *et al.*[5] in banded preparations of hamster chromosomes. The smaller centromeric C bands in the human complement (Fig. 1*d*), however, cannot be distinguished from the unstained promontories seen in these chromosomes in shadowed, but unstained, preparations.

The regions in which these stained, or unstained, dots appear are known to contain the paired disk-shaped kinetochores that are located in the centromere region on opposite sides of sister chromatids and which are clearly visible using the electron microscope[6]. Such studies have also shown that the paired fibrillar elements of the kinetochore have associated with them a less dense granular matrix which is also fibrillar in nature and which does not completely encircle the centromeric regions of the chromatids[7]. Moreover, although colcemid inhibits the full development of the spindle, the centromerically located spindle tubules are always present so that spindle proteins are concentrated in these regions and attached to the chromosome[8]. It would seem to us therefore that the stained, or unstained, dots which are seen under the light microscope to be specifically located in the centromere regions, may represent the kinetochores and particularly their associated proteins.

It is perhaps worth noting that although in man[9–11] and mouse[12] C band material is confined to the centromere regions in the autosomes, in cultured cell lines these materials are sometimes translocated away from the centromere. Thus, even in these materials, C banding does not provide a reliable method for detecting centromeres. On the other hand, a simple centromere staining method, such as Eiberg's, would, if consistent, provide a good method for locating centromeres and a useful tool in resolving the centromere states of D/D, D/G and G/G chromosome translocations in man and in screening for dicentric chromosomes in irradiated cells.

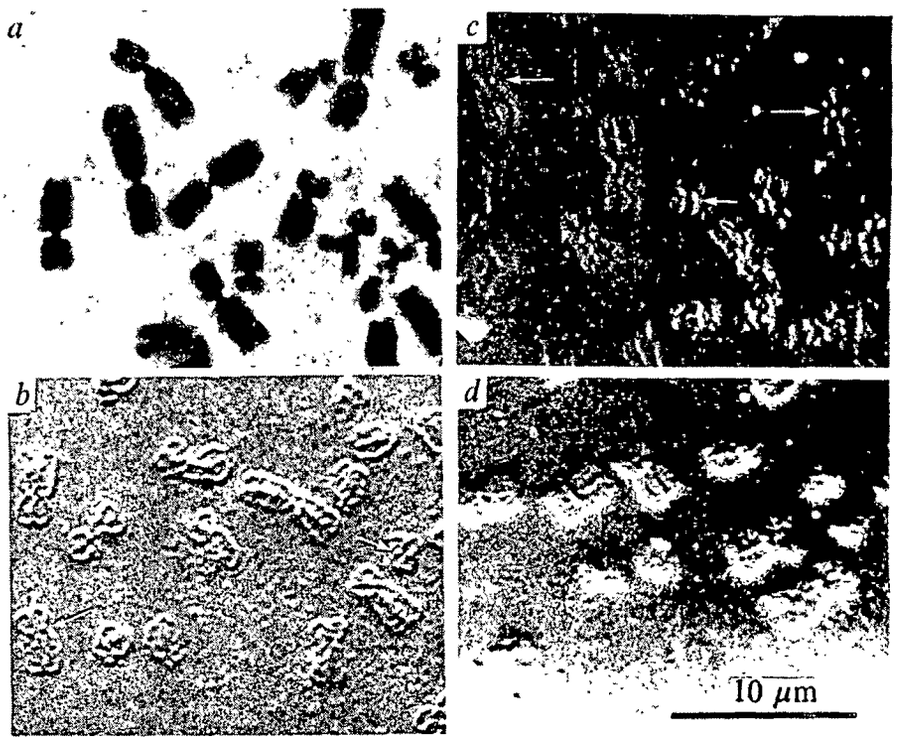


Fig. 1*a*, Human chromosomes stained with lactic-orcein and showing the pairs of unstained dots associated with the centromere. *b*, Unstained optically shadowed human chromosomes treated with trypsin as in Seabright's[4] G banding technique. Note the paired dots at the centromeres. *c*, Unstained and shadowed chromosomes treated with HCl, saline and $Ba(OH)_2$ as for C banding[3]. Note the paired dots at the centromeres. *d*, Shadowed chromosomes that have been stained for C banding. Compare the prominence at the centromere with *b* and *c*.

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1 Eiberg, H., *Nature*, **248**, 55 (1974).
2 Ross, A., and Gormley, I. P., *Expl Cell Res.*, **81**, 79 (1973).
3 Sumner, A. T., *Expl Cell Res.*, **75**, 304 (1972).
4 Seabright, M., *Lancet*, ii, 971 (1971).
5 Voiculescu, I., Vogel, W., and Wolf, U., *Chromosoma*, **39**, 215 (1972).
6 Jokelainen, P. T., *J. ultrastruct. Res.*, **19**, 19 (1967).

[7] Brinkley, B. R., and Stubblefield, E., *Chromosoma*, **19**, 28 (1966).
[8] Brinkley, B. R., Stubblefield, E., and Hsu, T. C., *J. Ultrastruct. Res.*, **19**, 1 (1967).
[9] Arrighi, F. E., and Hsu, T. C., *Cytogenetics*, **10**, 81 (1971).
[10] Chen, T. R., and Ruddle, F. H., *Chromosoma*, **34**, 51 (1971).
[11] Evans, H. J., Buckton, K. E., and Sumner, A. T., *Chromosoma*, **35**, 310 (1971).
[12] Pardue, M. L., and Gall, J. G., *Science*, **168**, 1356 (1970).

Random deviations or asynchronous DNA synthesis?

SIR,—In a study concerning the labelling of the X chromosomes of heterozygous Dp(1;f) AM female larvae, Bender, Barr and Ostrowski[1] compared the absolute amount of silver grains distributed over the 1AB region of the paired X chromosomes (compound X) with the absolute amount of labelling over the homologous region of the free duplication (fragment). In all 97 spot-labelled nuclei analysed, the 1AB region of the fragment and the compound X were asynapsed. In 65 of these nuclei a maximum of only two silver grains could be found over each of the two 1AB regions. The authors deemed this amount of labelling as unsignificant. Of the remaining 32 nuclei, 22 showed significant labelling over the compound X but an unsignificant labelling over the fragment. In contrast, only five nuclei indicated significant labelling over the fragment but unsignificant labelling over the compound X. In the remaining five nuclei, significant labelling was found both in the fragment and in compound X as well.

From these results the authors concluded that the fragment does not replicate the DNA in synchrony with the homologous region of the compound X chromosome. This conclusion is based on two assumptions. First, that labelling of less than a tight cluster of three silver grains over a given chromosome region is unsignificant and, second, that a synchronous DNA synthesis must be reflected by a constant ratio in the amount of silver grains of the two homologous chromosome sections without any random deviations.

On the basis of experiences with autoradiographic studies (ref. 2, and our unpublished results), we wish to mention that there is still another interpretation for these data. During the preparation of our manuscript, similar judgements have been published[3].

The data published in Fig. 1 of ref. 1 are repeated here, but are tabulated in a different way (Fig. 1 and Table 1). It is noteworthy that only seven (Fig. 1) of 97 nuclei show relatively strong deviations (more than three silver grains) from the axis of synchrony. In three of these cases the fragment synthesises its DNA earlier and in the remaining four later, than the compound X. Eleven nuclei show only one silver grain in compound X and none in the fragment. The remaining 49 labelled nuclei (including the seven exceptions) are distributed with 23 on each side of the axis of synchrony and three nuclei matching the axis of synchrony directly.

In light of the observation that of 97 nuclei with 194 1AB regions, 113 were unlabelled and the remaining 81 were distributed according to Table 1, it seems to be inconsequential, in our opinion to eliminate 44 cases of less than three silver grains as background labelling: in particular, because the authors claim that background labelling of the autoradiograms analysed was very low. Recent (unpublished) studies have shown that the restriction in the significance of labelling in respect to a tight cluster of three silver grains must be handled with caution, if slides with a very low background are used.

The authors' assumption, that the silver grains in the two corresponding 1AB regions have to indicate a constant 2:1 ratio according to their DNA amounts (or somewhat less than 2:1, because of the greater self absorption in the compound X), is based on the studies by Plaut and Nash[4]. During (an unpublished) reinvestigation of this problem, however, one of us (K. H.) has shown that the labelling in homologous bands of normally structured asynapsed chromosomes showed only a high degree of correspondence and not the absolute correspondence of Plaut and Nash[4]. Random deviations were to be found with common frequencies (Fig. 1). It must also be mentioned that 6.8–28.8% of 'exceptional patterns' are to be found in all phases of the replication cycle of normally structured chromosomes in *Drosophila melanogaster*[5–7]. Therefore, the seven nuclei with strong deviations (Fig. 1) may belong to those exceptional deviations. Also, on the basis of the arguments presented, we believe that the replication of the duplicated chromosomal region should better be interpreted as synchronous DNA synthesis.

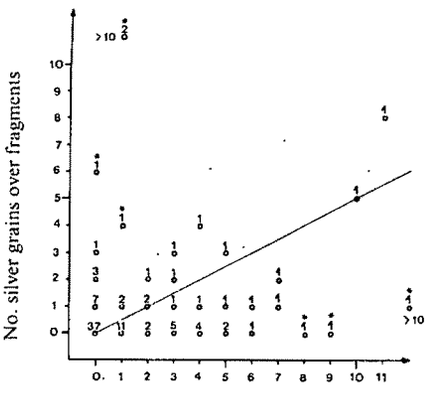


Fig. 1 Deviations of labelling intensity over the two 1AB regions in 97 salivary gland nuclei of Dp(1;f)AM female larvae, according to the data of ref. 1. The position of each circle indicates the absolute number of silver grains over the 1AB regions of the duplication (fragment) observed, as well as over the 1AB region of the two paired X chromosomes (compound X). An axis of synchrony is drawn through those circles which show a 2:1 ratio for the amount of silver grains. Figures on top of each circle indicate the number of cases observed. *Nuclei with strong deviations of labelling.

Table 1 Number and distribution of silver grains observed over each of the two 1AB regions in 97 nuclei of Dp(1;f)AM female larvae (changed after Bender, Barr and Ostrowski[1])

No. of silver grains observed over the 1AB regions	>10	11	10	9	8	7	6	5	4	3	2	1	0
Distribution of 1AB regions observed	3	1	1	1	2	2	3	5	8	11	11	33	113

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[2] Kalisch, W.-E., and Hägele, K., *Chromosoma*, **44**, 265 (1973).
[3] Rudkin, G. T., in *Results and Problems in Cell Differentiation*, **4**, (Springer Verlag, Berlin, 1973).

[4] Plaut, W., and Nash, D., in *The role of chromosomes in development*, 113, (Academic, New York, 1964).
[5] Plaut, W., Nash, D., and Fanning, T., *Molec. Biol.*, **16**, 85 (1966).
[6] Nash, D., and Bell, J., *Can. J. Genet. Cytol.*, **10**, 82 (1968).
[7] Lakhotia, S. C., and Mukherjee, A. S., *J. Cell Biol.*, **47**, 18 (1970).

Magnetic insulation

SIR,—The extravagant claims presented in a recent article by Winterberg[1], led me to look into the background of the idea of 'magnetic insulation'. The purpose of this letter is to point out that the scheme described in that article is unsound and will not work.

The basic idea in Winterberg's article is the prevention of electrical breakdown between conductors at different potentials using the interposition of a combination of a high vacuum and a high magnetic field parallel to the conductor faces. Electrons which could initiate the breakdown are supposed to be returned by the magnetic field to the electrode from which they were emitted. This, however, is essentially the configuration found in any number of magnetron devices. In forty years of experiments in this field it has been shown time and again that complete cutoff is not achieved for any magnetic field. The reason is that collective effects intervene; space charge instabilities always result in some current passing between the electrodes. Under these conditions simple orbit calculations no longer apply.

Winterberg's ideas were presented earlier in a longer article[2]. In that article he suggested that 'magnetic insulation' might make possible a transformer for 10^9 V. A year later the same objections that I have raised were published in some detail by Hirsch[3]. He concluded "The cutoff anomaly therefore renders 'magnetic insulation' impracticable . . . Clearly, the only way in which vacuum breakdown due to field emission from a given surface can be avoided is by reduction of the surface electric field." A reply by Winterberg[4] completely failed to answer Hirsch's objections.

Perhaps claimants for 'magnetic insulation' could be given the same requirement that is presented to inventors of perpetual motion machines. They are usually instructed to accompany their claims with a working model.

Yours faithfully,
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[1] Winterberg, F., *Nature*, **246**, 299 (1973).
[2] Winterberg, F., *Rev. scient. Instrum.*, **41**, 1756 (1970).
[3] Hirsch, E. H., *Rev. scient. Instrum.*, **42**, 1371 (1971).
[4] Winterberg, F., *Rev. scient. Instrum.*, **43**, 814 (1972).

DR WINTERBERG REPLIES: Contrary to Blewett's belief, magnetic insulation has not only been experimentally confirmed[2] since I proposed it several years ago[1], but is already utilised in the coaxial transmission lines of relativistic electron beam generators (for example, the MJ Aurora machine). The magnetic field needed for the insulation effect in this case is generated by the strong azimuthal self-induced field of the current pulse passing through the transmission line. In the Aurora machine for example, the pulse length is 100 ns and the feasibility of magnetic insulation is therefore confirmed, at least for this time scale. The same should therefore be true for the magnetically insulated high voltage transformer[3], at least for an a.c. frequency of the order 10 MHz as it is realised in a Tesla transformer.

Blewett argues that experience with magnetron devices speaks against the feasibility of magnetic insulation and that the device described in my article falls into the same category. This, however, is not quite correct, because the magnetron device is an open ended system. It therefore has quite similar end losses and instabilities as an open ended plasma confinement device. This contrasts with a closed toroidal system, which is proposed in my article. In a magnetron the electrons can drift freely in the axial direction, causing breakdown at the ends, by contrast with the proposed closed toroidal system in which this free motion follows a closed loop parallel to the circular torus axis.

Both the magnetically insulated coaxial transmission line and the high voltage transformer are also open ended systems but with an azimuthal field, rather than an axial field as in the magnetron. The azimuthal field leads to a much smaller axial drift motion than in the magnetron device. Here again, the analogy with the z pinch (azimuthal field) and the θ pinch (axial field) can be drawn. Therefore, for both the magnetically insulated coaxial transmission line and the high voltage transformer, larger time scales than in the magnetron can be expected. This prevents breakdown. A closed toroidal system, as it is proposed in my article should, however, lead to substantially larger breakdown times, quite analogous to the much longer plasma confinement times for closed than for open ended systems. It is also known, from toroidal plasma confinement devices, that even higher stability can be achieved by strong shearing of the magnetic field lines and average minimum B, as in the Stellarator or Tokamak. The same principle could, of course, be used in the proposed machine, a big shear field being produced with the external field coil, with an induced circular current in the central conductor or with a combination of both. In a closed, magnetically insulated system the only important collective instability which then remains is the diocotron instability which is suppressed by making $\omega_p^2 \ll 2\omega_c^2$ where ω_p is the electron plasma frequency and ω_c the electron cyclotron frequency. This is one of the conditions for magnetic insulation.

F. WINTERBERG

Desert Research Institute,
University of Nevada System,
Reno, Nevada 89507

[1] Winterberg, F., *Phys. Rev.*, **174**, 212 (1968).
[2] Miller, R., Rostocker, N., and Nebenzahl, I., *Bull. Am. phys. Soc.*, **17**, 1007 (1972).
[3] Winterberg, F., *Rev. scient. Instrum.*, **41**, 1756 (1970).

Bovine thymin

SIR,—Recently, Goldstein[1] reported on the isolation of two polypeptide hormones from bovine thymus. He suggests and extensively uses the terms thymin I and thymin II to designate these hormones.

The choice of these names seems unfortunate because of a possible confusion with thymine (2,4-dihydroxy-5-methyl-pyrimidine), a component of some nucleic acids. In French and German, there would be only one way of writing both thymine and thymin, so that they could not be distinguished from each other. The numbers I and II should also be considered as provisional ones, as the structures of the hormones are still unknown and it could be that II is the precursor of I.

It is to be expected that any new important biochemical compound will ultimately have its name defined on an international basis by a nomenclature commission of such a body as the International Union of Biochemistry (IUB) or the International Union of Pure and Applied Chemistry (IUPAC). As this is likely to take some time, it would be worthwhile if Dr Goldstein could reconsider the question of the names to be used for the new hormones, to make them unambiguous and compatible with existing recommendations.

Yours faithfully,
M. ROTH

Central Laboratory,
Hôpital Cantonal,
Geneva, Switzerland

[1] Goldstein, G., *Nature*, **247**, 11 (1974).

DR GOLDSTEIN REPLIES: The points made by Dr Roth are well taken. The name thymin was originally assigned[1] to designate a hormone of the thymus

by the traditional '-in' suffix (as for insulin and renin). At that time we avoided naming the hormone by its action as we were uncertain whether the neuromuscular effect was physiological; indeed, it now does not seem to be so.

Unfortunately, there is confusion between the polypeptide hormone thymin and the base thymine, which was also named for its original isolation from thymus. Would the name thymopoietin be more acceptable? This would indicate the action of the hormone in inducing stem cell differentiation to, or 'producing', thymocytes (as in erythropoietin and granulopoietin).

I agree that the numbers I and II are provisional and that this will be resolved by structural work, which is in progress.

Department of Pathology,
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[1] Goldstein, G., and Hofmann, W. W., *Clin. exp. Immun.*, **4**, 181-189 (1969).

(Na + K)-ATPase activity in cattle red cells

SIR,—Schatzmann[1] has described a positive correlation between ouabain-sensitive ATPase activity and red cell K levels in cattle, cells with higher K levels giving a greater enzyme activity. These determinations were made with Na=150 mM, K=10mM. If ouabain-sensitive ATPase is determined as a function of K concentration (at K levels >5 mM to saturate the outside pump site) in fragmented red cell ghosts from individual cattle with different erythrocyte K levels, it can be seen that, as in other ruminant red cells[2], the enzyme shows a variable degree of K inhibition (Fig. 1). Thus, membranes derived from cells having an original K level of 26 mmol per 1 cells are 50% inhibited at K=20 mM, whereas membranes from cells originally having 77 mmol K per l cells are not 50% inhibited even at K=100 mM. From the 26 cows so far investigated a continuous spectrum of K sensitivities has been found, with the degree of K inhibition correlating well with the resting K level of the original red cells. It therefore seems likely that the internal affinity for K of the ouabain-sensitive ATPase in cattle red cells may be the dominant determinant of resting cell K levels, and not the amount of enzyme activity measured at relatively low K concentrations.

Yours faithfully,

J. C. ELLORY

ARC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT, UK

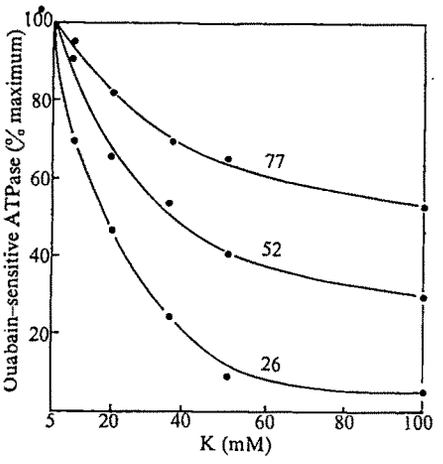


Fig. 1 Ouabain-sensitive ATPase activity in cattle red cell ghosts as a function of K concentration. Ghosts prepared and assayed as previously described[3]. Incubation conditions: NaCl 150 mM, $MgCl_2$ 1.25 mM, Na_4-ATP 1.25 mM, 10 mM Tris *p*H 7.7 at 37° C, (KCl + choline Cl) 100 mM, ouabain, when present, 0.1 mM. Ghost concentration equivalent to 25% haematocrit, original packed cells. All conditions measured in quadruplicate. Curves shown for cells from three individual cattle with red cell K 77, 56, 26 mmol per 1 cells. Data were normalised as % maximum ouabain-sensitive ATPase activity (at 5 mM K). Actual maximum ouabain-sensitive ATPase activities at 5 mM K; upper curve, (77 mmol K per 1 original cells), ATPase 375 nmol P_i per mg protein per h; middle curve, (52 mmol K per 1 original cells), ATPase 660 nmol P_i per mg protein per h; lower curve, (26 mmol K per I original cells), ATPase 260 nmol P_i per mg protein per h.

[1] Schatzmann, H. J., *Nature*, **248**, 58 (1974).
[2] Ellory, J. C., Glynn, I. M., Lew, V. L., and Tucker, E. M., *J. Physiol. Lond.*, **217**, 61P (1971).
[3] Fortes, P. A. G., Ellory, J. C., and Lew, V. L., *Biochim. biophys. Acta.*, **318**, 262 (1973).

DR SCHATZMANN REPLIES: The marked variability of the K affinity of the internal Na site found by Dr Ellory renders untenable my tacit assumption that the ATPase activity measured at 10 mM K and 100 mM Na reflects the number of pump sites per cell. But unless our conditions of Na concentration, *p*H and ionic strength considerably enhance the effect due to different affinities it does not seem to account completely for the variability of the Na+K−ATPase which we reported. From the regression in our experiments cells with $[K]_{cells} = 77$ mmol l^{-1} might be expected to have 4.5 times the ATPase activity of those with 26 mmol l^{-1}, whereas the curves shown above only yield a factor of about 1.4 at 10 mM K. Yet I fully agree with the conclusion that the internal K affinity seems more important than the number of pump sites per cell in determining the cellular K concentration.

University of Bern, Switzerland

No chromosome 9q+ in Ph¹-negative CML

SIR,—To demonstrate that in Ph¹-positive cells the additional band at the end of the long arms of chromosome 9 (9q+) stems from chromosome 22, Rowley[1] suggested the examination of metaphases derived from bone marrow from patients with Ph¹-negative chronic myelocytic leukaemia (CML). We studied the chromosomes of three such patients, two of whom had symptoms considered to be characteristic for Ph¹-negative CML[2], that is, mild leukocytosis, anaemia, severe thrombocytopenia and discrete splenomegaly.

Case 1 died of a cerebral haemorrhage 3 months after first presentation. Case 2 entered blastic phase and died 20 months after CML had been diagnosed; chromosomes were studied during blastic phase. Case 3 has typical CML and is well 3 yr after the diagnosis has been established.

The chromosome banding pattern was obtained by the trypsin-saline-Giemsa method. The karyotype of case 1 was normal, 46,XY. Case 2 had two clones; the major clone (60%) had the karyotype 47,XY, 20q-,+21 and the minor clone (40%) had 46,XY,20q-. In case 3 the marrow cells revealed a missing Y on several occasions; the karyotype was 45,XO while the PHA-stimulated lymphocytes were chromosomally normal.

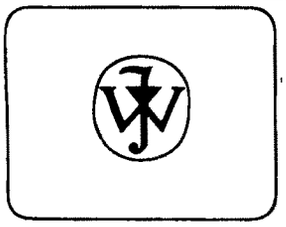
Chromosome 9q+ could not be detected in any metaphase of the Ph¹-negative cases. Thus, these findings corroborate Rowley's assumption[1] that the additional material on chromosome 9 comes from chromosome 22. Furthermore, an abnormal banding pattern which would characterise Ph¹-negative CML could not be seen. This may be related to the finding that patients with Ph¹-negative CML represent a rather heterogenous group[3].

Yours faithfully,

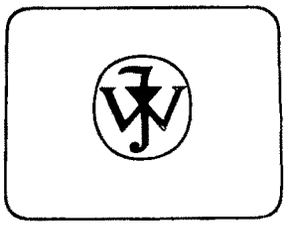
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Federal Republic of Germany

[1] Rowley, J. D., *Nature*, **243**, 290-293 (1973).
[2] Ezdinli, E. Z., Sokal, J. E., Crosswhite, L. H., and Sandberg, A. A., *Ann. int. Med.*, **72**, 175-182 (1970).



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book reviews

Builders of the Universe

The Astronomical Revolution: Copernicus–Kepler–Borelli. By Alexandre Koyré. Translated by R. E. W. Maddison. Pp. 531. (Hermann: Paris; Methuen: London; Cornell University: Ithaca, July 1973.) £6.50.

The Cosmology of Giordano Bruno. By P. H. Michel. Translated by R. E. W. Maddison. Pp. 306. (Hermann: Paris; Methuen: London; Cornell University: Ithaca, July 1973.) £4.50.

THE writings of the late Alexandre Koyré never fail to reveal the intimacy of the union between the history and the philosophy of science. His interpretation of *The Astronomical Revolution*, first published in French in 1961 and now in an English translation is, to quote the author's own expression "the history of the evolution and transformation of the key concepts" (page 9) in astronomy as opposed to a history of astronomy. This distinction arises from the fact that Koyré took no account of advances in observational methods and techniques, since he was of the opinion that these had virtually no influence upon the metaphysical revolution which he is consciously seeking to epitomise through Copernicus, Kepler, and Giovanni Alfonso Borelli. He has, as it were, defined practical astronomy out of existance as being irrelevant to his purpose of exposing both "hidden and acknowledged motives and incentives" (page 18) in the literary legacies of these three men.

The fact that he himself regarded this dichotomy between theory and observation as "rather strange" (page 9) indicates that he might have been prepared to concede the importance of observational astronomy if he had not been bound by his own ontological terms of reference. He cites Rheticus's view that it was Copernicus's success in explaining the lunar theory without equants which encouraged him to carry his explanation further, without stressing that the latter's initial dissatisfaction with Ptolemy's lunar theory was founded upon the observation that the Moon's parallax and angular diameter "differ little, or not at all, from those that are observed at conjunction and opposition" (page 40). Though no one would deny that Copernicus's own observations seem to have been few and their accuracy no better than those of Ptolemy, there are some who are not prepared to accept the inference that observational astronomy had little significance upon the former's belief in the Earth's axial rotation (see J. R. Ravetz, *Scientific American*, **215** 88-98, 1966) Since Koyré placed his entire emphasis upon the heliocentric hypothesis and neglected the question of the Earth's diurnal motion he cannot be challenged on this score, but one might well query the justification of according such an unequal status to these historically inseparable axioms of Copernican astronomy.

The author's interpretation of Kepler's achievements occupy roughly two thirds of this book. Kepler's discovery that the planetary orbits are elliptical with the Sun at one focus—his first law—is attributed to the fact that he was trying to explain discrepancies between theory and observation within the framework of a celestial physics conceived long before he had access to Tycho Brahe's data. On this ground the latter are regarded as having been of little importance to Kepler's rejection of Ptolemy's time-honoured kinematical model of circles and spheres. It is nevertheless true that Kepler derived all three of his laws empirically after years of trial and error in trying to fit Brahe's planetary observations to his physically erroneous model of the solar system. Had the observations of Mars, in particular, been intrinsically less reliable, more dispersed spatially, or more restricted temporally, Kepler—despite his unquestionable genius—would now be featuring much less boldly in the history of science.

The significance attached by Koyré to Borelli is due principally to the latter's recognition that circular motions produce centrifugal forces. Here the thought is considered to be more important than the deed, since Borelli's speculations in his *Theoricae Mediceorum Planetarum* (Florentiae, 1666) were not sufficiently clear to lead to any predictable results. He did not analyse the phenomenon of curvilinear motion into its constituent components and gave no explanation of the origin of the centrifugal tendency, merely seeking to establish the necessity of a constant central force by an appeal to experiment. Koyré sees in him a true disciple of Galileo, who adopts the same methodological approach of using theory to guide experiment "which confirms, or invalidates, the theory, and provides firm data relating to the matter under investigation" (page 469). Stillman Drake has recently cast doubts upon whether this image of Galileo which Koyré himself, in *Études galiléenes*, has done much to propagate, comprehends a true reflection of his epistemology (see *Scientific American*, May, 84-92; 1973) but whether this will affect future assessments of Borelli's work is difficult to say. The latter's deductive approach is here attributed to "the sterility of observation, pure and simple" (page 471), and the telescopic observations which he had been making for years on Jupiter's satellites are supposed merely to have provided an excuse for publishing his theory of their motions. But one cannot escape the fact that this theory was developed from the ellipticity of planetary orbits—a 'fact' derived observationally by Kepler and accepted by Borelli on observational grounds (as an extract from his writings quoted on page 474 clearly reveals). Thus here again it ought to be recognised that Koyré's philosophical distinction, though heuristically convenient, is historically inadequate.

In another of his writings, Koyré once claimed that the greatest achievement of the scientific revolution was the destruction of the cosmos which it had been the greatest achievement of the Greeks to create. The determinative factor in this dramatic ontological transformation was the collapse of the finite bounded Aristotelian Universe and the consequent rejection of a central point. This notion originated not from Copernican astronomy but from an admixture of neoplatonic philosophy and commentaries such as that of Nicolas of Cusa upon the Aristotelian vision of the heavens. *The Cosmology of Giordano Bruno* is concerned with this metaphysical revolution which precedes yet transcends the astronomical revolution of Copernicus, Kepler and Borelli. Even the Sun loses its privileged position in an infinite Universe, but the Copernican (and Pythagorean) postulate of the Earth's diurnal rotation can be seen as a logical corollary to the observed fact that all the stars seem to be carried round the heavens at the same uniform speed in a westerly direction. Thus a belief in Bruno's cosmology, with its emphasis on the infinity of the Universe might have acted as a stimulus to the acceptance of the new astronomy, and *vice versa*. Here, however, the late Paul-Henri Michel is not interested in exploring possible connections between the seventeenth

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century reaction against Bruno's heresies and the slow initial reception of Copernican astronomy, but rather with examining the internal consistency of the cosmological ideas in Bruno's writings; it being the central thesis of his book that the latter embody a systematic philosophy of nature. The work is consequently likely to have a greater appeal to philosophers of science than to historians of science specialising in Copernican studies.

Though drawing upon statements from all Bruno's extant cosmological and moral writings—these date from 1582 to 1592—the author is concerned principally with the contents of the three vernacular dialogues *La cena de le ceneri, De la causa, principis et uno, De l'infinito immenso et mondi* of 1584, and the trilogy of Latin poems *De minimo, De monade, and De immenso* composed seven years later. These, in his opinion, "have in common a scholarly, careful and almost meticulous method of utterance" (page 50). Yet his attempts to justify his belief in the compatibility of Bruno's statements require all his powers of exigesis and are at times less than convincing. For example, he dismisses an inconsistency between Bruno's views in *De la causa* and the moral treatise *Eroica furori* regarding the possibility of attaining supreme metaphysical knowledge, as resulting from "two complementary aspects of a single consistent thought, rather than two different modes" (page 58). He confesses that Bruno's ideas concerning the indivisibility of the soul are "scattered, and sometimes contradictory" (page 116); admits that Bruno's answer to the question of continuity in the material Universe "was not vigorously asserted" (page 132); and tells us that "we must resign ourselves" to the fact that Bruno's conception of an aether "retains its ambiguous and mysterious character" (page 140). Moreover, he remarks upon Bruno's "unfitness for certain kinds of mathematic reasoning" (page 145); and so on. It would seem much less difficult to defend the opposing thesis that Bruno was not quite up to the task of producing a consistent poetic, polemic, and aphoristic description of the Universe; and that his philosophical utterances on its infinity and homogeneity, the plurality of worlds, and animism in nature, were little more than a regurgitation of ill-digested ideas from Albertus Magnus's and St Thomas Aquinas's discussions of the doctrines of David of Dinant, and from works by Nicolas of Cusa and Marsilio Ficino.

Dr Maddison's translations of both Koyré's and Michel's books are excellent, and misprints are few. In the former, the year of Tiedemann Giese's letter to the magistrates of Nuremberg complaining of Osiander's breach of faith to Copernicus is wrongly printed on page 37 as 1523 (instead of 1543). In the latter (page 36), the twentieth century French scholar Etienne Gilson is said to have been writing in 1628, and a rather odd mistake on the book jacket is the statement that Bruno was burnt as a heretic in Rome in 1589 (rather than 1600). But these are but minor errors. English-speaking historians and philosophers of science will undoubtedly be pleased to have these well documented studies of sixteenth and seventeenth century thought accessible in their native language, at relatively modest prices.

ERIC G. FORBES

Rockefeller Research

The Arthropod-Borne Viruses of Vertebrates: An Account of the Rockefeller Foundation Virus Program, 1951–70. By M. Theiler and W. G. Downs. Pp. xxviii+578. (Yale University: New Haven and London, June 1973.) $25; £10.50.

THE agent causing yellow fever was shown to be a virus only in 1927. By 1950 about 20 other arthropod viruses, or arboviruses, had been discovered, mostly as a result of work primarily on yellow fever, but now more than 300 such viruses are known. This great increase in the numbers of known arboviruses is largely due to the Division of Medicine of the Rockefeller Foundation. Workers from here first studied yellow fever in 1914 and played a major part in the isolation of the virus, in elucidating the epidemiology of the disease, and in devising control measures, recognised by the award of a Nobel Prize in 1951 to the late Dr Theiler for work on the development of an effective vaccine.

Because of a lack of knowledge about the significance of arboviruses other than yellow fever in causing morbidity and mortality in man or his domestic animals, the foundation decided in 1950 to start a major research programme to investigate them, particularly in tropical countries where they are prevalent. In addition to the existing yellow fever laboratories supported by it in Entebbe, Lagos and Rio de Janeiro, the foundation, over several years, established new field laboratories in Poona, Cairo, Johannesburg, Ibadan, Port of Spain, Belem and Cali. Although staff members were assigned to these laboratories, the subprofessional staff were recruited almost entirely from local people, and an integral part of the programme was the training of workers from the coun-

tries themselves so that they could eventually take over the laboratories. As well as financial support, travel grants and fellowships were given to laboratories working on arboviruses in many other countries.

The Rockefeller Foundation Virus Laboratory, New York, which moved to Yale in 1964 as the Yale Arbovirus Research Unit, acted as a coordinating laboratory, as a centre for training and for the evolution of new techniques, and as a reference laboratory for identification and classification of the spate of new arboviruses which were isolated. This intensive research continued for a period of some 20 years but, with a change of emphasis in the activities of the foundation, has recently been curtailed, some would think prematurely.

The purpose of this book is chiefly to give an account of the activities of the central and, to a lesser extent, of the field laboratories. Part 1, consisting of four chapters, deals with the development, application, and interpretation of the classical techniques of neutralisation, haemagglutination-inhibition, and complement fixation tests as applied to the diagnosis of arbovirus infection in man and animals and the identification of isolates. Previously unpublished material is used to illustrate some points but scant reference is made here, or elsewhere, to the development of new techniques such as the use of mammalian or insect cell cultures for arboviruses.

In the second and largest part, comprising ten chapters, the principles of the method of classification of arboviruses into antigenic groups, evolved at the Rockefeller Foundation Laboratories, is explained. This is followed by a systematic description of most of the arboviruses known at the time of writing, from Group A to the ungrouped viruses. Details are given of the isolation, of the classification and of the epidemiology of the viruses in man and animals, as far as this is known, and of their importance as pathogens. Many are still in search of a human disease with which they can be identified. In the third part, of seven chapters, there are general discussions about the world distribution of arboviruses, and of their vectors and vertebrate hosts, which give useful summaries. These are followed by brief discussions of their significance for man and clinical manifestations. Finally, the specific problems of dengue and yellow fever in the Caribbean are described.

As a monument to the notable work of the Rockefeller Foundation in the field of arboviruses this book fulfils its primary objective. As a reference book for the serious worker it is less satisfactory. Much is omitted about fundamental work, some of the epidemiology is dealt with perfunctorily, and there is little reference to recent manifestations such as the dengue shock syndrome. The most useful sections of the book are the second part and some of the third which, with the extensive bibliography, are ready sources of knowledge.

C. C. DRAPER

Pituitary

The Pituitary Gland: A Comparative Account. By R. L. Holmes and J. N. Ball. Pp. x+397. (Biological Structure and Function.) (Cambridge University London, February 1974.) £10; $28.50.

THE first chapter of this book deals briefly, but adequately, with a general appraisal of the embryology, morphology and physiology of the pituitary gland. The next few chapters deal with morphology and cytology of the various parts of the gland in mammals beginning with the pars distalis. Perhaps the most confusing aspect of pars distalis cytology lies in the nomenclature used to describe different cell types. Although a functional name is to be preferred, for example thyrotroph for TSH cells, in many cases this is not possible, and the use of other systems of nomenclature, though no fault of the authors, makes this a difficult chapter to read.

A major fault in this chapter, which also deals with staining techniques, and the ultrastructure of the pars distalis, lies in the omission of any reference to the use of the peroxidase-labelled antibody method for identifying cell types. This technique has been widely used in both light and electron microscope studies in the last few years.

The beauty of the opening colour plate whets the appetite for the subsequent micrographs; the identification of cell types in electron micrographs is certainly adequately described, but nearly all of the electron micrographs are of poor quality.

Chapter 4 deals with the pars tuberalis and pars intermedia, serving to point out the lack of knowledge of these two subdivisions of the pituitary. A general outline of the neurohypophysis, which includes the median eminence, follows in chapter 5, with chapter 6 covering the basis for a neural control of the pars distalis. There is a certain amount of repetition of chapter 1 here, but the objective approach of the authors makes this chapter very useful for anyone looking for a brief appraisal of the historical and comparative evidence, for and against, a neurovascular control of pars distalis function.

The next eight chapters are concerned with a comparative account of pituitary structure and function, with a greater emphasis on structure, and it is in these chapters that the book becomes an invaluable information source for both research workers and teachers. The importance of the portal vessels is not diminished, but to be able quickly to refer to those members of the animal kingdom which have an anterior pituitary gland, but no portal vessels, demonstrates that our dogmas sometimes need deflating.

In each of these eight chapters, information is divided under subheadings; for example in the chapter on teleosts a general paragraph is followed by sections covering the overall structure in several teleost species. This is followed by a description of the histophysiology of the pars distalis, with very useful sections devoted to different cell types from both a morphological and a physiological viewpoint. Hypothalamic control of the teleost pars distalis is then considered from the physiological viewpoint, with several subsequent pages devoted to the pars intermedia. A consideration of neural lobe and hypothalamus follows, and anatomical aspects of hypothalamic control of the anterior pituitary end the chapter. I feel that if these subdivisions had been tabulated, either in the list of contents, or at the beginning of each chapter, the use of this book as a reference source would have increased further.

But any book which goes into such detail in describing the pituitary gland in vertebrate groups from fish to man will be a useful addition to libraries.

H. M. CHARLTON

Not only frog and rabbit

Comparative Vertebrate Morphology. By Douglas and Molly Webster. Pp. xiv+517. (Academic: New York and London, January 1974.) $32. £15.35.

THIS is an interesting new book on vertebrate morphology, set in clear, readable type and well illustrated. The adjective "comparative" in the title is fully justified, each organ system being illustrated for an example of each extant class or order, as appropriate, and the text similarly trying to cover each extant group. The book certainly does not follow the usual 'dogfish–frog–rabbit' format. Moreover, the text is heavily slanted towards a functional morphological approach, which will undoubtedly make more interesting reading for students than several of the rival texts. The book concentrates on extant vertebrates, though the phylogenetic relationships expressed by current palaeontological opinion are fully appreciated, and used frequently in diagrams. There are a

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few minor errors, for example the ball-and-socket joint is a characteristic of many placoderms, not just *Dinichthys* (page 22), and the description of the mole's digging musculature (page 146) is decidedly misleading. But errors seem to be reassuringly few in number, and the book can at least be commended as an informative text.

The preface says that the book has been written for students attending a one semester course on vertebrates, and before one can recommend it as such, it must be considered in relation to its rivals, especially Romer's *Vertebrate Body* which I recommend to my students. In this comparison, the Webster's book scores heavily on its functional approach—Romer is by contrast rather dry reading. Romer's book is, not surprisingly, better grounded in palaeontology, and gives a better account of the skeletal systems. The Websters on the other hand, give a better account of the nervous system. In value, however Romer's book is so superior that the comparison is hardly worth making—it contains about 35% more figures, 16% more pages, a much better biblography, yet costs only 30% of the Websters' book. It is difficult to know quite why this new text should be so expensive, but the 45 mm margins to the text are wasteful and the insert of six unhelpful colour plates seems an extravagance. Romer's use of colour on ordinary text figures is far more satisfactory.

In summary, then, this text is sufficiently different from its rivals to warrant a place in university libraries. On simple economic arguments, it cannot possibly be commended to students.

D. W. YALDEN

Snake bite

Toxicology and Pharmacology of Venoms from Poisonous Snakes. by John H. Brown. Pp. xiv+184. (Thomas: Springfield, September 1973.) $13.75 cloth; $9.95 paper.

I FIND little merit in this book. For one thing the production is poor. The reproduction of most of the 84 colour plates is bad, of some very bad and of two upside down. It is claimed to be encyclopaedic but the widespread and dangerous viper, *Echis carinatus*, receives scant mention and none in the chapter on symptomatology and treatment. Snakebite in England is mentioned but not in the subcontinent of India!

I cannot make out to whom the book is addressed. The chapter on pharmacology is in technical language and contains information likely to interest only research workers. The factors which make the distinctions between those known to be dangerous, those only potentially dangerous and harmless snakes are only vaguely indicated. There is a full list of poisonous snakes around the world but not a hint on how to go about identifying a specimen. Those who do not already know snakes will learn little.

The best parts deal with yields and preparation of venom and assessment of toxicity. These seem, however, unlikely to justify the purchase price to many readers.

GARTH UNDERWOOD

Above the atmosphere

Radio Wave Propagation. By Armel Picquenard. Pp. vi+343. (Macmillan: London and Basingstoke, April 1974.) £10.

THIS year is the fiftieth anniversary of the experimental proof of the existence of the ionosphere, so that there is a special interest in the subject of Professor Picquenard's new book. It is refreshing to be able to welcome such a book from a Brazilian author, when there have been so many books on related subjects from more northerly parts of the American continent.

The whole range of subjects in radio propagation is reviewed. The early chapters are concerned with the troposphere, including both stratified media and scatter propagation, and with the Earth's surface, including both smooth and irregular terrain and diffraction by obstacles. The chapter on the ionosphere deals with its formation and temporal variation and discusses propagation mainly for an isotropic plasma.

The object is to show how to assess a given radio communication link. More than one third of the book is devoted to the last chapter entitled "Practical Calculation of Radio Links", but much of this space is occupied by charts and diagrams, for example a series of 24 maps of the world showing how the intensity level of atmospherics varies with frequency and with time of day, through the year.

This book is for the practical communications engineer who wants to know the answers and is prepared to take the theory on trust. It is not for the more serious scientist who wants to understand the theory and apply it to new situations. There are errors in some of the formulae although many of them are only minor. On the whole it is a useful book.

K. G. BUDDEN

Announcements

Awards

H. H. Lamb has been given the **Murchison Award** of the Royal Geographical Society for his work in the field of climatic change.

Graham Highman has been awarded the **De Morgan Medal** and **Paul M. Cohen** the **Senior Berwick Prize** of the **London Mathematical Society.**

Errata

In the article "Effects of bicuculline on functions of inhibition in visual cortex" by D. Rose and C. Blakemore (*Nature*, **249**, 375; 1974) the following corrections should be made: the last sentence of para 1 on page 376 should read 'The erratic mechanical stability of the preparation for topical application used in some experiments, has meant that few cells have been fully analysed so far.'; line 12, para. 6 should read '. . . best orientation. This suggests the presence . . .'; and in Table 1, in the column "cortical layer" the first entry should be VI, the third V and the fourth IV.

In the article "Do D-glucosamine and D-galactosamine form a part of the specific receptor sites for the competence substance on the cell wall of *Pneumococcus*?" by M. Kohoutova and J. Kocourek (*Nature*, **247**, 277; 1974) the first sentence of the paragraph under Fig. 4 should read 'The presence of a 2-N-acetamido-4-amino-2,4,6-trideoxyhexose was also reported in the C substance[13]', and not as printed.

In the article "Behavioural and physiological bases of drinking inhibition in water deprived rats" by E. B. Blass and W. G. Hall (*Nature*, **249**, 485; 1974) the first word of the fourth column of Table 1 should be drink and not pre-load.

International meetings

August 25–30, **9th Meeting of the Federation of European Biochemical Societies** (the Secretariat of the 9th FEBS Meeting, H–1502 Budapest, P.O. Box 7, Hungary).

August 27–September 3, **International Symposium on Discharge of Sewage from Sea Outfalls** (Water Pollution Research Laboratory, Elder Way, Stevenage, Herts SG1 1TH, UK).

September 1–6, **International Symposium on Bread** (James Hadley, Executive Director, The Rank Prize Funds, 152 Grosvenor Road, London SW1V 3JL).

September 1–6, **15th Congress of the International Society of Haematology** (Fifteenth Congress, International Society of Haematology, P.O. Box 12060, Jerusalem, Israel).

September 1–7, **1st Intersectional Congress of the International Association of Microbiological Societies** (Dr Daizo Ushiba, Secretary General, First Intersectional Congress of IAMS, c/o Science Council of Japan, 7–22–34, Roppongi, Minato-ku, Tokyo 106, Japan).

September 1–14, **UNESCO-UNDP-ICRO Training Course on The Cell Nucleus** (Professor F. B. Straub, Biological Research Center, Hungarian Academy of Science, H-6701 Szeged, P.O.B. 521, Hungary).

September 2–6, **3rd International Congress on Photosynthesis** (Photosynthesis Secretariat, Department of Biochemistry, Weizmann Institute of Science, Rehovot, Israel).

September 2–6, **Residential School Binding Processes Involving Biopolymers** (Miss J. M. Usher, The Chemical Society, Burlington House, Piccadilly, London W1V 0BN).

September 2–6, **6th Conference on Molecular Spectroscopy** (Mr C. H. Maynard, The Institute of Petroleum, 61 New Cavendish Street, London W1M 8AR).

September 2–7, **British Association for the Advancement of Science Stirling Annual Meeting** (British Association, 23 Saville Row, London W1X 2AA).

September 2–7, **4th International Congress on Hormonal Steroids** (Congress Secretariat, Dr J. L. Mateos, Apartado postal 73-132, México, D.F.).

September 2–7, **12th International Congress for Fat Research** (Società Italiana per lo Studio delle Sostanze Grasse, Twelfth ISF Congress, c/o Stazione Sperimentale Oli e Grassi, Via G. Colombo 79, 20133 Milano, Italy).

September 3–6, **5th Canadian Medical and Biological Engineering Conference** (Dr Chas. A. Laszlo, Technical Program Chairman, Bio-Medical Engineering Unit, McIntyre Medical Sciences Center, McGill University, 3655 Drummond St, Montreal, Quebec, H3G 1Y6, Canada).

September 4–6, **Conference on Elementary Particle Physics at Ultra High Energies** (Dr V. A. Bull, Department of Physics, Westfield College (University of London), Kidderpore Avenue, London NW3 7ST).

September 6–9, **Course on the Biological Basis of Ageing** (Director of Special Courses, Department of Adult Education and Extramural Studies, University of Leeds, Leeds LS2 9JT, UK).

September 7–10, **28th Annual Meeting of The Society of General Physiologists** (The Society of General Physiologists, Box 257, Woods Hole, Massachusetts 02543).

September 8–10, **International Symposium on Foams** (Dr D. Seaman, Imperial Chemical Industries Ltd, Jealott's Hill Research Station, Bracknell, Berkshire RG12 6EY, UK).

September 8–13, **National Meeting of The American Chemical Society** (American Chemical Society News Service, 1155 16th St., N.W. Washington D.C. 20036).

September 8–14, **1st International Congress of Ecology** (Secretariat 1st International Congress of Ecology, P.O. Box 9000, The Hague, The Netherlands).

September 8–14, **International Solvent Extraction Conference,** 1974 (Dr A. Naylor, British Nuclear Fuels Limited, Windscale and Calder Works, Seascale, Cumberland, CA20 1PG, UK).

September 9–11, **Neurophysin Proteins: Carriers of Peptide Hormones** (New York Academy of Sciences, 2 East 63rd Street, New York 10021).

September 9–11, **4th Meeting of the European Connective Tissue Clubs** (Dr A. J. Bailey, Agricultural Research Council, Meat Research Institute, Langford, Bristol BS18 7DY, UK).

September 9–11, **International Symposium on Radiation Protection in Mining and Milling of Uranium and Thorium** (Symposium Joint Secretariat, c/o Occupational Safety and Health Branch, International Labour Office, CH-1211 Geneva 22, Switzerland).

September 9–11, **17th International Conference on the Biochemistry of Lipids** (Dr Giovanni Galli, Institute of Pharmacology and Pharmacognosy, University of Milan, Via A. Del Sarto 21, 20129 Milano, Italy).

September 9–12, **3rd International Conference Gas Discharges** (Conference Department, Institution of Electrical Engineers, Savoy Place, London WC2R 0BL).

September 9–13, **7th International Conference on Water Pollution Research** (SOCFI, 7 rue Michel Ange, 75018 Paris).

September 9–13, **4th International Symposium on Medicinal Chemistry** (Secretariat 4th International Symposium on Medicinal Chemistry, c/o Merck Sharp and Dohme B.V., Waarderberg 39, P.O. Box 581, Haarlem, The Netherlands).

September 9–14, **18th Ampere Congress on Magnetic Resonance and Related Phenomena** (Professor E. R. Andrew, Department of Physics, The University, University Park, Nottingham NG7 2RD, UK).

September 10–14, **Biostereometrics '74 Symposium** (Dr R. E. Herron, Biostereometrics Laboratory, Texas Institute for Rehabilitation and Research, Baylor College of Medicine, 1333 Moursund Ave., Houston, Texas 77025).

September 10–14, **3rd International Conference on Ferroelectricity** (Dr H. Montgomery, Physics Department, The University, King's Buildings, Mayfield Road, Edinburgh EH9 3JZ, UK).

September 11-14, **2nd International Congress of Collegium Internationale Activitas Nervosae Superioris** (2nd International Congress of CIANS, Czechoslovak Medical Society, J. E. Purkyne, Sokolska 31, 120 26 Praha 2, Czechoslovakia).

September 13–14, **Conduction Electron Scattering in Metals** (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1 8QX).

September 15–21, **3rd International Congress on Muscle Diseases** (Professor J. N. Walton, Charman of the Organising Committee of the Third International Congress on Muscle Diseases, Regional Neurological Centre, Newcastle General Hospital, Newcastle, UK).

September 15–21, **11th International Congress on High Speed Photography** (11th International Congress on High Speed Photography, c/o Central Unit for Scientific Photography, Royal Aircraft Establishment, Farnborough, Hants, UK).

September 16–18, **International Symposium Theory and Practice of Emulsion Technology** (Dr D. Seaman, Imperial Chemical Industries Ltd, Jealott's Hill Research Station, Bracknell, Berkshire, RG12 6EY, UK).

September 16–20, **5th European Symposium on Fluorine Chemistry** (Dr John G. Gibson, The Chemical Society, Burlington House, London W1V 0BN).

September 16–21, **6th International Conference on Magnetic Resonance in Biological Systems** (Professor K. Wüthrich, Institute of Molecular Biology and Biophysics, ETH, 8049, Zürich, Switzerland).

September 16–21, **6th L. H. Gray Conference on the Initial Shapes of Cell Survival Curves: Clinical and Theoretical Implications** (Dr Tikvah Alper, Medical Research Council, Experimental Radiopathology Unit, Hammersmith Hospital, London W12 0HS).

September 17–19, **3rd European Conference on Hard Magnetic Materials** (Professor K. Hoselitz, Mullard Research Laboratories, Cross Oaks Lane, Redhill, Surrey RH1 5HA, UK).

September 17–19, **Stress Vibration and Noise Analysis in Vehicles** (T. H. Richards, Department of Mechanical Engineering, University of Aston, Gosta Green, Birmingham B4 7ET, UK).

September 17–20, **International Symposium on Practical Applications of Ergonomics in Industry, Agriculture and Forestry** (Organising Committee for the International Symposium on Ergonomics, Ministry of Labour, Str. Scaune No. 1-3, Bucharest, Rumania).

September 18–19, **Electrical Properties of Polymers** (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX).

September 18–19, **Seminar on Electrostatics: Fundamentals, Application and Hazards** (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX).

September 18–20, **Interim Colloquium on Field Simulation** (Dr Yakup Paker, Polytechnic of Central London, 115 New Cavendish Street, London W1M 8JS).

September 18–27, **General Assembly of International Council of Scientific Unions** (Associate Secretary General of IUPAP, c/o Institute of Theoretical Physics, Fack, S-402 20, Göteborg 5, Sweden).

Reports and Publications

Great Britain

The Ciba Foundation for the Promotion of International Cooperation in Medical and Chemical Research. Annual Report for 1973. Pp. 58. (Lodon: The Ciba Foundation, 1974.) [15

International Tin Research Council. Annual Report for 1973. Pp. 48. (Greenford, Middx.: Tin Research Institute, 1974.) [15

Bulletin of the British Museum (Natural History). Entomology. Vol. 30, No. 3: A Catalogue of the Family-Group and Genus-Group Names of the Coleophoridae (Lepidoptera). By K. Sattler and W. G. Tremewan. Pp. 183–214. (London: British Museum (Natural History), 1974.) £1.50. [25

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 276, No. 1257: The Place of Astronomy in the Ancient World—a Discussion. Edited by F. R. Hodson. Pp. 1–276. Vol. 276, No. 1258: Derivation and Testing of a Molecular Orbital Description of Ligand Field Spectra. By B. D. Bird, E. A. Cooke, P. Day and A. F. Orchard. Pp. 277–339. (London: The Royal Society, 1974.) [25

The Year Book of the Royal Society of London 1974. (No. 78). Pp. 466. (London: The Royal Society, 1974.) £2.10. [35

Proceedings of the Royal Irish Academy. Vol. 74, Section A. No. 6: Generalized Shape Theory. By T. Porter. Pp. 33–48. 38p. No. 7: The Conformal Properties of a Poincare Invariant Fiedl Theory. Pp. 49–60. 26p. No. 8: On the Expansion of Closed Universes. By P. Yodzis. Pp. 61–66. 18p. Vol. 74, Section B. No. 6: The Donegal Granite—a Gravity Analysis. By D. G. C. Young. Pp. 63–74. 21p. No. 7: The Lower Palaeozoic Stratigraphy of the Northern Part of the Leinster Massif. By P. M. Bruck, T. L. Potter and C. Downie. Pp. 75–84 + plate 2. 27p. No. 8: Littoral and Benthic Investigations on the West Coast of Ireland—III. (Section A: Faunistic and Ecological Studies). The Bivalves of Galway Bay and Kilkerrin Bay. By B. F. Keegan. Pp. 85–123. 50p. (Dublin: Royal Irish Academy, 1974.) [75

Strategic Survey 1973. Pp. 104. (London: The International Institute for Strategic Studies, 1974.) [85

Central Electricity Generating Board. Annual Report 1972–73, Vol. 2: Statistical Digest and Detailed Accounts. Pp. 43. (London: CEGB, 1974.) £1 net. [95

Other Countries

Canada: Department of Energy, Mines and Resources. Coal in Canada. Pp 36. (Ottawa: Information Canada, 1974.) [295

Smithsonian Contributions to Paleobiology No. 15: Permian Brachiopods of West Texas, II. By G. Arthur Cooper and Richard E. Grant. Pp. vii + 233–793 (plates 24–191) (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) $9.65. [295

International Labour Office, Geneva. Occupational Safety and Health Series, No. 26: Occupational Health Problems of Young Workers. By S. Forssman and G. H. Coppée. Pp. v + 143. (Geneva: International Labour Office, 1974.) [305

Australia: Department of Supply. Annual Report of the Defence Standards Laboratories 1972/1973. Pp. 88. (Maribyrnong, Victoria: Defence Standards Laboratories, 1974.) [305

Commonwealth Scientific and Industrial Research Organization, Australia. Annual Report 1972–73. Division of Animal Physiology. Pp. 85. (Melbourne: CSIRO, 1974.) [315

Endocrine Research Communications, Vol. 1 No. 1, 1974. Edited by Jerome Kowal. Pp. 1–116. Subscription:- Vol. 1 (6 issues) $35. (Students and Individual professionals $15. (New York: Marcel Dekker Journals.) [315

Australian Journal of Plant Physiology, Vol. 1 No. 1, March 1974. Pp. xi + 1–175. Subscription $A10 per annum. (4 issues) (Melbourne: Commonwealth Scientific and Industrial Research Organization. 1974.)

Government of India. Report of the Department of Agricultural Research and Education, 1973/1974. Pp. ii + 142. (New Delhi: Department of Agricultural Research and Education, Ministry of Agriculture, 1974.) [36

National Cancer Program. Report of the President's Cancer Panel, January 1973, submitted to the President of the United States. Pp. 15. (Bethesda, Md.: US Department of Health, Education and Welfare. Public Health Service, National Institute of Health, National Cancer Institute, 1974.) [36

Machine Diagnosis and Information Retrieval in Medicine in the USSR. (A publication of the Geographic Health Studies, John E. Fogarty International Center for Advanced Studies in the Health Sciences.) Pp. viii + 138. (Bethesda, Md.: US Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, 1974.) [63

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APPOINTMENTS VACANT

MARINE PHYSIOLOGIST

The Rosenstiel School of Marine and Atmospheric Science has an opening for a whole animal physiologist with at least a secondary interest in environmental physiology, beginning as early as January, 1975. Responsibilities will include teaching a graduate level course on the physiology of marine animals. Appointment will be at a rank and salary commensurate with experience and qualifications. Applicants must have completed the Ph.D. degree. Send curriculum vitae and names of five referees to Dr Barrie F. Taylor, Chairman of Search Committee, University of Miami, R.S.M.A.S., 4600 Rickenbacker Causeway, Miami, Florida 33149. Applications close on August 31, 1974. (2509)

DIRECTOR

The Jackson Laboratory invites applications for the position of Director of the Laboratory, which will become open on October 1, 1975. Qualified applicants are invited to submit a curriculum vitae and summary of experience to one of the following:

Dr James D. Ebert, Chairman, Search Committee, Marine Biological Laboratory, Woods Hole, Massachusetts 02543;
Dr James F. Crow, Member, Search Committee, Department of Medical Genetics, University of Wisconsin, Madison, Wisconsin 53706;
Dr Douglas L. Coleman, Secretary, Search Committee, The Jackson Laboratory, Bar Harbor, Maine 04609.

AN EQUAL OPPORTUNITY EMPLOYER. (2508)

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF BOTANY
LECTURER IN APPLIED ECOLOGY

Applications are invited for the post of Lecturer in applied ecology commencing on October 1, 1974. Applicants should possess a good honours degree and postgraduate experience in plant ecology, soil science or agronomy. An interest or experience in applying ecological principles to solving practical problems of the environment such as land reclamation or pollution would be an advantage.

There will be excellent opportunities for collaborating with a very active research group in environmental rehabilitation which has strong links with industry and local government.

Initial salary within the range £2,118 to £2,412 on a scale rising to £4,896 per annum.

Applications together with the names of three referees should be received not later than July 19, 1974 by the Registrar, The University P.O. Box 147, Liverpool L69 3BX from whom further particulars may be obtained. Quote ref. RV/N/276073. (2495)

UNIVERSITY OF MANCHESTER
DEPARTMENT OF MEDICAL BIOCHEMISTRY

Applications are invited for a Postdoctoral RESEARCH ASSOCIATESHIP financed by the British Heart Foundation. The project involves the isolation of endothelial cells from major blood vessels, their establishment in culture and studies of the synthesis of the collagen and glycoprotein components of the endothelial basement membrane. Experience in cell culture and/or protein synthesis would be advantageous. The appointment from October 1, 1974 is tenable for two years. Salary range p.a.: £2,118 to £2,412 plus F.S.S.U. Applications giving the names of two referees should reach the Secretary, Department of Medical Biochemistry, University of Manchester Medical School, Manchester M13 9PT, as soon as possible. (2481)

Ministry of Agriculture, Fisheries and Food
Food Science Division, Norwich

Chemist

■ **Join Unit studying reactions of additives and storage effects on food** ■ **Initially work involves investigating changes in baking quality of flour during storage** ■ **Help identify basis for variability in storage stability.**

□ Degree, HNC or equivalent in chemistry-based subject □ At least 2 years' post-graduate experience □ Some biochemical, food or natural product orientation □ Age under 30 □ Appointment as Higher Scientific Officer (£2221–£2854, under review, plus threshold payments) □ Ref: SB/10/AE.
□ Application forms (for return by 18 July 1974) from Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, telephone Basingstoke 29222 ext. 500 or London 01-839 1992 (24 hour answering service).

Ministry of Agriculture, Fisheries and Food
Food Science Division, London

Food Scientist/ Technologist

■ **In Information Unit** ■ **Collect, distribute and use scientific and technical information on various aspects of food science and technology, nutrition and toxicology.**

□ Degree, HND in Food Science/Technology □ Experience of information work or work in food industry an advantage □ Age under 27 □ Appointment as Scientific Officer (£1663–£2557, under review, plus threshold payments) □ Ref: SB/9/AE.
□ Application forms (for return by 18 July 1974) from Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, telephone Basingstoke 29222 ext. 500 or London 01-839 1992 (24 hour answering service).

British Museum (Natural History)

Assistant Cytotaxonomist

■ **Routine organisation of laboratory and cultural facilities at Chelsea Physic Garden** ■ **Undertake cytological and other biosystematic investigation in support of research projects.**

□ Degree, HNC or equivalent in Botany □ Practical experience in relevant fields desirable □ Age under 27 □ Appointment as Scientific Officer (£1662–£2557, under review, plus threshold payments) □ Ref: SB/28/DK.
□ Application forms (for return by 22 July 1974) from Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, telephone Basingstoke 29222 ext. 500 or London 01-839 1992 (24 hour answering service).

Science group
CIVIL SERVICE

(2668)

PROFESSOR OF BIOMETRY OR BIOSTATISTICS

Applicants are invited to apply for a new senior faculty position in the Statistical Science Division, Department of Computer Science, SUNYAB, starting in September 1975. Applicants should have an outstanding record of research, training and collaboration in the Health and Statistical Sciences. Responsibilities include teaching, developing curriculum for undergraduate and graduate courses in Biometry and Statistical Science for specialists and non-specialists, and active participation in the collaborative activities within the Statistical Laboratory. Applications from women and minority candidates will be especially welcome. Please send curriculum vitae to:

Professor Marvin Zelen, Chairman
Search Committee
Statistical Laboratory
SUNY at Buffalo
4230 Ridge Lea Road
Amherst, New York 14226 (2569)

PORTSMOUTH POLYTECHNIC
DEPARTMENT OF CHEMISTRY

Application are invited for a

TEMPORARY LECTURESHIP IN MATERIALS TECHNOLOGY

from graduates with a specialised knowledge of the chemistry and application of PLASTICS/PAINTS and other PROTECTIVE COATINGS.

The appointment will be made either at Lecturer Grade I or Lecturer Grade II level according to qualifications and experience.

Salary scales: Lecturer Grade I: £1,800 to £3,045 per annum; Lecturer Grade II: £2,700 to £3,474 per annum.

Application forms and further particulars may be obtained from the Staff Officer, Portsmouth Polytechnic, Alexandra House, Museum Road, Portsmouth, PO1 2QQ, to whom completed applications should be returned by July 15, 1974. Please quote reference D50. (2577)

HARWELL

Physics of Materials

The Physics Divisions at Harwell covera broad range of activities reflecting the Laboratory's breadth of interests and knowledge, with particular expertise and responsibilities in the application of new techniques to nuclear and non-nuclear materials problems, in neutron beam research and in laser applications.

Applications areas of particular interest and under development are:

Liquid Physics
Image Analysis
Laser Applications

Our LIQUIDS experience is in the study of liquid movement, and flow-induced vibration using optical techniques including lasers.

The IMAGE ANALYSIS studies are developing new electronic techniques of image processing and analysis.

In LASER APPLICATIONS our interests cover the use of both low and high power lasers for the study of the physics of materials.

We are looking for people with post-graduate experience in related areas with interest in physics application, and the energy to pursue and develop them. We will appoint either to our permanent staff or to three year Fellowships in a salary or stipend range of £2000-£3500 p.a.

Applications should be sent to:
Appointments Section 'A',
AERE, Harwell, Oxfordshire OX11 0RA.

(2605)

RESEARCH POST AT THE OXFORD HAEMOPHILIA CENTRE

Applications are invited from medically qualified graduate scientists with some experience of research in some aspect of haemostasis for appointment as a member of a small team of Medical Research Council staff working at the Oxford Haemophilia Centre, under the direction of Dr Rosemary Biggs. The successful candidate will be expected to co-operate with other members of staff on projects already progressing at the Centre and to initiate a research project or projects in the general field of interest of the work at the Centre. The work of the M.R.C. staff is integrated with that of the N.H.S. Clinical Unit, many projects being undertaken jointly, and an Honorary Clinical contract with the United Oxford Hospitals may be sought at a level appropriate for the successful candidate. The M.R.C. and N.H.S. staff also work in close co-operation with the staff of the Lister Institute Plasma Fractionation Laboratory and joint research projects may be planned. The appointment will be to the M.R.C. staff on the appropriate scientific grade and will initially be for a period of five years with the possibility of renewal at the end of this period. Superannuation provision.

Applications should be addressed to Dr Rosemary Biggs, Director, Oxford Haemophilia Centre, Churchill Hospital, Headington, Oxford OX3 7LJ. (2477)

ASSISTANT PROFESSOR OF HUMAN GENETICS

Applications are invited from qualified Ph.D. and/or M.D. with educational research background in genetics, biochemistry, and cell biology. Candidate must have significant research accomplishments and teaching capabilities in all of the above areas. Preference will be given to those whose research focus is either in mammalian chromosome structure and function, or biochemistry of genetic variation in eukaryotic (preferably human) organisms.

Please send complete curriculum vitae and three references to: Chairman, Department of Human Genetics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19174, U.S.A.

An equal opportunity—affirmative action employer. (2573)

RESEARCH ON TRANSFERABLE DRUG RESISTANCE ENTERIC REFERENCE LABORATORY

Applications are invited for two posts in the Enteric Reference Laboratory, Public Health Laboratory Service, Colindale, directed by Professor E. S. Anderson. Successful candidates will join a team investigating transferable drug resistance.

(1) A microbial geneticist to study epidemiological and ecological aspects of transferable drug resistance in enterobacteria.

(2) A microbial or molecular geneticist for research on the genetic and molecular characterisation of plasmid transfer systems.

Candidates with a 1st or 2nd class honours degree or a Ph.D. will be considered. Starting salary in accordance with age and experience.

Application with full curriculum vitae and names of 2 referees to Personnel Officer, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT. (2517)

UNIVERSITY OF NOTTINGHAM
SCHOOL OF AGRICULTURE
DEPARTMENT OF PHYSIOLOGY AND ENVIRONMENTAL STUDIES
EXPERIMENTAL OFFICER

Applications are invited for the post of Experimental Officer within the Plant Physiology Section. The duties involve carrying out research on the structure and properties of tobacco. The post would be suitable for a recent graduate in Biology or Biochemistry, who has expertise in preparation of sections for light microscopy and electron microscopy. Determination of nicotine content is also part of the work, and thus experience or at least interest in gas chromatography would be an added advantage. The post is for an initial period of one year, with possible extension to a total period of 2 years, 10 months. Starting salary within the range £1,569; £1,647; £1,725, under review. Application forms from the Staff Appointments Officer, The University, Nottingham, quoting reference 379. Closing date July 7, 1974. (2580)

UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF CANCER STUDIES

Research Technician, Grade III, is required to work on a project involving a study of the mechanism of carcinogenesis by chemical compounds. Biochemical work, tissue culture work and some animal experimentation will be involved. Preference will be given to candidates with experience in either biochemistry or tissue culture techniques. Applicants must have an ONC in an appropriate subject and several years laboratory experience. Salary will be on the scale £1,650-£1,920. Applications should be sent with a curriculum vitae and the names of two referees to the Personnel Department, University of Birmingham, Birmingham, B15 2TT. Ref. 450/B/264. (2546)

PORTSMOUTH POLYTECHNIC
SCHOOL OF PHARMACY
RESEARCH ASSISTANTSHIP

Applications are invited from graduates with a good honours degree in Pharmacy or a subject which includes bacteriology. Applicants about to graduate will be considered. The successful applicant, who will be expected to register for a higher degree, will work in the general area of competitive growth in mixed cultures of interest to medical microbiology.

Salary scale: £1,320 to £1,420 per annum.

Application forms and further particulars may be obtained from the Staff Officer, Portsmouth Polytechnic, Alexandra House, Museum Road, Portsmouth, PO1 2QQ, to whom completed applications should be returned by July 15, 1974. Please quote reference R73. (2576)

THE UNIVERSITY OF NEWCASTLE UPON TYNE
CHAIR OF PHYSICS

Applications are invited from experimental physicists for the Chair which was held until his death by Professor W. R. Hindmarsh. This is the third Chair in the School of Physics (the others being Geo- and Planetary Physics, and Theoretical Physics). Salary in accordance with the Professorial Scale (£5,625 by £96 to £5,721 by £195 to £6,501) with membership of F.S.S.U.

Further particulars may be obtained from the Registrar, the University of Newcastle upon Tyne, 6 Kensington Terrace, NE1 7RU, with whom applications (15 copies), giving the names of not more than three referees, must be lodged not later than August 31, 1974. (Applicants from outside the British Isles may submit one copy only.) (2584)

UNIVERSITY OF DURHAM
DEPARTMENT OF GEOLOGICAL SCIENCES
SENIOR DEMONSTRATOR IN GEOPHYSICS

Applications are invited for the post of Senior Demonstrator in Geophysics in the above Department from October 1, 1974 for two years in the first instance with possible renewal for a third and final year. Preference will be given to candidates with a good single or joint Honours degree in either Geology or Physics and with post-graduate research training in solid-earth geophysics.

Salary will be at an appropriate point on the scale £1,908 to £2,820 with F.S.S.U.

Applications (3 copies) naming three referees, should be sent by July 15, 1974 to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained. (2582)

UNIVERSITY OF NOTTINGHAM
SCHOOL OF AGRICULTURE
Department of Applied Biochemistry and Nutrition

Applications are invited for a post of Lecturer in Plant Biochemistry within the Department of Applied Biochemistry and Nutrition of the University of Nottingham. Preference will be given to graduates with initial degrees in Biochemistry or Chemistry and with postgraduate experience in Plant Biochemistry. Facilities for research can be provided in several areas of Plant Biochemistry. The salary will be within the range of £2,118 to £4,896 per annum, but the appointment will initially be made within the first three points of the scale. Application forms and further details can be obtained from the Staff Appointments Officer, The University, Nottingham, quoting Reference 378. Closing date July 7, 1974. (2581)

MEMORIAL UNIVERSITY OF NEWFOUNDLAND
FACULTY OF MEDICINE

A new medical school in Canada is expanding its faculty and will make post-doctoral and faculty appointments in the field of Human Biochemical Genetics. Candidates must be prepared to work on human biochemical variation in the context of collaborative population studies though this does not preclude the pursuit of other research interests. Interested individuals should submit applications accompanied by a detailed curriculum vitae to Dr K. B. Roberts, Associate Dean for Basic Medical Sciences, Memorial University of Newfoundland, St. John's, Newfoundland, A1C 5S7, CANADA. (2570)

UNIVERSITY OF RHODESIA
ENGINEERING

A Faculty of Engineering has been established by the University of Rhodesia in Salisbury, to provide four-year Honours degrees in Civil, Electrical, Mechanical, or Inter-disciplinary Engineering, with G.C.E. "A" level entry. Faculty buildings are in process of erection. Professors have already been appointed in the fields of Electrical and Civil Engineering, and applications are now invited for the following:

CHAIR in the field of MECHANICAL ENGINEERING.

LECTURERS/SENIOR LECTURERS with experience in one or more of the following areas (the symbols c, e, or m, imply primary application in the fields of Civil, Electrical or Mechanical Engineering respectively): Building Science (c); Control Systems (e, m); Dynamics (m); Electronic Circuit and System Design (e); Electrical Machines and Power (e); Electromagnetics (e); Fluid Mechanics (c, m); Geotechnology (c); Materials Science (m, e); Mechanical Design (m); Public Health and Water Utilisation (c); Solid Mechanics (m); Structures (c); Telecommunications (e); Thermodynamics (m); Transportation Intrastructures (c).

Minimum qualifications: university degree plus substantial postgraduation experience. (Corporate membership of a Professional Engineering Institution desirable).

Salary Scales (Approx. stg. equiv.): Professor: £7,430 by £277 to £8,812; Senior Lecturer: £5,400 by £216 to £7,128; Lecturer I: £4,905 by £181 to £5,633; Lecturer II: £3,024 by £156 to £3,491 by £173 to £3,836 by £181 to £4,562 by £173 to £4,735. Commencing salary according to qualifications and experience.

Family passages and allowances for transport of effects; installation loan; travel allowance for Sabbatical and Biennial (Professor) or Triennial (Lecturers) visits; superannuation and medical aid schemes. For persons recruited outside Rhodesia, unfurnished accommodation on or near the Campus, and within easy reach of good Junior and Senior Schools, is guaranteed for up to three years.

The University is prepared to consider a Secondment/Contract appointment.

Applications: Six copies, giving personal particulars (including full names, place, and date of birth), qualifications, experience, and publications, and naming three referees, should be sent by August 31, 1974 to the Assistant Registrar (Science), University of Rhodesia, P.O. Box MP. 167, Mount Pleasant, Salisbury, Rhodesia. Applicants from outside Southern Africa should send an additional copy to the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, from whom further particulars may be obtained. Some appointments are envisaged for 1974, others for 1975; applicants should state the earliest date available for duty, and also the preferred date. (2587)

UNIVERSITY OF GLASGOW

Applications are invited for the post of Project Engineer to the Kelvin Laboratory. The Engineer is responsible for the maintenance and operation of the 130 MeV electron linear accelerator which is used for research in nuclear structure physics. The techniques involved include high power s-band microwave generation, ultra high vacuum, electromagnetic beam analysis and control systems for electrical and fluid supplies.

The duties involve direction of the highly experienced technical staff in these activities, diagnosis and elimination of faults, and development leading to improved performance of the accelerator in experiments.

The post is remunerated on the new University Lecturer's scale between £3,462 and £4,896 per annum, which will be effective from October 1, 1974, the appointment depending on experience and age.

Further details will be supplied on request. Applications, with the names of three referees, should be sent to Professor G. R. Bishop, Kelvin Laboratory, N.E.L., East Kilbride, not later than July 15, 1974.

In reply please quote Ref. No. 3486M (2597)

BRITISH MUSEUM Natural History

Educational Technologist

To join a team of scientists and designers engaged in the development and production of a comprehensive new exhibition scheme for natural history including biology and geology.

The successful candidate will assist the team to conceptualize and organize the subject matter in order to define objectives, and will be expected to devise methods of presenting complex information and to evaluate exhibits and related publications.

Appointment will probably be as Senior Scientific Officer, requiring a good honours degree in a scientific subject and at least 4 years postgraduate experience. Graduates with a minimum of 2 years experience will, however, be considered for appointment as Higher Scientific Officer. Preference will be given to those with a broad knowledge of biological sciences and experience as an educational technologist.

SALARY: SSO, £3026-£4123; HSO, £2449-£3082.

For further details and an application form (to be returned by 12 July 1974) write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone Basingstoke 29222 ext. 500 or 01-839 1992 (24 hour answering service). Please quote SB/29/DK. (2686)

POWER STATION CHEMIST

(salary: HK$6,415 to HK$7,010 per month. Sterling £6,350 to £6,950 a year)

required by

HONG KONG GOVERNMENT

Office of the Water Authority

Applications are invited from suitably qualified and experienced Power Station Chemists for appointment as Senior Waterworks Chemist (Desalting) to take charge of all chemical services at the Lok On Pai Desalting Plant. The plant is the largest and most modern of its kind in the world operating on a multistage flash distillation process, producing 40 MGD of fresh water from the sea. The plant will comprise power station equipment, in addition to the evaporators.

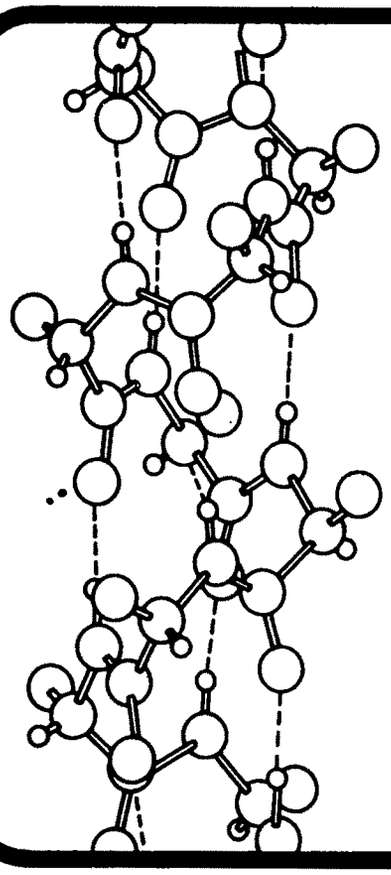
Duties will include the setting up of laboratory services, analyses of boiler water, fuel, lubricating oils, sea water etc., corrosion monitoring and pollution surveys. The Senior Chemist will head a team of assistants who will carry out this work and other duties associated with water treatment works.

In addition to the salary quoted, there are extra benefits, including free family passages, children's school passages, paid leave, children's educational allowances, subsidised accommodation, free medical attention, income tax at low local rates and a terminal gratuity of 25 per cent. Appointment will be on agreement for 2½ years.

For further particulars you should apply, giving brief details of experience to:

crown agents

M Division, 4 Millbank, London SW1P 3JD, quoting reference number M/3B/731207/NE. (2594)



Research opportunities for graduates

The Laboratory. Our Research Laboratory of 1,350 needs to keep pace with the requirements of the Unilever world-wide food business. We require three first degree graduates, with or without additional experience, to work at Welwyn in our Dairy Division, which has a staff of about 70 people and services the needs of the Chilled Products and Ice Cream businesses.

Relevant scientific disciplines: Chemistry, Biochemistry, Physics, Food or Dairy Science.

Vacancy CWA 198. One vacancy occurs in product development to assist in devising and processing frozen or chill concepts to pilot plant scale. This work will require the successful candidate to acquire an understanding of the scientific principles of the products, since an important objective in the totality of our work is to relate product development with the background work also being undertaken.

Vacancy CWA 203. It is in the background area where the other two vacancies occur. One involves assisting in a study of the role and function of proteins, whereas the other is assisting with studies of the interactions of the various components in product systems through processing and in the final product. No previous experience in any of these areas is necessarily required, since suitable training will be given on the job. A good scientific approach and clarity and accuracy in communications are, however, required.

Location. Welwyn, Hertfordshire, is approximately twenty miles north of London.

Applicants. should write for an application form and further details to: The Personnel Officer, Unilever Research, Colworth/Welwyn Laboratory, The Frythe, Welwyn, Herts.

Unilever Research
Colworth/Welwyn Laboratory

(2692)

UNIVERSITY OF WARWICK

RESEARCH TECHNICIANS—GRADE 3

The Department of Biological Sciences has vacancies for research technicians in the Virus Research Laboratory. The work involves the tissue culture of animal cells and the isolation and characterisation of nucleic acids. A knowledge of biochemical techniques will be a definite advantage. The posts are for contract periods of one to three years. Salary on the scale £1,650 by £54 to £1,920 p.a. Applications in writing as soon as possible, to the Academic Registrar, University of Warwick, Coventry CV4 7AL, quoting Ref. No. 46/2T/74. (2575)

MASSEY UNIVERSITY

PALMERSTON NORTH, NEW ZEALAND

LECTURER/SENIOR LECTURER IN VETERINARY SURGERY

Applications from graduates in Veterinary Science are invited for a teaching position in Veterinary Surgery in the Department of Veterinary Clinical Sciences to commence as soon as possible. Applicants should have had postgraduate experience in veterinary surgery and, preferably, should hold a postgraduate qualification. Preference will be given to candidates who have been working in the field of anaesthesiology.

The successful applicant will be required to teach veterinary anaesthesiology to students in the third, fourth and fifth years of the course and will be responsible to the Head of the Department of Veterinary Clinical Sciences. The appointee will be expected to participate in other aspects of the course in veterinary surgery and will have some clinical responsibilities in the general servicing of the veterinary clinics and hospitals. Opportunities for research in the surgical field are available and the appointee may be required to supervise postgraduate students and conduct postgraduate courses in the area of his speciality.

Salary: Lecturer NZ$6,753 to NZ$8,568, Senior Lecturer NZ$8,718 to NZ$10,232. Reader/Associate Professor NZ$8,718 to $10,232.

Conditions of appointment and further details may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University.

Applications close on August 16, 1974 (2589)

WITWATERSRAND

JOHANNESBURG, SOUTH AFRICA

LECTURER IN SEDIMENTOLOGY

Applications are invited for appointment to the above post. Duties are to be assumed on October 1, 1974.

Duties attached to the post will include teaching of sedimentology and aspects of oceanography. Research experience in advanced techniques of sedimentology will be a recommendation for appointment.

Salary will be in the range: R5,520 to R7,935, the initial salary will be determined according to qualifications and experience. Benefits include an annual bonus, pension and medical aid facilities, and a housing subsidy, if eligible.

Intending applicants should obtain the information sheet relating to this post from the Registrar, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, with whom applications should be lodged not later than July 31, 1974. Applicants in the U.K. may obtain the information sheet from the London representative, University of the Witwatersrand, 278 High Holborn, London WC1 to whom a copy of the application should be sent. (2613)

UNIVERSITY OF SYDNEY

LECTURESHIP IN VETERINARY PHYSIOLOGY

Preference may be given to an applicant interested in Endocrinology and Reproduction in the Female. Teaching duties, however, will cover any part of the undergraduate physiology course.

The appointment will be from January 1, 1975 or shortly thereafter. It is a permanent position but may be filled for three years in the first instance, with the possibility of permanency after that time, or in certain cases return fares.

Salary range, $A9,002 to A$12,352 p.a.

Applications including curriculum vitae, list of publications and names of referees to the Registrar, University of Sydney, NSW 2006, Australia, by July 31, 1974, from whom further information is available. Information also available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. (2593)

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL

(University of London)

DEPARTMENT OF PHYSICS AS APPLIED TO MEDICINE

A Research Assistant is required for a period of one year to work with group investigating solid state (Xeroradiography and Ionography) X-ray imaging methods. After completion of one year the position may be transferred to that of Lecturer in the Department with special responsibilities for development work in the field of diagnostic radiology and teaching trainee radiologists, radiographers and M.Sc. students. Applications stating age, qualifications and experience together with curriculum vitae and the names and addresses of two referees to Professor J. F. Tait, Department of Physics as Applied to Medicine, (N) The Middlesex Hospital Medical School, Cleveland Street, London W1P 6DB. (2591)

MASSEY UNIVERSITY

PALMERSTON NORTH, NEW ZEALAND

LECTURER IN BIOPHYSICS OR PHYSICS

Applications are invited for appointment to a Lectureship in Biophysics of Physics.

The appointee will be required to assist with teaching and research in Physics or Biophysics within the Department of Chemistry, Biochemistry and Biophysics. The Department offers Science degree units in Physics, Honours Science degree units in Biophysics, and appropriate Physics courses for the applied faculties of Agricultural and Horticultural Science, Food Science and Biotechnology, and Veterinary Science.

Preference could be given to applicants with research experience in the field of molecular biophysics.

Salary: NZ$6,753 to NZ$8,568.

Conditions of appointment and details concerning the University may be obtained from the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University.

Applications close in New Zealand on August 10, 1974. (2588)

JAMES COOK UNIVERSITY OF NORTH QUEENSLAND

Applications are invited for the following appointments:

LECTURER IN ENGINEERING (SYSTEMS)

Applicants should have a higher degree, preferably a Ph.D., with industrial and/or research experience in some fields of systems engineering. Some preference will be given to applicants with expertise in one of the following: Industrial Engineering, Process Engineering, and Environmental Engineering. The appointee will assist with undergraduate and postgraduate courses in Systems Engineering and undertake research in an appropriate field of study.

Applications close July 19, 1974.

LECTURER IN BOTANY

Candidates should have a special interest and research experience in some aspect of Plant Genetics, including Cytogenetics, Plant Breeding, Population Studies or some related field. The appointee will conduct undergraduate courses in the area of his specialisation and assist in the general teaching of the Department.

Applications close July 26, 1974.

Salary range: $A9,002 to A$2,352 p.a. plus locality allowance of $A142 p.a. for a married male, or $A71 p.a. for a single appointee. Conditions of appointment include F.S.S.U. superannuation, invalid pension scheme, housing assistance, study leave and allowance for travel and removal expenses on appointment.

Further details and application forms obtainable from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. (2592)

ROYAL POSTGRADUATE MEDICAL SCHOOL

SENIOR TECHNICIAN

required in research laboratory of the Diagnostic Radiology Department, in collaboration with the Respiratory and Cardiovascular Research Laboratories. The research carried out in this department is largely of a physiological and radiological nature and there will be opportunity for further study, including work for a higher degree. Qualifications H.N.C. in physiological measurement/BSc. or equivalent; radiographic experience not essential. Salary in range £1,578 to £2,448 according to qualifications and experience.

Applications to the Secretary, R.P.M.S., Hammersmith Hospital, Du Cane Road, London W12 0HS, quoting ref. no. 12/102/N. (2590)

LIVERPOOL POLYTECHNIC

SCHOOL OF PHARMACY

SENIOR LECTURER OR LECTURER II IN PHARMACOLOGY

Application are invited from graduates in Pharmacy, Pharmacology or Toxicology for the post of Senior Lecturer or Lecturer II in Pharmacology. A higher degree is essential and relevant postdoctoral experience would be an advantage.

The person appointed will be expected to teach pharmacology to honours degree students and to engage actively in research, for which excellent facilities are available.

Salary: Senior Lecturer £3,525 to £4,212 p.a.; Lecturer Grade II £2,700 to £3,474 p.a.

Details available from Staff Office, Dept. N, Liverpool Polytechnic, Richmond House, 1 Rumford Place, Liverpool, L3 9RH. (2599)

UNIVERSITY OF NEWCASTLE UPON TYNE

DEPARTMENT OF MEDICINE

RESEARCH ASSOCIATE

Applications are invited for a postdoctoral post as Research Associate to assist in research into thyroid and pituitary physiology to be carried out in the Department of Medicine. Candidates should have experience in organ and tissue culture. Previous endoctrine experience would be an advantage. The appointment will be for a period of up to three years. Salary on the scale £2,118 by £129 to £2,247 by £165 to £2,412 per annum.

Further information may be obtained from Dr D. C. Evered, Department of Medicine, University of Newcastle upon Tyne, Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP, to whom applications providing the names of two referees should be submitted within three weeks of this advertisement. (2601)

Reckitt & Colman

Nutritionist

To join the Medical Department of a well established and expanding Pharmaceutical Division of this major British Company.

The successful applicant will join a highly qualified team of experienced professionals and will provide his or her professional contribution on all subjects relating to nutrition. Initial work will be in the field of infant nutrition, particularly premature infants an enonates, and will involve liaison with clinical research workers in centres throughout the U.K.

The demands of this job will require the successful applicant to be well qualified and experienced, preferably with a research orientated career background which will enable him or her to function effectively in an atmosphere of new ideas and creative work.

The appointment is located in Hull and the salary will be attractive.

Please write quoting Ref. U.83 for an application form to:

R. A. Sandow,
Divisional Personnel Manager,
Reckitt & Colman,
Pharmaceutical Division,
Dansom Lane, Hull HU8 7DS (2669)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF BIOCHEMISTRY

Applications, are invited for the post of Demonstrator or Senior Demonstrator in the Department of Biochemistry. The post is tenable from October 1, 1974, for one year in the first instance, renewable yearly though not normally more than twice. Salary scale £1,761 to £2,367 per annum with F.S.S.U. benefits.

Applications, together with the names of three referees, should be received not later than July 12, 1974 by the Registrar, The University, P.O. Box 147, Liverpool, L69 3BX, from whom further particulars may be obtained. Quote ref RV/N/276097. (2598)

UNIVERSITY OF SOUTHAMPTON FACULTY OF MEDICINE MICROBIOLOGY

Senior Chief Technician

to establish a Research and Teaching Laboratory. The appointee will be aged between 25-40 and have had experience in one or several of the following fields; Immunochemistry, Virology, Chemical Microbiology or Clinical Microbiology. Whitley Council Conditions of Service and salary will apply. Further information from: Professor P. J. Watt, Faculty of Medicine-Microbiology, University of Southampton Medical & Biological Sciences Building, Bassett Crescent East, Southampton SO9 3TU. Telephone Southampton 559122, Ext. 8411.

Application forms and job description from the Personnel Officer, Southampton Health District, Tremona Road, Southampton SO1 6HU. Telephone 777222, Ext. 3651. Closing date: July 20, 1974. (2596)

ST. BARTHOLOMEW'S HOSPITAL, LONDON EC1A 7BE

SENIOR GRADE PHYSICIST

Applications are invited for the post of Senior Grade Physicist in the Department of Radiation Physics. The successful applicant will be deeply involved in the clinical work of the Radiotherapy Department, which employs a wide range of irradiation facilities (10kV to 15MeV X-rays and electrons). An interest in the advancement of treatment planning and the use of simulators is essential. A considerable portion of time will be spent on relevant research and development work. Lecturing to students is involved. Some experience of computers would be advantageous.

Salary will be in the range £2,964 to £3,843 per annum plus London Weighting.

Application (to be returned by July 12) to Personnel Department, (quoting ref PT/4564/N). (2603)

THE UNIVERSITY OF ADELAIDE

invites applications for appointment as a

READER IN THE FIELD OF ENVIRONMENTAL STUDIES

The University proposes to offer a postgraduate inter-disciplinary course leading to a Master's degree in Environmental Studies. The Reader will be Director of the course in Environmental Studies, and will have the responsibility of co-ordinating the teaching and research programmes of students taking the course.

Applicants should have academic attainments at a level that would fit them for appointment as a Reader in a relevant field in the sciences, (including the applied sciences) social sciences or the humanities. An interest and some experience in multi-disciplinary studies is essential.

Salary scale: $A16,389, wth superannuation on the F.S.S.U. basis.

Further information, including list of particulars required in an application, is available from the Registrar of the University or from the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications should reach the Registrar, G.P.O. Box 498, Adelaide, South Australia 5001 not later than **July 26, 1974.** (2615)

Experimental Officer

Biochemistry

Pharmaceuticals Division have a vacancy for an Experimental Officer to work with a senior biochemist on rheumatoid arthritis.

We are seeking a candidate with a university degree or similar qualification, preferably with wide experience in biochemical and/or immunological techniques.

Pharmaceuticals Division is attractively situated in rural North Cheshire, within easy reach of main road and rail routes. Assistance given to married men in moving to this area is designed to attract and retain staff of high calibre.

Applications in writing, requesting an application form should be sent to:

Mr. M. F. Losse, Personnel Officer,
ICI Pharmaceuticals Division, Mereside,
Alderley Park, Macclesfield, Cheshire.

ICI Pharmaceuticals Division

(2694)

FOOD HYGIENE LABORATORY

A.I.M.D.T. with experience in Bacteriology. The laboratory is concerned with the routine surveillance of foodstuffs and the investigation of problems associated with food hygiene and food poisoning. The successful applicant will be encouraged to take part in research projects which include work on the many types or organisms now associated with food poisoning.

The laboratory offers good facilities for study towards the special examination in bacteriology.

Further information or arrangements to visit the laboratory can be made by telephoning the Head Technician, 205 7041, Extension 38. Application forms can be obtained from the Personnel Officer, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT. (2600)

CHELSEA COLLEGE
UNIVERSITY OF LONDON
DEPARTMENT OF PHYSIOLOGY

We are looking for two GRADE 4 TECHNICIANS to take part in research and teaching activities. The main responbilities will be in the field of human physiology and in the organisation and running of the teaching laboratories. We would particularly welcome applications from persons with experience in human or animal physiology and from those who have an understanding of electronic apparatus and an ability to design and make both electronic and mechanical apparatus. Salary Scale: £2,023 to £2,338 per annum (including £175 London Allowance). Application forms from the Personnel Officer, (N) Chelsea College, Manresa Road, London SW3 6LX. (2602)

UNIVERSITY OF BATH
SCHOOL OF BIOLOGICAL SCIENCES
ASSISTANT EXPERIMENTAL OFFICER
ECOLOGY TEACHING PROJECT

An Assistant Experimental Officer is required for one year to assist in the development of materials and teaching methods for an introductory ecology course at University level. Applicant must have a degree in a Biological subject.

Salary in the range £1,524 to £1,794 per annum

Application forms, available from The Registrar(S), University of Bath, Bath BA2 7AY, quoting reference 74/105, should be returned as soon as possible. (2618)

THE UNIVERSITY OF ADELAIDE

Invites applications for appointment as a

SENIOR LECTURER IN SOCIAL BIOLOGY (HUMANITIES OR SCIENCES)

The appointment will be in a department appropriate to the interests of the successful applicant who will initially be required to teach a third-year part subject to students interested in both the biological and the humanities.

Salary scale: $A12,643 to $417 (1) to $416 (4) to $14,724; with superannuation on the F.S.S.U. basis. Initial salary will be fixed within the scale in accordance with qualifications and previous experience.

Further information, including list of particulars required in an application, is available from the Registrar of the University or from the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications, should reach the Registrar, G.P.O. Box 498, Adelaide, South Australia 5001, not later than **July 26, 1974.** (2617)

SUFFOLK AREA HEALTH AUTHORITY
IPSWICH HEALTH DISTRICT
THE IPSWICH HOSPITAL
PHYSICS DEPARTMENT
PHYSICIST

at the Ipswich Hospital, Anglesea Road Wing. Duties will be principally concerned with radiotherapy and nuclear medicine. Salary £1,497 to £2,694 p.a. Further details may be obtained from the Principal Physicist, telephone Ipswich 51021. Application forms are available from the Hospital Secretary, Ipswich Hospital, Anglesea Road Wing, Ipswich, Suffolk. Reference ARW/P/16 Nature. (2626)

UNIVERSITY OF GLASGOW
DEPARTMENT OF PATHOLOGICAL
RESEARCH ASSISTANT

Applications are invited for a research assistantship (3 years appointment) in the above department at a salary of £1,395 per annum. The sucessful candidate will be involved in an investigation of the metabolism of human plasma lipoproteins in health and disease, and should have a good honours degree in biochemistry or chemistry. The applicant may be enrolled for a Ph.D. in the University of Glasgow.

Applications, including a brief curriculum vitae and the names of two referees, should be sent to:
Professor H. G. Morgan,
Department of Pathological Biochemistry,
Royal Infirmary,
Glasgow G4 0SF. (2614)

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL
(UNIVERSITY OF LONDON)

Department of Physics as Applied to Medicine

Applications are invited from good honours graduates or those expecting to graduate this year in Biophysics or related sciences, to study receptor mechanisms in single mammalian cells. An M.R.C. training scholarship is available and the successful applicant may register for a postgraduate degree. Applications including curriculum vitae and the names and addresses of two referees, to Professor J. F. Tait, (N) Department of Physics as Applied to Medicine, The Middlesex Hospital Medical School, Cleveland Street, London W1P 6DB by July 9, 1974. (2623)

WESTFIELD COLLEGE
(UNIVERSITY OF LONDON)
CHEMISTRY DEPARTMENT

Experimental Officers (two) are required for varied and interesting work, including steroid synthesis, on the Medical Research Council's Steroid Reference Collection. New graduates, holders of G.R.I.C. or H.N.C., or school-leavers (A-level) with qualifications including chemistry are invited to apply. Salaries: scales £1,679 to £1,939 (graduate) or £1,211 to £1,679 (junior). Applications, including names of two referees, or requests for further information, should be addressed to Dr. D. N. Kirk, Chemistry Department, Westfield College, (N), Kidderpore Avenue, Hampstead, London NW3 7ST. (2622)

Senior Pharmacologist

Our client is a major British Research Organisation with extensive expertise in the field of biomedical sciences. Its function is to employ the latest skills, facilities and equipment to carry out research projects under contract for industry, government agencies and international bodies.

The laboratories are already well established, and are committed to a substantial growth and development programme over the next few years. In this connection we seek an experienced pharmacologist to spearhead the existing pharmacology projects, and also to identify and exploit new business opportunities in the UK and overseas.

Much importance is attached to publicising the specialised techniques developed in the unit, which currently include facilities for advanced psychopharmacological studies in primates, sensitive test systems for assessing the abuse-potential of new drugs and cardiovascular pharmacology. The successful candidate will be encouraged to develop interrelationships with other specialist research units, both within the organisation and outside it.

Applicants should be graduates in pharmacology or a closely related subject, ideally with a Ph.D. and several years' post-graduate experience in industrial pharmacology. A specific knowledge of behavioural pharmacology would be of particular interest to our client. In addition, candidates should have an adaptable and outgoing personality, with the ability to sell their ideas and services.

Salary is negotiable within wide limits and will reflect the importance of this position. Benefits include a progressive contributory pension scheme with free life insurance, four weeks holiday and generous assistance with relocation expenses.

The laboratories are situated in pleasant countryside near a major University city.

Please write in complete confidence for an application form and further literature, or 'phone if you would like to discuss the position:

Mr. R. A. Forbes-Leith, Talentmark Limited,
King House, 5/11 Westbourne Grove,
London W2 4UA. Tel: 01-229 2266.

Talentmark
Biomedical and Scientific Consultants

(2685)

LONDON BOROUGH OF BROMLEY
ORPINGTON COLLEGE OF FURTHER EDUCATION

Required for September 1, 1974.

(1) Technician for BIOLOGY laboratory and to assist in the Chemistry laboratory.

(2) Technician for PHYSICS laboratory and to assist in the college workshop.

Salary: Technician Grade III scale, £1,416 to £1,644 by £105 London Weighting (under review). 36 hours per week Monday to Friday including one evening per week.

Further details and application forms from The Principal, Orpington College of Further Education, The Walnuts, High Street, Orpington, Kent. Telephone Orpington 39336. (2606)

SIR GEORGE WILLIAMS UNIVERSITY
MONTREAL, QUEBEC, CANADA
DEPT. OF BIOLOGICAL SCIENCES
THREE FACULTY POSITIONS.

Three faculty positions avalable, July 1, 1974; 1 Associate Professor ($15,400 to $17,000), 2 Assistant Professors ($12,000 to $15,00) depending upon experience and qualifications. Research oriented persons qualified to teach in one or more of the following areas will be considered: Embryology Plant Ecology, Biostatistics, Introductory Biology, Ph.D. required. Send resume and names of three references to: **Dr. H. Enesco, Chairman, Dept. of Biological Sciences, Sir George Williams University, Montreal, Que. H3G 1M8.** (2647)

TECHNICIAN (Qualified)

required for work in immunology in relation to renal disease. Experience in this field is not essential. Apply in writing stating age and giving details of education and experience, to the Secretary, Guy's Hospital Medical School, London Bridge SE1 9RT, quoting Ref. R.U.3. (2646)

LA TROBE UNIVERSITY
MELBOURNE, AUSTRALIA
LECTURER/SENIOR LECTURER IN BIOCHEMISTRY

The Department is interested in making an appointment in one of the following areas: Biochemistry of Hormone Action, Biochemistry of Development or Differentiation.

Salary: Senior Lecturer — $A12,643 to 1 by $A417 to 4 by $A416 to $A14,724; Lecturer $A9,002 to 4 by $479 to 3 by $A478 to $A12,352.

Professor B. A. Stone, the Chairman of the Department of Biochemistry, will be in the United Kingdom from July 15, to July 26 and may be contacted through the Association of Commonwealth Universities.

Further information and application forms are available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar, La Trobe University, Bundoora, Victoria, Australia, 3083.

Applications, close on **July 27, 1974** (2659)

THE EDINBURGH SCHOOL OF AGRICULTURE
RESEARCH ASSISTANT

Applications are invited from Honours graduates in Microbiology or a related discipline for a research assistantship to work in the microbiology and biochemistry departments on silage deterioration studies financed by the A.R.C. The assistant will be expected to register for a higher degree.

Salary £830 by £40 to £910 plus F.S.S.U. and fees.

Further particulars from The Secretary, The Edinburgh School of Agriculture, West Mains Road, Edinburgh EH9 3JG. (2636)

ROYAL HOLLOWAY COLLEGE
(UNIVERSITY OF LONDON)
EGHAM HILL, EGHAM, SURREY.
RESEARCH TECHNICIAN— CHEMISTRY DEPARTMENT

The following Research post supported by the F.R.C. and supervised by Dr. Paul Powell is available from October 1, 1974.

Technician Grade 3 with experience in the routine synthesis of organic compounds to work on mechanisms of reactions of nucleophiles with organic ligands co-ordinated to transition elements. Salary £1,650 by £54 to £1,758 plus superannuation. Applications together with names and addresses of referees should be sent to the Personnel Officer (N) as soon as possible from whom further details may be obtained. (2616)

UNIVERSITY OF GLASGOW
DEPARTMENT OF NATURAL PHILOSOPHY
DATA ANALYSIS UNIT RESEARCH TECHNOLOGIST

The unit has an IBM 370/145, and IBM 7040 and four Data General computers. To these are attached various devices to assist in the analysis of data from high energy physics experiments (bubble chambers, spark chambers, etc.)

A vacancy exists for a responsible post as deputy to the physicist in charge of the unit, to assist him in the design of such equipment, its implementaion and testing, and to help supervise the construction of the hardware. It is anticipated that the successful candidate will have a degree or similar qualification in electronics and several years experience designing computer hardware. Alternatively the position might attract a physicist with experience of similar work. Salary will be within the range £2,637 to £3,174 per annum of the Research Technologist scale of £2,637 to £3,921.

Applications, giving relevant particulars and the names of two referees, should be sent to Professor I. S. Hughes, Department of Natural Philosophy, The University, Glasgow G12 8QQ, before July 15, 1974.

In reply please quote Ref. No. 3494 M. (2662)

Experienced histopathologist in research

Join a team conducting histopathological research and safety evaluation tests on our new ethical and proprietary drugs – a critical factor in their development.

You will have considerable involvement in the planning of safety evaluation studies as well as contact with other departments covering a wide range of biological research. In your own field advanced techniques such as histochemistry and electron microscopy are available.

The products of our research have an international reputation; we also have a reputation for advanced thinking in professional development. We can therefore promise that your experience and abilities will be fully recognised, and that further training where appropriate in pathology and other aspects of safety evaluation, with opportunities to attend international meetings, will be available to broaden and progress your career.

Salary will be attractive, and benefits are excellent: contributory pension, welfare schemes, and expenses toward relocation with the choice of reasonably priced houses in town – Nottingham, Loughborough, Leicester – or in pleasant surrounding countryside.

Please write with details or telephone: John Mills, Fisons Limited, Pharmaceutical Division, Bakewell Road, Loughborough, Leicestershire. Loughborough 66361.

FISONS

(2684)

MEDICAL RESEARCH COUNCIL
NATIONAL INSTITUTE FOR MEDICAL RESEARCH

BIOCHEMIST

Postdoctoral Position in Division of Molecular Pharmacology

A post-doctoral biochemist is required to participate in a study of the mechanism of action of dihydrofolate reductase and its inhibition by folate analogues. Applicants should have experience in techniques of protein chemistry. The appointment, which is for up to three years, is available from October 1, 1974 in the salary range £2,223 to £3,378 per annum plus £162 p.a. London Allowance. Superannuation provision under F.S.S.U.

Applications giving details of qualifications, research experience and the names of two professional referees should be sent to The Director, National Institute for Medical Research, Mill Hill, London NW7 1AA. (2638)

UNIVERSITY OF SOUTHAMPTON
DEPARTMENT OF ELECTRONICS

RESEARCH ASSISTANT

Applications are invited for the post of Research Assistant to work for a project on Computer Modelling Techniques. The research will involve development of computer models and optimisation studies of electro-chemical systems.

Applicants should possess or be about to complete, a good honours degree or equivalent in engineering, physics or chemistry. Salary will depend upon qualifications and experience, and could be up to £1,500. The successful applicant will be expected to register for a higher degree.

Applications, citing two referees, should be addressed to the Deputy Secretary's Section, The University of Southampton, Southampton SO9 5NH. Closing date July 12, 1974. Please quote ref. 247/R/Na. (2640)

ROYAL HOSPITAL FOR SICK CHILDREN
and
QUEEN MOTHER'S HOSPITAL
GLASGOW.

ASSISTANT CYTOGENETICIST, science graduate, required for Glasgow and West of Scotland Genetic Advisory Service, to work on the diagnosis of human chromosome aberrations. Experience in cytogenetics and cell culture desirable but not essential.

Further details may be obtained from the University Department of Medical Genetics (041-339 8888, Ext. 7117).

Applications, with full details of qualifications and experience, and names of two referees to Medical Administration Office, Royal Hospital for Sick Children, Yorkhill, Glasgow G3 8SJ. (2666)

THE UNIVERSITY OF SHEFFIELD
C.A.S.E. AWARD IN PHYSICAL CHEMISTRY OF MICELLES

Applications are invited from good honours graduates in chemistry or chemical physics, or those about to graduate in these subjects, for a C.A.S.E. award under the joint supervision of Dr. I. A. McLure (University of Sheffield) and Dr. D. G. Hall (Unilever Research, Port Sunlight).

The project will involve initially study of distribution of suitable solutes between an aqueous solution of a surfactant and an immiscible and unsolubilized solvent with the object of gaining an improved understanding of the nature of the interior of micelles. Applications, consisting of curriculum vitae and names of two referees, should be sent to Dr. I. A. McLure, Department of Chemistry, The University, Sheffield S3 7HF, from whom further details can be obtained. Please quote reference R101/G. (2632)

UNIVERSITY OF EDINBURGH
ROYAL (DICK) SCHOOL OF VETERINARY STUDIES
DEPARTMENT OF VETERINARY PHARMACOLOGY

LECTURER

Applications are invited from holders of registerable veterinary degrees for the post of LECTURER in the Department of Veterinary Pharmacology.

Salary will be on the scale £2,118 to £4,896 with placement according to qualifications and teaching experience, and with superannuation benefits.

This post involves teaching pharmacology and chemotherapy to under- and postgraduate veterinary students. Appropriate clinical and/or research experience is desirable. The successful candidate may if necessary, register for a higher degree.

Further particulars may be obtained from the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh EH8 9YL with whom applications (three copies) giving the names of two referees, should be lodged not later than July 27, 1974. Please quote reference number 1028. (2635)

UNIVERSITY OF YORK
DEPARTMENT OF BIOLOGY

TRIALS OFFICER

Applications are invited for the post of Trials Officer, vacant from October, 1974 for a period of three years, from graduates in agriculture, biology or forestry. The successful applicant will join a team concerned with various aspects of colliery spoil reclamation. The research will be supported by the Department of the Environment and will be directed by Dr. M. J. Chadwick.

The work involves the establishment and maintenance of field trials on colliery spoil sites throughout England and Wales and the main responsibility of the Trials Officer will be supervision of these.

Salary within the range £1,761 to £1,995, with F.S.S.U.

Three copies of applications, naming two referees, should be sent as soon as possible to the Registrar, University of York, Heslington, York YO1 5DD, from whom further particulars may be obtained. Please quote reference number 11/7053.

TWO POST DOCTORAL RESEARCH FELLOWSHIPS

Applications are invited for two post-doctoral Research Fellowships from those with previous research interests in ecological aspects of soil-plant relationships. The successful applicants will work as members of a team concerned with various aspects of colliery spoil reclamation.

The research will be supported by the Department of the Environment for a period of three years from October 1974 and will be directed by Dr. M. J. Chadwick.

Salary within the range £1,878 to £2,580, with F.S.S.U.

Three copies of applications, containing a curriculum vitae and the names of two academic referees should be sent as soon as possible to the Registrar, University of York, Heslington, York YO1 5DD. Please quote reference number 11/6021. (2627)

LIVERPOOL POLYTECHNIC
DEPARTMENT OF BIOLOGY

RESEARCH ASSISTANT

To investigate the invertebrate fauna of industrial waste heaps under the supervision of Dr. M. Luxton.

The successful applicant will be expected to register for a higher degree.

Salary scale: £1,353 to £1,644 per annum.

Application forms available from Staff Office, Dept. N, Liverpool Polytechnic, Richmond House, 1 Rumford Place, Liverpool, L3 9RH to be returned within 10 days of the appearance of this advertisement. (2667)

UNIVERSITY OF SUSSEX

RESEARCH FELLOWSHIP IN OBSERVATIONAL EXTRA-GALACTIC ASTRONOMY

Applications are invited for one post of POSTDOCTORAL RESEARCH FELLOW in OBSERVATIONAL EXTRAGALACTIC ASTRONOMY

from October 1, 1974, tenable for a period of one to three years.

The Research Fellow will be expected to collaborate with visiting faculty members based at the Royal Greenwich Observatory in carrying out spectrometric and photometric observations of extragalactic radio and X-ray sources in the Southern Hemisphere.

Commencing salary will be in the range £2,247 to £2,931, with F.S.S.U. benefits.

Further information can be obtained from Professor L. Mestel, Astronomy Centre, Physics Building, University of Sussex, Falmer, Brighton BN1 9QH, U.K., to whom applications, with the names of two or three referees, should be sent by July 19, 1974.

(2631)

UNIVERSITY OF LONDON

READERSHIP IN VETERINARY PHARMACOLOGY

at the Royal Veterinary College

The Senate invite applications for the above Readership. Salary scale £4,707 to £5,844 a year, plus £162 London Allowance. Applications (10 copies) should be received not later than August 23, 1974, by the Academic Registrar (N), University of London, Senate House, London WC1E 7HU, from whom further particulars may be obtained. (2672)

UNIVERSITY OF EDINBURGH

MARY DICK CHAIR OF VETERINARY ANATOMY

The University Court invites applications for the above Chair. The Department, of which the Professor is the Head, is in the Faculty of Veterinary Medicine and is one of eight Departments in the Royal (Dick) School of Veterinary Studies.

Applicants must have a full range of knowledge of Veterinary Anatomy including Veterinary Histology and Embryology and would also be expected to have specialised knowledge in one or more sections of this wide field.

The salary will be within the professorial range. Superannuation under F.S.S.U.

Terms and conditions of appointment together with further particulars may be obtained from the undersigned, with whom applications (twelve copies) giving the names of three referees, should be lodged not later than August 15, 1974. A candidate from overseas may submit one copy of his application.

Applications should be sent to The Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh EH8 9YL. Please quote reference number 2/74. (2637)

Senior Toxicologist

The Toxicology area of the Research Department is expanding and an experienced Toxicologist is required to take charge of a new section. This will entail the planning, conduct and assessment of acute and chronic tests in rodents and some general pharmacological investigations. The person appointed will be expected to maintain close liaison with other staff concerned in the safety evaluation of new Medical, Animal Health and Crop Protection products, and to contribute towards technical documents for registration of such products in the U.K. and overseas. Publication of results and attendance at scientific meetings are encouraged.

The modern, well-equipped research laboratories and associated facilities are located in Nottingham.

Applicants must have a good honours degree in pharmacology or relevant biological science and, preferably, a postgraduate qualification. Experience of working in Toxicology and a high standard of scientific report writing are essential.

Starting salary is negotiable and will reflect qualifications and experience. Conditions of employment are attractive and include good profit sharing and contributory pension schemes. Assistance with relocation expenses will be granted where appropriate.

Please write to :—J. F. Pattison, Employment Services,

Boots

The Boots Company Ltd.,
Station Street,
Nottingham NG2 3AA.

(2641)

THE MACMILLAN COMPANY OF INDIA

Managing Editor

We are looking for a man to take over the direction of our rapidly expanding publishing programme in English and Hindi. We expect that the right candidate will have an Indian background, good university qualifications, some publishing experience and a knowledge of Hindi. Drive, organising ability, entrepreneurial flair and sound judgement are the most important qualifications. The post is based in Delhi.

Please apply in confidence to:—

Adrian Soar,
Macmillan Publishers Ltd.,
Little Essex Street,
London WC2R 3LF.

Sharad Wasani,
Macmillan Company of India Ltd.,
2/10A Ansari Road,
Daryaganj, Delhi 6.

(2671)

AARHUS UNIVERSITY
(DENMARK)

At the Department of Chemistry (in the newly formed Division of Biostructural Chemistry) an adjunkt/lektor position will be vacant on August 1st, 1974.

A molecular biologist/biochemist is wanted for the position to complement work in the biostructural group in cooperation with a.o. professor B.F.C. Clark. Applicants with research experience in the field of protein biosynthesis, and/or in enzymology would be preferred.

The successful applicant must, on equal terms with the other members of the group, share the teaching obligations—from first-year level to supervision of graduate students.

The appointment is on a scale equivalent to assistant/associate professor with a salary range 65.000–115.000 Dkr.

Applications containing curriculum vitae, information on scientific and educational activities, a list of publications plus wherever possible 3 copies of published material, and any additional information relevant for the evaluation of the applicant's scientific and educational qualifications should be sent to the Faculty of Science, Aarhus University, Ndr. Ringgad 1, 8000 Aarhus C., Denmark, before August 1, 1974.

UNIVERSITY OF WESTERN AUSTRALIA
PERTH

PHYSICAL SCIENCES LIBRARIAN

A person with suitable qualifications and experience is required towards the end of 1974 for the above position which is rendered vacant by the present holder proceeding overseas. The Physical Sciences Librarian is responsible, under the direction of the University Librarian, for the Mathematics and Physical Sciences Library and the Science Reading Room. The Mathematics and Physical Sciences Library contains the bulk of the University's holdings in those subjects and is designed primarily for the needs of senior students and staff. The Reading Room holds the undergraduate collection.

Ideally, candidates should possess a degree in science or engineering, professional library qualifications and experience in a university or science library, but consideration will be given to graduates in other fields who possess considerable experience in scientific libraries or to science graduates without library experience who have a genuine interest in scientific information services. As the appointee will be expected to keep in touch with new developments in scientific information, experience or interest in the application of computers to libraries will be an advantage. Further details of duties and responsibilities can be obtained from the University Librarian.

The appointment will be made according to qualifications and experience either in the Librarian's salary range (based on that of a Lecturer), at present $A9,002 to $A11,396 pa. but currently under review, or in the Assistant Librarian's range of $A7,247 to $A9,578 p.a. Benefits include superannuation similar to F.S.S.U. fares to Perth for appointee and dependent family, removal allowance, annual and long service leave plus, in special circumstances for a Librarian, study leave.

Applications in duplicate stating full personal particulars, qualifications and experience should reach the Staffing Officer, University of Western Australia, Nedlands, Western Australian, 6009, by **July 13, 1974.** Candidates should request three referees to write immediately to the Staffing Officer.

Conditions of appointment may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. (2657)

Hormones and Liver Glycogen Project

Assistant Research Officer required to join in study of Hormonal Control of Liver Meabolism. Post would suit graduate with interest in biochemistry. Initial salary £1,296 p.a. (scale under review). Written applications to Departmental Superintendent, Biochemistry Department, Imperial College, London SE7 2AZ. (2642)

THE UNIVERSITY OF OXFORD POST-DOCTORAL ASSISTANTSHIP IN ORGANIC CHEMISTRY

Applications are invited for an S.R.C. supported postdoctoral post concerned with the synthesis of functional surfactants for selective catalysis of organic reactions. A background in organic synthesis or mechanism is desirable. Appointment would be for one year in the first instance, commencing around October 1, 1974, on the salary scale £1,821 to £2,058 (according to age) (under review) plus F.S.S.U. Applicants should forward a curriculum vitae together with the names of two referees to Dr. J. M. Brown, The Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY, as soon as possible, and before August 15, 1974. (2689)

UNIVERSITY OF DUNDEE
DEPARTMENT OF BIOLOGICAL SCIENCES

Applications are invited for two posts of

RESEARCH ASSISTANTS

to work on Nutrient Cycling in Freshwater Ecosystems.

Starting date October 1, 1974. Salary around £1,500 but applicants registering as full-time students for a higher degree will be paid at the studentship equivalent rate.

Further particulars are available from The Secretary, The University, Dundee DD1 4HN, to whom applications (quoting Ref. Est/43/74J and naming 2 referees) should be sent as soon as possible. Informal enquiries may be made to Professor W. D. P. Stewart at the Department of Biological Sciences. (2678)

PORTSMOUTH POLYTECHNIC
SCHOOL OF PHARMACY

LECTURER II IN PHARMACOLOGY

Applications are invited from pharmacists or pharmacologists to participate mainly in the teaching of Pharmacology to students of the Pharmacy degrees and the Honours degree in Pharmacology, and to assist with the teaching of other courses in the School of Pharmacy. A higher degree together with teaching or research experience is desirable. Excellent research facilities are available.

Salary scale: £2,700 to £3,474 per annum.

Application forms and further particulars may be obtained from the Staff Officer, Portsmouth Polytechnic, Alexandra House, Museum Road, Portsmouth PO1 2QQ, to whom completed applications should be returned by July 8, 1974. Please quote reference R76. (2663)

PRODUCTIVE DEVELOPMENT SCIENTIST

Required by Manufacturers producing mainly beverages with malt extract and also new instant meals.

Successful applicant will be responsible for reviewing and improving recipes of these products. Salary £3,000 up. Location: Herts.

Please apply to:
TUDOR APPOINTMENTS
11 Muswell Hill, N10 3TH
Tel: 01-444 9121 (24 hrs)
(2664)

PAPUA NEW GUINEA UNIVERSITY OF TECHNOLOGY
DEPARTMENT OF CHEMICAL TECHNOLOGY

SENIOR LECTURER

The Department of Chemical Technology offers a first degree course in Chemical Technology with specialisation in food technology, mineral processing and industrial chemistry. It is also responsible for the teaching of Chemistry to engineering students and for the operation of the University's Analysis Laboratory, which offers a wide range of analytical services on a commercial basis.

In 1975 the Department will be housed in a new biulding and associated with the Department of Forestry in a School of Natural Resources and with the Faculty of Agriculture of the University of Papua New Guinea.

Applications are now invited for the position of senior Lecturer, which is available immediately. Applicants should have a good degree in either Chemical Engineering or Applied/Industrial Chemistry as well as considerable industrial experience.

The successful candidate will be expected to play a major role in the development of the Chemical Technology degree course and in the setting up of various pilot plant operations. The Department already operates a small tannery and food and mineral processing programmes are being drawn up. In-service training of students is stressed in the degree course and staff are encouraged to form close working links with those organisations and companies involved in the training programme.

Research work in the Department is mainly concerned with environmental impact studies or the development of original processing techniques. The Department is also interested in Community Development and Small Business Development work.

Salary range: $A9,967 to $A11,589 per annum. Allowances totalling a further $A860 per annum are payable in certain circumstances. Modern furnished housing is provided at a nominal rental. Superannuation may be provided.

Applications in duplicate should include particulars of age, nationality, maritl status, family if any, qualifications, experience, present post and the names and addresses of three referees from whom confidential enquiries may be made, and are required by **July 26, 1974.** They should be sent to The Registrar, The Papua New Guinea University of Technology, P.O. Box 793, LAE, Papua New Guineaa. An additional copy should be sent to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, from whom further information may be obtained. (2658)

LECTURER IN EXPLORATION GEOCHEMISTRY

Imperial College of Science and Technology wishes to appoint a Lecturer in Exploration Geochemistry with effect from October 1, 1974. Practical and/or research experience in this field is essential and preference will be given to candidates who have gained this experience Post-Doctorally.

Commencing salary, according to age, qualifications and experience in the scale £2,118 to £4,896 p.a. plus £162 London Allowance and FSSU.

Applications, including curriculum vitae and the names of two referees should be made by July 31, to Professor J. S. Webb, Applied Geochemistry Research Group, Geology Department, Imperial College, London SW7 2BP. (2639)

UNIVERSITY OF BRISTOL

Postdoctoral Protein Crystallographer

Applications are invited for an S.R.C. post to work in the crystal structure of pyruvate kinase or phospho glucose isomerase. The research will involve the development of computer programs to handle data collected by an oscillation camera. The appointment is for a period of two years with an initial salary of up to £2,058 per annum depending on experience. Previous research experience in crystallography is essential.

Applications, including a brief curriculum vitae and names and addresses of two referees, should be sent to Dr. H. Muirhead, Department of Biochemistry, University Walk, Bristol BS8 1TD. (2651)

PLYMOUTH POLYTECHNIC

SCHOOL OF ENVIRONMENTAL SCIENCES

SENIOR LECTURER/LECTURER II IN POLLUTION ECOLOGY

LECTURER II IN ANIMAL PSYCHOLOGY

LECTURER II IN GEOLOGY

(1 year temporary appointment)

Preference will be given to candidates with teaching and research interests in air pollution.

Salary: £2,700 to £3,474 p.a. Lecturer II £3,535 to £3,915 to (3 by 90) to £4,212 p.a. Senior Lecturer.

Further details and application forms from:

The Establishment Officer
Plymouth Polytechnic
Plymouth PL4 8AA.

to be returned by July 9, 1974. (3661)

UNIVERSITY OF STRATHCLYDE

DEPARTMENT OF PURE AND APPLIED CHEMISTRY

C.A.S.E. AWARD

Applications are invited from candidates wishing to study for a Ph.D. with Professor N. B. Graham in Applied Chemistry on a programme in co-operation with the I.C.I. Corporate Research Laboratory at Runcorn. The Science Research Council have agreed to sponsor a candidate under their programme of Co-operative Awards in Science and Engineering to study "The utra-violet treatment of fabricated polyethylene".

The successful candidate, who must have a first or second class honours degree, will receive training and pursue researchIs in photochemistry and polymer fabrication and characterisation at the University of Strathclyde and at I.C.I.

Applications to Professor N. B. Graham, Department of Pure and Applied Chemistry, Thomas Graham Building, University of Strathclyde, 295 Cathedral Street, Glasgow G1 1XL. (2654)

Unique opportunities in Ireland

SCIENTIFIC AND TECHNOLOGICAL ANALYSTS

The National Science Council of Ireland invites applications from suitably qualified persons for employment as Scientific and Technological Analysts within its Secretariat.

Successful candidates will carry out detailed techno-economic analyses and prospective studies on the scientific and technological infrastructure in Ireland, aids and incentives for the development of science and technology-based industry and science and technology policies necessary to develop important areas of the economy.

Successful applicants will be 27-35 years of age with a University degree or equivalent in an appropriate discipline and at least five years relevant working experience.

Appointments will be on a contract of up to two years duration and remuneration will be in the range of £4,000-£5,500 per annum. These positions will be particularly attractive to Irish citizens interested in returning to a job in Ireland offering a high level development perspective. Opportunities exist for working closely with Industry, Research Institutes, Universities and other agencies concerned with scientific and technological development in Ireland, and with relevant organisations in Europe. Applicants who can and wish to make a contribution within the Secretariat before moving to another position within Ireland will be afforded every facility and encouragement to do so.

Interested applicants should write for further information to:—

THE SECRETARY GENERAL,
NATIONAL SCIENCE COUNCIL,
ST. MARTIN'S HOUSE,
WATERLOO ROAD,
DUBLIN, 4, IRELAND

All applicants and enquiries will be treated confidentially.

Closing date for receipt of applications: 1st September 1974. (2652)

OXFORD UNIVERSITY

DEPARTMENT OF EXPERIMENTAL PSYCHOLOGY

Applications are invited for the post of

RESEARCH ASSISTANT

to work on the relation between brain activity and behaviour. The post offers wide opportunities for a person interested in neurophysiology, pharmacology and behaviour. The salary will be on the D.R.A. II scale and the starting point will depend on qualifications and experience.

Applications and requests for further details should be sent in writing to Dr. E. T. Rolls, Department of Experimental Psychology, South Park Road, Oxford OX1 3PS. (2676)

DEPARTMENT OF BIOLOGY

AMERICAN UNIVERSITY OF BEIRUT

is recruiting teaching research faculty for Fall (1974) in the following areas: general ecology, marine microbiology, plant physiology, and animal physiology (vertebrate and invertebrate). More than one opening is anticipated at Assistant or Associate Professor rank. Ph.D. required, post doctoral and teaching experience preferred. Travel, relocation and fringe benefits included. Applications with letters of reference should be addressed to Dr. Philip Basson, Chairman, Department of Biology, American University of Beirut, Beirut, Lebanon, with application copies to Personnel Services, American University of Beirut, 380 Madison Avenue, 22nd Floor, New York, N.Y. 10017. (2688)

Scientific Officers (Biometrics)

Applications are invited for two permanent and pensionable posts in the Biometrics Division of the Department of Agriculture.

Candidates must be under 27 years of age on 31 December, 1974, and have (i) a Degree, HNC or equivalent qualification in Computing, Mathematics or Statistics; or (ii) Part II of the Examination leading to Associateship of the Institute of Statisticians.

The Biometrics Division supplies a consultative service to research workers of the Department on the design and analysis of experiments and surveys. The posts in question will be concerned with (a) the programming of work in connection with computer-based sampling schemes to aid the study and control of Mastitis and Brucellosis; and (b) the application of computers to solve analytical problems and with documentation of computer packages.

Salary Scale (under review): £1,435 — £2,329.

Commencing salary will be related to qualifications and experience.

There are prospects of promotion to Higher Scientific Officer (£2,221 — £2,854), Senior Scientific Officer (£2,798 — £3,895) and Principal Scientific Officer (£3,715—£4,895).

Please write or telephone for an application form, quoting Ref. SB 175/74/N to Civil Service Commission, Clarendon House, Adelaide Street, Belfast BT2 8ND (telephone 44300, ext. 26). Completed forms should be returned to arrive not later than 16 July, 1974.

(2644)

NORTHERN IRELAND CIVIL SERVICE

UNIVERSITY OF STRATHCLYDE

DEPARTMENT OF APPLIED PHYSICS

LECTURESHIP

Application is invited for this appointment from physicists, preferably with a background in Solid State Physics or in Instrumentation.

The successful candidate will be expected to take part in the setting up of a research group in one of these fields. This group is to complement the present main research work of the Department, which is in the application of physics to problems of the natural environment and of (macro) biology. Some Industrial experience would be desirable.

Salary scale: £2,118 to £4,896 with placing according to qualifications and experience. F.S.S.U. benefits.

Application forms and further particuars (quoting 31/74) may be obtained from the Registrar, University of Strathclyde, Royal College Building, 204 George Street, Glasgow G1 1XW with whom applications should be lodged by July 8, 1974. (Applications from overseas candidates will be accepted up to July 16, 1974). (2655)

ROSKILDE UNIVERSITY CENTRE

DIVISION OF NATURAL SCIENCES

CHAIR OF BIOLOGY

Applications are invited for the chair of Biology from zoo-physiologists with experience in general physiology and human physiology.

Applicants with experience in physically oriented physiology will be preferred.

For further information, please write:

Division of Natural Sciences,
Roskilde University Centre,
P.O. Box 260,
4000 Roskilde/Denmark,

Deadline of applications August 2, 1974.

(2650)

CHRISTIE HOSPITAL AND HOLT RADIUM INSTITUTE, MANCHESTER M20 9BX PATERSON LABORATORIES

(Director: Professor L. G. Lajtha, M.D., D.Phil., F.R.C. Path.)

A vacancy exists for a person with a good honours degree in the biological sciences to investigate the nature of the target cells in experimental murine leukaemogenesis using a variety of techniques including surgery, transplantation and immunological procedures. The person appointed would work in collaboration with the divisions of Carcinogenesis, Haematology and Immunology. There will be an opportunity to register for a higher degree and the salary will be according to current MRC scale for research studentships.

Applications in writing, together with the names of two referees, should be sent forthwith to the Director. Ref. 74/26.

(51704) (2681)

ULSTER: THE NEW UNIVERSITY

EXPERIMENTAL OFFICERS

Applications are invited for two posts:

SCHOOL OF BIOLOGICAL AND ENVIRONMENTAL STUDIES
Ref: 74/93

The Experimental Officer will manage the School's scanning and transmission electron microscope facilities and will be expected to maintain the equipment, instruct users and assist in research programmes.

Applicants should be graduates (or equivalently qualified) and postgraduate experience in biological electron microscopy would be an advantage.

SCHOOL OF PHYSICAL SCIENCES (CHEMISTRY)
Ref: 74/94

The Experimental Officer in Chemistry will participate in a programme of biosynthetic research in collaboration with Professor M. F. Grundon and Dr D. M. Harrison.

Applicants should have a Ph.D. in organic chemistry or equivalent research experience.

Salary scale (under review with F.S.S.U.): £1,929 to £2,718 per annum.

Further particulars and application forms (quoting appropriate reference number) should be obtained from The Registrar, The New University of Ulster, Coleraine, Northern Ireland to whom completed applications, including the names and addresses of three referees, should be returned not later than July 31, 1974. (2653)

N.M.R. SPECTROSCOPIST

The role of the Analytical Chemistry Department within our Central Research Division is to devise and develop new techniques to control potential new drugs from discovery to marketing. A principal aim is the generation of technical data in support of submissions to world-wide regulatory agencies.

To maintain the high standard of supporting data required, we need an additional postgraduate Chemist, whose main responsibility will be the supervision of two 60 MHz Spectrometers in the NMR laboratory. Planned expansion includes 100 MHz proton and fourier transform facilities for carbon—13 work. The job involves working with a large number of research and development staff on a variety of problems arising from synthetic organic chemistry programmes.

The successful applicant will have a Ph.D. in Organic/Physical Chemistry; most probably with a heavy emphasis on NMR Spectroscopy, and a keen interest in the application of NMR Spectroscopy to the structural elucidation of complex organic molecules. A reasonable knowledge of other modern analytic techniques is desirable.

Our laboratories are situated in a pleasant rural/coastal area at Sandwich, Kent.

Our conditions of employment include a free pension and death benefit scheme, assistance with removal costs (where appropriate), bonus and flexible working hours.

Pfizer

Applications are invited from Chemists with not more than two years post-doctoral experience, and should be addressed to:
D. W. Sells, Personnel Manager, Central Research,
PFIZER LIMITED, Sandwich, Kent CT13 9NJ.

(2695)

AUSTRALIA
PUBLIC SERVICE OF VICTORIA
DEPARTMENT OF CROWN LANDS AND SURVEY
ROYAL BOTANIC GARDENS

SENIOR BOTANIST

REF. NO. (S/O1)

Yearly Salary: $A10,449 minimum; $A11,017 maximum.

Duties: To identify, classify and survey plants and to assist in the preservation and care of herbarium specimens. To conduct research and other duties as directed.

Qualifications: An approved degree in Science with a major in Botany and research experience in biosystematics and taxonomy is preferable.

Applications, quoting reference number (S/O1), should be addressed to the Secretary, Public Service Board of Victoria, State Public Offices, No. 1 Treasury Place, Melbourne, 3002, Australia, by not later than 9.30 a.m. on Wednesday, 7 August 1974, together with statements of experience and qualifications and date and place of birth. (2648)

INSTITUTE OF NEUROLOGY

requires TECHNICIAN to assist in Muscular Dystrophy Dept. in Biochemical Studies of Muscle.

Qualifications: H.N.C. preferable but not essential. Salary: University scale from £1,440 to £1,845 plus £126 L.A. Applications in writing giving qualifications, experience and names of two referees to: Dr. R. Yasin, Muscular Dystrophy Research Laboratories, National Hospital, Queen Square, London WC1N 5BG. (2673)

JUNIOR TECHNICIAN

required for Department of Histopathology to assist in research on multiple sclerosis and immunity in relation to the nervous system. O.N.C. or Science A levels an advantage. Apply in writing to the Secretary, Guy's Hospital Medical School, London Bridge SE1 9RT, giving details of age, qualifications and experience, if any, and quoting Ref. H.P.I. (2687)

THE INTERNATIONAL LABORATORY OF MARINE RADIOACTIVITY OF THE INTERNATIONAL ATOMIC ENERGY AGENCY IN MONACO HAS AN OPENING AT THE P-1 LEVEL.

The successful candidate should have a knowledge of and some experience in Marine Radioecological Research. Familiarity with radiochemical techniques employed in the determination of transuranic radio-nuclides is desirable. Ph.D. or equivalent in chemistry or chemical oceanography necessary.

Emoluments in the region of U.S. $10,994. Allowances for dependent spouse and children. Travel and removal expenses paid. Fixed term appointment two years. Send curriculum vitae to Division of Personnel, International Atomic Energy Agency, P.O. Box 590, A-1011 Vienna, Austria, not later than thirty days from today. (2677)

UNIVERSITY OF STRATHCLYDE

DEPARTMENT OF PURE AND APPLIED CHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for the position of Postdoctoral Research Assistant in the Chemical Technology Section. The successful candidate will work with Professor N. B. Graham on a research programme involving synthetic and morphological studies of novel polymeric systems.

The position will appeal to candidates with both enquiring minds and experimental skills who like to pursue basic research which is clearly relevant to industrial or biomedical applications.

The position is initially tenable for two years with a salary of £2,247 during the first year. F.S.S.U. benefits.

Applications (quoting R19/74) with names of three referees to Professor N. B. Graham, Department of Pure and Applied Chemistry, Thomas Graham Building, 295 Cathedral Street, Glasgow G1 1XL. (2656)

UNIVERSITY OF READING

RESEARCH ASSISTANT

required for two years in Department of Botany to work under the direction of Dr. M. W. Dick, on the ecology of Mucorales in aquatic ecosystems. Applicants should have a good honours degree in Botany or Microbiology. Salary approximately £1,300 p.a. depending upon age and experience. Apply as soon as possible with curriculum vitae and names of two referees and quoting ref.: MN 27, to Assistant Bursar (Personnel), University of Reading, Whiteknights, Reading RG6 2AH, from whom further particulars may be obtained. (2683)

Queensland Agricultural College

(A Multi-discipline College of Advanced Education)

APPLICATIONS: are invited for appointment to the following positions in the Department of Plant Industries:—

LECTURER IN ORNAMENTAL HORTICULTURE

LECTURER IN HORTICULTURAL AGRONOMY

SALARY RANGE: Min. $8,698—Max $11,982 per annum.

(A commencing salary above the minimum of the range may be paid depending on qualifications and experience of the successful applicant)

QUALIFICATIONS: For both positions, the possession of an appropriate degree is essential, with major studies in Horticultural Science and/or allied subjects. Applicants should have had professional experience in ornamental or environmental horticulture in the one case and horticultural agronomy, preferably with vegetable crops, in the other.

Preference may be given to an applicant who holds a higher degree and who has completed a relevant and recognised course of training as apart from studies within a basic degree course. General personal suitability and ability in teaching are also required.

GENERAL: The College is located 90 km west of Brisbane in the rich sub-tropical Lockyer Valley. Tropical and temperate, arid and humid, environments are within easy access. There is extensive vegetable production for processing and the fresh market in the Lockyer Valley and adjacent areas.

The College will pay fares and assist with removal expenses for the succcessful applicant and family, and may assist in providing temporary accommodation.

APPLICATIONS: Containing full particulars of name, address, date of birth, marital status, qualifications, experience, present employment and furnishing the names of two refereees should be forwarded to the Registrar, Queensland Agricultural College, Lawes, Q'ld, 4345 by August 10, 1974.

The successful applicant will be required to take up duties prior to the commencement of the 1975 academic year in February. (2670)

THE ROYAL VETERINARY COLLEGE

(University of London)

Division of Paraclinical Studies

DEPARTMENT OF PATHOLOGY

LECTURER IN VIROLOGY

Applications are invited from veterinary and science graduates for the newly-created post of Lecturer in Virology which is available from October 1, 1974.

Candidates should have considerable postgraduate experience in animal virology and possess a Ph.D. or equivalent degree. The appointee will be required to participate in teaching virology and virus diseases at the undergraduate and postgraduate levels. Strong research interests are essential; good facilities available, including supply of gnotobiotic animals. Prospective applicants are invited to contact Professor W. Plowright for further information.

Salary scale: 2,118 to 4,896 plus London Allowance (under review) at present £162 per annum, plus threshold supplement. Initial salary according to qualifications and experience. Superannuation under F.S.S.U.

Application form and further details available from Assistant Secretary (Personnel) (N). The Royal Veterinary College, Royal College Street, London NW1 0TU. **Closing date for applications:** August 19, 1974. (2674)

FELLOWSHIPS AND STUDENTSHIPS

UNIVERSITY OF BIRMINGHAM FACULTY OF MEDICINE AND DENTISTRY

DEPARTMENT OF PHYSIOLOGY

Applications are invited for a POSTDOCTORAL RESEARCH FELLOWSHIP to collaborate in a study of the physiological and pharmacological regulation of renin release under the supervision of Dr B. Singer.

Starting salary up to £2,553 p.a. plus F.S.S.U., depending on age and experience.

Applications, with curriculum vitae, naming two referees, should be sent to Professor S. M. Hilton, Head of the Department of Physiology, The Medical School, Birmingham L15 2TJ. (2476)

RESEARCH STUDENTSHIPS

Applications are invited for two three-year Research Studentships, tenable from October 1, 1974, at the M.R.C. Dunn Nutritional Laboratory, Cambridge, in the following areas:

(1) The relationship between protein metabolism and hormonal status in animals subjected to protein-energy malnutrition.

(2) The biochemical effects of riboflavin deficiency in an experimental animal, to relate the known parameters of subclinical riboflavin deficiency with biochemical lesions which may have pathological consequences. These studies will form part of a new programme, the aim of which is to examine various aspects of human nutritional status in relation to the water-soluble vitamins.

Applicants should have, or be expecting this summer, a first or upper second class honours degree, preferably in Biochemistry or Nutritional Science, and should send their curriculum vitae and names of two referees for (1) to Dr P. G. Lunn and (2) to Dr C. J. Bates, at the Dunn Nutritional Laboratory, Milton Road, Cambridge CB4 1XJ, not later than June 30, 1974. (2556)

UNIVERSITY OF EXETER

DEPARTMENT OF ENGINEERING SCIENCE

RESEARCH STUDENTSHIP

The Science Research Council has allocated a C.A.S.E. (Co-operative Award in Science & Engineering) studentship for three years (starting October 1974) to suitably qualified graduates interested in carrying out at the University of Exeter a "Study of deformation and failure of surface coatings on metals".

The co-operating body is the British Railways Board, Research & Development Division, Derby.

The value of the award is not less than £695 or £540 (for students living in parental home).

Applicants should possess, or expect to obtain in the Summer of 1974, a first or upper second class honours degree in Materials, Metallurgy, Physics or Engineering.

Further details and application forms can be obtained from:

Dr C. A. Brookes,
Department of Engineering Science,
University of Exeter,
North Park Road,
Exeter EX4 4QF.

Applications should be sent in as soon as possible. (2583)

North East London Polytechnic

Department of Biological Science

SRC Research Studentship in Plant Tissue Culture

Applications are invited for a **Science Research Council Studentship tenable from October 1, 1974.**

Applicants should be interested in working with plant tissue cultures. Areas in which work is in progress include the effect of cytokinin on protein synthesis and ultrastructure in soybean callus tissue; and on organogenesis and the isolation of variant cell clones in legumes and cereals. The successful candidate would be expected to work on one of the above projects under the supervision of Dr K. C. Short.

Further details may be obtained from the **Head of Biological Science Department, Ref. S/CF130, North East London Polytechnic, Romford Road, London, E15 4LZ.** (01-555 0811), to whom applications (curriculum vitae and names of 2 referees) should be made as soon as possible. (2607)

UNIVERSITY OF SOUTHAMPTON

DEPARTMENT OF CHEMISTRY

POSTDOCTORAL FELLOWSHIP IN SYNTHETIC ORGANIC CHEMISTRY

A fellowship is available now for a one-year period, although a starting date in October or later could be arranged. The appointment will be renewable for a second year. Salary in the usual range, £1,929 to £2,223 (under review) plus F.S.S.U.

Applications including the names of two referees should be sent as soon as possible to Professor R. C. Cookson, F.R.S., Department of Chemistry, The University, Southampton SO9 5NH, from whom further particulars may be obtained. (2579)

UNIVERSITY OF WARWICK

POSTDOCTORAL FELLOWSHIP IN BIOLOGICAL SCIENCES

Applications are invited for the above post to work on the isolation of messenger RNA for the small sub-unit of Fraction I protein. The work will be carried out in the Chloroplast Research Group under Dr R. J. Ellis. The appointment is for two years commencing October, 1974.
Salary in the range £2,118 by £129 to £2,412 p.a. with FSSU. Further details and application forms may be obtained from the Academic Registrar, University of Warwick, Coventry CV4 7AL, to whom completed applications should be returned by July 12, 1974. Please quote Ref. No. 46/2Q/74. (2611)

UNIVERSITY OF ST. ANDREWS

RESEARCH STUDENTSHIP

GATTY MARINE LABORATORY

Applications are invited for an S.R.C. studentship (£695) in the Laboratory from intending graduates interested in the study of invertebrate nervous systems; non-nervous conduction; sense organs; innervation of fish heart; pharmacology of small neurones. Application not later than July 5, 1974 to Professor M. S. Laverack, Gatty Marine Laboratory, St. Andrews. (2604)

Dunstaffnage Marine Research Laboratory

Applications are invited for a three year studentship to work on the role of meiofauna, particularly protozoa, in processes originating from the breakdown of cellulose fibre in marine sediments. The studentship carries with it a substantial equipment allowance. The successful applicant would be expected to read for a Ph.D. and would be required to satisfy University regulations for a higher degree. Application forms and further information can be obtained from the Director, Dunstaffnage Marine Research Laboratory, P.O. Box 3, Oban, Argyll PA34 4AD. (2568)

UNIVERSITY OF SOUTHAMPTON

FACULTY OF MEDICINE
CHEMICAL PATHOLOGY

RESEARCH STUDENTSHIP

Application are invited from biochemistry or physiology graduates, or from those who expect to graduate this summer for a Research Studentship leading to a higher degree. Applicants should have or expect to gain a good Honours degree. The successful candidate will work under the supervision of Professor K. G. M. M. Alberti in the area of intermediary metabolism in experimental diabetes or experimental liver disease. The post gives an excellent opportunity to gain experience in medical biochemistry.

The value of the award is £695 per annum plus approved fees but this is currently under review.

Applications and enquiries to Professor K. G. M. M. Alberti, Chemical Pathology, Southampton General Hospital, Tremona Road, Southampton SO9 4XY. (2578)

UNIVERSITY COLLEGE, CARDIFF

DEPARTMENT OF APPLIED MATHEMATICS AND ASTRONOMY

S.R.C. POSTDOCTORAL FELLOWSHIP IN ASTROPHYSICS

Applications are invited for an S.R.C. financed post-doctoral fellowship. The work is in collaboration with an experimental group at U.W.I.S.T. and is on the theoretical investigation of the optical properties of likely constituents of intersteller dust. The salary is commensurate with the lectureship scale and the post is for three years.

Applications including the names of two referees, to The Registrar, University College, P.O. Box 78, Cardiff CF1 1XL. Closing date two weeks from date of this advertisement. Please quote ref. 0597. (2630)

HERIOT-WATT UNIVERSITY

Applications are invited for a

CASE STUDENTSHIP

in the Department of Chemistry. The successful applicant will work in collaboration with Organon Laboratories Ltd. on new amino steroids in a series already established as possessing interesting pharmacological and clinical properties. The project will involve working both in the University and the industrial laboratories.

The University is situated in a parkland campus just outside Edinburgh.

The student, who should have a good Honours degree, will register for the degree of Ph.D. Enquiries should be sent to Dr M. M. Campbell, Department of Chemistry, Heriot-Watt University, Riccarton, Midlothian EH14 4AS. (2585)

SCIENCE RESEARCH COUNCIL

UK 48″ Schmidt telescope unit

vacancies for two Research Fellows

The Unit operates a 48-inch Schmidt telescope at Siding Spring, Australia. The primary purpose of the telescope, which has been in operation since July, 1973 is to survey the Southern sky to very faint limits in a blue waveband but time in conditions not suitable for survey work is used for other approved programmes.

POST I—SENIOR RESEARCH FELLOW/ JUNIOR RESEARCH FELLOW

An astronomer with research experience is required to join the group operating the Telescope at Siding Spring Observatory. The appointment will be at Senior Research Fellow or Junior Research Fellow level.

The primary duty of the person appointed will be to share in the demanding task of carrying out the survey to high standards of quality, including operation of the telescope and daytime photographic work. Ample opportunity exists, however, to make use of the survey material and non-survey telescope time for research purposes, and the Anglo-Australian 150-inch telescope nearby is expected to become available during 1975.

Return fares and removal expenses to Australia paid. Cost of living allowances are payable in Australia.

POST II—SENIOR RESEARCH FELLOW

An experienced astronomer is required at Edinburgh to be responsible to the Head of the Unit for the organisation and pursuit of research programmes and for some administration.

Survey plates are copied for world-wide distribution in an Atlas to be published jointly with the European Observatory who are carrying out a complementary survey in a red waveband. A limited number of glass and film copies of both surveys and of a quick ESO blue survey to less faint limits are also being distributed quickly to ESO and UK Observatories and Universities.

Copies of survey plates and original non-survey plates are returned to Edinburgh where they are available on application for loan to UK astronomers for research purposes. Some twenty research programmes are under way, many of them involving the Unit on a cooperative basis.

The duties of the astronomer appointed will be to ensure that existing research programmes are pursued with vigour, to initiate new research preferably in cooperation with University groups, and to administer the receipt, recording and distribution of plate material. A plate librarian is to be appointed to assist with the latter task together with routine photographic work.

Qualifications: Candidates for either post should possess a good honours degree in astronomy, together with research experience.

Salary: The fellowships will be for three years and will attract a fixed salary, determined according to age, qualifications, and experience on the appropriate scale: SRF £2,794 to £3,408
JRF £1,914 to £2,498

Provision can be made for F.S.S.U.

Application forms, which should be returned by July 5, 1974, are obtainable from: Mr A. Carter, Science Research Council, c/o British Rail Engineering Ltd., Swindon Works, Swindon SN1 5BW.
Tel: Swindon 26222 Ext. 22. (2624)

UNIVERSITY OF WARWICK

POSTDOCTORAL FELLOWSHIP IN BIOLOGICAL SCIENCES

Applications are invited for the above post to work on the mechanism of interferon induction by polynucleotides. The appointment is for three years commencing on October 1, 1974. Salary in the range £2,118 by £129, £2,247 by £165 to £2,412 p.a. with FSSU. Further details and application forms may be obtained from the Academic Registrar, University of Warwick, Coventry CV4 7AL, to whom completed applications should be returned by July 19, 1974. Please quote Ref. No. 46/Q/74. (2612)

UNIVERSITY COLLEGE, CARDIFF

DEPARTMENT OF APPLIED MATHEMATICS AND ASTRONOMY

Applications are invited for an S.R.C. financed post-doctoral fellowship on the theory of disordered semiconductors. The post is for three years and the salary will be commensurate with the lectureship scale.

Applications, including the names of two referees, to The Registrar, University College, P.O. Box 78, Cardiff CF1 1XL. Please quote ref. 0596. (2629)

UNIVERSITY OF STRATHCLYDE

Department of Pharmaceutical Chemistry

S.R.C. RESEARCH STUDENTSHIP ENZYME CHEMISTRY AND TECHNOLOGY

Applications are invited from candidates who have or expect to attain first or upper second class Honours degrees in Chemistry, Biochemistry or related disciplines. The research will be in one of the following areas: adsorption and micro-encapsulation of enzymes; membrane-dependence of intracellular enzymes; applications of circular dichroism; genetically determined enzyme polymorphism.

Applications and enquiries to Professor J. B. Stenlake, Department of Pharmaceutical Chemistry, Royal College, University of Strathclyde, 204 George Street, Glasgow, G1 1XW. (2610)

THE MICHAEL SOBELL CANCER RESEARCH FELLOWSHIPS OF THE CANCER RESEARCH CAMPAIGN

IN ASSOCIATION WITH THE ROYAL COLLEGE OF PATHOLOGISTS

Applications are invited for the above two Fellowships, endowed through the generosity of Sir Michael Sobell and intended for young graduate research workers to undertake laboratory research into some aspect of cancer. The Fellowships would normally be held for a period of from three to five years, and could include a period of work abroad. The salary would be appropriate to the age, qualifications and experience of the successful candidates, and funds would be made available for the running expenses of the work undertaken, and also, if applicable, for a limited amount of travel.

Applicants should submit their curriculum vitae and full details of their proposed project, and name both the Institute at which the work will be carried out and the Director who would supervise it. These details, with the names of three referees, should be sent to:

The Secretary General
Cancer Research Campaign
2 Carlton House Terrace
London SW1Y 5AR

and must reach him not later than July 29, 1974.

(2620)

POST DOCTORAL RESEARCH FELLOWSHIP

Applications are invited for a post-doctoral fellowship, tenable for one year, starting October 1974.

The project, directed by Dr. C. Descoins and Dr. P. Carle, involves an investigation on aggregating pheromones of European bark beetles. Candidates with a training in biochemistry or organic chemistry and a general interest in entomology preferred. Salary 30,000 French Francs per annum, plus 5,000 F.F. travel allowance.

The work will be carried out at Ecole Normale Supérieure (Paris) for 10 months and at Station de Recherches Forestières (Avignon) for 2 months.

Application, with curriculum vitae citing two referees, should be sent to Dr. Descoins, Chemistry Department, Ecole Normale Supérieure, 24 rue Lhomond, 75231-Paris, cedex 05, France. (2675)

UNIVERSITY OF BRISTOL

DEPARTMENT OF PHYSICAL CHEMISTRY

S.R.C./C.A.S.E. RESEARCH STUDENTSHIPS

Applications are invited for two Ph.D. Research Studentships available from October 1, 1974.

1 Non-destructive characterisation of equilibrated alloy surfaces as a function of their bulk composition. This project involves Auger and photoelectron spectra of alloy surfaces and a new method in which the alloy surface is titrated using physical adsorption of gas. Supervisor—Dr. D. F. Klemperer
Co-operating Body—AERE, Harwell.

2 An investigation of the physical chemistry of moisture movement in porous media, with particular reference to the slip-casting process. Supervisor—Dr. J. M. Haynes
Co-operating Body—English China Clays, St. Austell, Cornwall.

Candidates must have a First or Upper Second Class Honours degree (or its equivalent) in chemistry or physics.

Write to the Secretary,
Department of Physical Chemistry,
University of Bristol,
Cantock's Close,
Bristol BS8 1TS (2619)

UNIVERSITY OF GLASGOW

Applications are invited for a research studentship to work on aspects of bacterial electron transport or nitrogen fixation. Candidates should have, or expect to obtain this year, a first or upper second class Honours degree in an appropriate subject, and the successful applicant will be expected to register for a Ph.D.

Applications stating age, qualifications, experience, and the names of two academic referees, should be sent as soon as possible to Dr. R. M. Daniel, Department of Cell Biology, University of Glasgow, Glasgow G11 6NU.

In reply please quote Ref. No. 3491M. (2634)

UNIVERSITY OF GLASGOW

DEPARTMENT OF NEUROLOGY

Institute of Neurological Sciences, Southern General Hospital, Glasgow.

RESEARCH STUDENTSHIPS

Applications are invited for two Research Studentships in the University Department of Neurology. The award is for one year initially with the possibility of renewal, at Research Council rates (increase pending). It is intended that the holder shall work for a higher degree of the University of Glasgow, (M.Sc. or Ph.D) and fees will be paid.

The holder will work upon biochemical and physiological changes associated with neurological diseases. Current projects include studies of anticonvulsants in epilepsy, and exercise. Applicants should have relevant qualifications. Candidates who have graduated in 1973 or are due to graduate in 1971 will be considered.

Applications, with curriculum vitae and the names of two referees, should be addressed to Dr. R. H. Johnson, Senior Lecturer, University Department of Neurology, Institute of Neurological Sciences, Southern General Hospital, Glasgow, G51 4TF.

In reply please quote Ref. No. 3492M. (2633)

A POSTDOCTORAL FELLOWSHIP (M.R.C.)

is available on a grant held jointly by the Departments of Physiology and Chemistry, to study mechanisms of sugar transport through biological membranes, and related phenomena. The major technique to be used will be N.M.R. Spectroscopy. Candidates with a chemical or bio-chemical background, preferably with N.M.R. experience, will be preferred. Please address enquiries to Professor M. C. R. Symons, Department of Chemistry, The University, Leicester LE1 7RH. (2628)

MEDICAL RESEARCH COUNCIL MINERAL METABOLISM UNIT, LEEDS

Applications are invited for an M.R.C. research studentship from graduates with a first or upper second class honours degree in physiology, biochemistry or pharmacology who wish to work for a Ph.D. The post will be tenable for three years from October 1, 1974 and will involve a study of calcium transport in muscle and bone.

Applications, giving details of age and curriculum vitae and quoting the names of two referees, should be made to Dr. W. G. Robertson, M.R.C. Mineral Metabolism Unit, The General Infirmary, Leeds LS1 3EX, before July 16, 1974. (2665)

STUDENTSHIPS

WYE COLLEGE
(UNIVERSITY OF LONDON)

Applications are invited from U.K. graduates or from those due to graduate in the summer of 1974 for Science Research Council postgraduate studentships tenable from October 1, 1974. A First or Upper 2nd Class Honours degree will be expected. The fields of research are as follows:

Department of Horticulture

Mechanisms controlling the inhibition of flowering or Studies on the role of leaves in photoperiodic reactions.

Department of Physical Sciences

A graduate in Chemistry to work on the synthesis of biologically active compounds.

Department of Biological Sciences

Detection of plant viruses in whole plants and protoplasts by fluorescence and immunology.

Department of Poultry Research

A graduate in physiology or zoology to work on the role of central transmitter substances in avian thermoregulation.

Value award: £695 per annum plus appropriate allowances and expenses.

Application to the Registrar, Wye College, Wye, Ashford, Kent TN25 5AH as soon as possible, and in any case not later than July 10, 1974. (2660)

IMPERIAL COLLEGE

DEPT. OF ZOOLOGY AND APPLIED ENTOMOLOGY

RESEARCH STUDENTSHIPS

(PH.D./M.PHIL)

Applications are invited from persons interested in postgraduate studentships (M.R.C. or S.R.C. funded) tenable in the department as from October 1, next. These studentships will be in the following broad fields and application should be made to the member of staff concerned at Dept. of Zoology, Imperial College, London SW7.

Protozoology, apply to Dr E. U. Canning

Insect behaviour, apply to Professor J. S. Kennedy

Helminthology, apply to Professor J. D. Smyth

Biology of leafhopper, apply to Dr N. Waloff

Applications by July 10.

(2643)

FRESHWATER BIOLOGICAL ASSOCIATION RIVER LABORATORY

N.E.R.C. RESEARCH STUDENTSHIP

A studentship is available from October 1, 1974, for research into the ecology of periphytic bacteria and yeasts in rivers under the supervision of Dr. J. H. Baker. Applicants must have or expect to obtain a good honours degree or the equivalent in an appropriate subject. Applications with curriculum vitae and names of referees, as soon as possible to the Secretary, Freshwater Biological Association, The River Laboratory, East Stoke, Wareham, Dorset BH20 6BB. (2679)

UNIVERSITY OF READING

DEPARTMENT OF PHYSILOGY AND BIOCHEMISTRY

The Ministry of Agriculture, Fisheries and Food is prepared this year to offer to a suitable candidate a Studentship tenable in the Department of Physiology and Biochemistry in the University of Reading. The value of the MAFF award in 1974 will normally be £695 per annum. The subject for research leading to a Higher Degree will be either: protein and amino acids in the growing rabbit or citrate and malate metabolism in the pregnant ewe. Work should start in October 1974 and applicants should hold at least an Upper Second Class degree in a suitable subject or hope to qualify this Summer. Further particulars may be obtained from: Professor C. Tyler, Department of Physiology and Biochemistry, The University Whiteknights, Reading RG6 2AJ. Ref. M.N.18. (2682)

UNIVERSITY COLLEGE CORK

Applications are invited for the following research appointment in Physical Chemistry, supported by external funds and tenable for one year from August 1974.

POST-DOCTORAL FELLOWSHIP IN DYNAMIC MASS SPECTROMETRY

Qualifications: Ph.D. in physical science, and additional research experience, preferably in the application of Rapid Scanning Mass Spectrometry or in interfacing mass spectrometers with a computer.

Duties: Full time research in the development of dynamic mass spectrometry and the application of mass spectrometry to study heterogeneously catalysed reaction.

Stipend: Within the range £1,800 to £2,250.

Applications with Curriculum Vitae and names of two referees to:

Professor of Physical Chemistry, University College Cork, Ireland.

Closing date July 15, 1974. (2690)

KING'S COLLEGE, CAMBRIDGE

FELLOWSHIP IN APPLIED PROBABILITY THEORY IN RELATION TO POPULATION BIOLOGY

Applications are invited for Fellowships in the above field, including population and human genetics, ecology, evolutionary theory, demography and stochastic diffusion processes to be held (from October 1975 or by arrangement) in the King's College Research Centre where computing facilities are available. Senior (age limit of 32) and Junior (age limit of 28 years and not more than about 3 years research experience) fellowships are available, together with an unrestricted fellowship which could be held by a senior worker on sabbatical leave. Maximum tenure, 4 years. Closing dates: **October 1, 1974,** for seniors and open; **November 15, 1974,** for juniors.

Salary: This is based on age and linked to the University lecturer scale. For an unmarried Fellow who is provided with free-accommodation in the College it ranges at present from about £1,500 at age 24 or below to £3,450 at age 36. Married Fellows receive £500 more, £625 if they have a dependent child or children.

Details of accommodation, research facilities, etc., from: The Convener, King's College Research Centre, King's College, Cambridge CB2 1ST, England. (2680)

MISCELLANEOUS

WEDDING

Bullard, Edward Crisp to Curnow, Ursula at Braintree Registry Office, June 17, 1974. (2693)

LECTURES AND COURSES

UNIVERSITY OF STRATHCLYDE
DEPARTMENT OF BIOCHEMISTRY

M.Sc. in BIOCHEMISTRY OF REPRODUCTION

A 12 months' course in Biochemistry and Physiology of Reproduction in the female mammal commencing October 1974.

The Course is designed for graduates in a Life Science, including Medicine and Veterinary Medicine, and consists of lectures, seminars and practical work in biochemistry and endocrinology as applied to the Study of Reproduction.

M.R.C. Scholarships available for suitably qualified applicants.

Applications should be made to Professor P. J. Heald, Department of Biochemistry, University of Strathclyde, 204 George Street, Glasgow, G1 1XW, from whom further information can be obtained. (2609)

FEBS Advanced Course No. 28

Rapid Reaction in Biochemistry

Rome, October 21—26, 1974

This Advanced Course, for 20-25 participants, will consist of lectures and practical demonstrations on methods for studying rapid reactions, application to biochemical systems, and analysis of kinetic data.

The Course fee of about DM 300 will include accommodation and meals.

Applications should be sent as soon as possible to: FEBS Advanced Course, c/o Prof. E. Antonini, Istituto di Chimica Biologica, Città Universitaria, 00185 Roma, Italy, with a copy to Prof. M. Gruber, Biochemisch Laboratorium, Zernikelaan, Groningen, The Netherlands. (2608)

PROTEIN SEQUENCING

EMBO and the Max-Planck-Gesellschaft sponsor jointly a course in protein sequencing to be held at the Max-Planck-Institut für Biochemie in Munich during November 10-16, 1974.

A purpose of the course is to assist laboratories wanting to take up sequencing, particularly automatic sequencing with sequenators. The course will consist of lectures and laboratory exercises and should impart a working knowledge of commonly used techniques.

Qualified to participate in the course are those who intend to do protein sequencing in the near future and who possess academic qualifications equivalent to or higher than a Master of Science degree.

Because of the intensive nature of the course the number of participants has been restricted to 12.

Board and lodging will be provided free of charge at the guest house of the Institute. Funds are available to cover substantially the travelling costs from and to the home country.

Applicants should give a brief curriculum vitae including academic qualifications, and should state the reason for wishing to participate in the course.

Enquiries and applications should be directed to the Course Director, Dr Agnes Henschen, Max-Planck-Institut für Biochemie, 8033 Martinsried bei München, West Germany.

Deadline for applications is September 1, 1974. (2672)

UNIVERSITY OF BRADFORD

Postgraduate School of Studies in Pharmacology

M.Sc. COURSE IN EXPERIMENTAL PHARMACOLOGY

Applications are invited from graduates in pharmacology, pharmacy or other appropriate subjects, for admission to the course starting in October, 1974. The course is of one year in duration and consists of four parts concerned with quantitative pharmacology, toxicology, psychopharmacology and a research project. The aim is to provide instruction and experience of research methodology as appropriate to the investigation of mechanisms of action of drugs. M.R.C. advanced course studentships are available for suitably qualified applicants. Further information and application forms are obtainable from the Secretary of the School of Studies in Pharmacology, University of Bradford, Yorkshire BD7 1DP. (2645)

GRANTS & SCHOLARSHIPS

THE WEST OF SCOTLAND AGRICULTURAL COLLEGE RESEARCH SCHOLARSHIPS

Applications are invited from graduates with a 1st or 2nd class Honours degree in an appropriate subject for a Research Scholarship leading to a University higher degree. Suitable projects are available in Agronomy, Animal Husbandry, Agricultural Economics and Marketing, Agricultural Botany, and Agricultural Zoology. The current value of the awards, which will be tenable for 2 or 3 years, is £750 p.a. plus fees and approved travel expenses.

Applications, stating age, qualifications and experience, together with the names of two academic referees, should be sent as soon as possible to the Secretary, the West of Scotland Agricultural College, Auchincruive, Ayr, KA6 5HW. (2574)

UNIVERSITY OF ABERDEEN MILNER SCHOLARSHIPS

Applications are invited from men only for two MILNER SCHOLARSHIPS tenable at the University of Aberdeen for research in:

(1) BACTERIOLOGY.

(2) PATHOLOGY (to join one of the research groups investigating "control of the mitotic cycle", "degradation of immunoglobulins" or "serum globulins and malignant cells".)

Applicants for the Bacteriology Scholarship must hold a good Honours Degree in Biochemistry or Microbiology; those for the Pathology Scholarship in Biochemistry or Zoology/Biology.

The Scholarships, each of £1,000 per annum, are tenable for one year from October 1974 in the first instance, but are renewable; any fees due from the holder will also be paid.

Applications (2 copies) to the Secretary, The University, Aberdeen, by July 12, 1974, stating qualifications and giving names of two referees. (2586)

MEDICAL RESEARCH COUNCIL

BLOOD PRESSURE UNIT

(GLASGOW)

Applications are invited for a Medical Research Council Training Scholarship in the above Unit. The award is for up to £695 per annum and the work caried out eligible for a Ph.D. The research envisaged is the biochemical investigation of peptide hormones (in particular antidiuretic hormone) produced by malignant neoplasms.

Applications, with curriculum vitae, to Dr. J. J. Morton, Medical Research Council Blood Pressure Unit, Western Infirmary, Glasgow G11 6NT. (2625)

MEETINGS

NUDE MICE—THEIR HUSBANDRY AND USE

An informal one-day meeting on the husbandry and use of the Nude mouse will be held at the Medical Research Council, Laboratory Animals Centre, Woodmansterne Road, Carshalton, Surrey, SM5 4EF (Tel: 01-643 8000) on Tuesday, July 16, 1974. Applications to attend this meeting (for which there will be no charge) are invited from interested research workers. Numbers will be restricted to a total of 40 people. Copies of the programme are available from Dr. M. Festing at the above address. (2691)

The THIRD INTERNATIONAL SYMPOSIUM

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JULY 6–12, 1975

Glycoconjugates: Functions in Animals

The programme will include symposia on the control of synthesis, glycoconjugate production by cultured animal cells, pathology of glycoconjugate metabolism, glycoconjugates in the neoplastic transformation.

In addition Workshops will include the chemistry and biology of transplantation antigens, glycoconjugates of viruses, nervous tissue and cell receptors.

Information is available from:

Dr R. C. Hughes
National Institute for Medical Research
Mill Hill, London NW7 1AA

Dr R. D. Marshall
Department of Chemical Pathology
St Mary's Hospital Medical School
Praed Street, London W2

(2571)

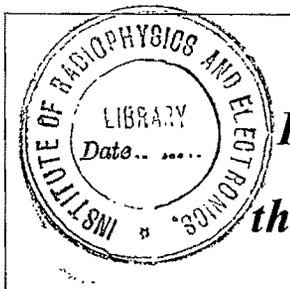
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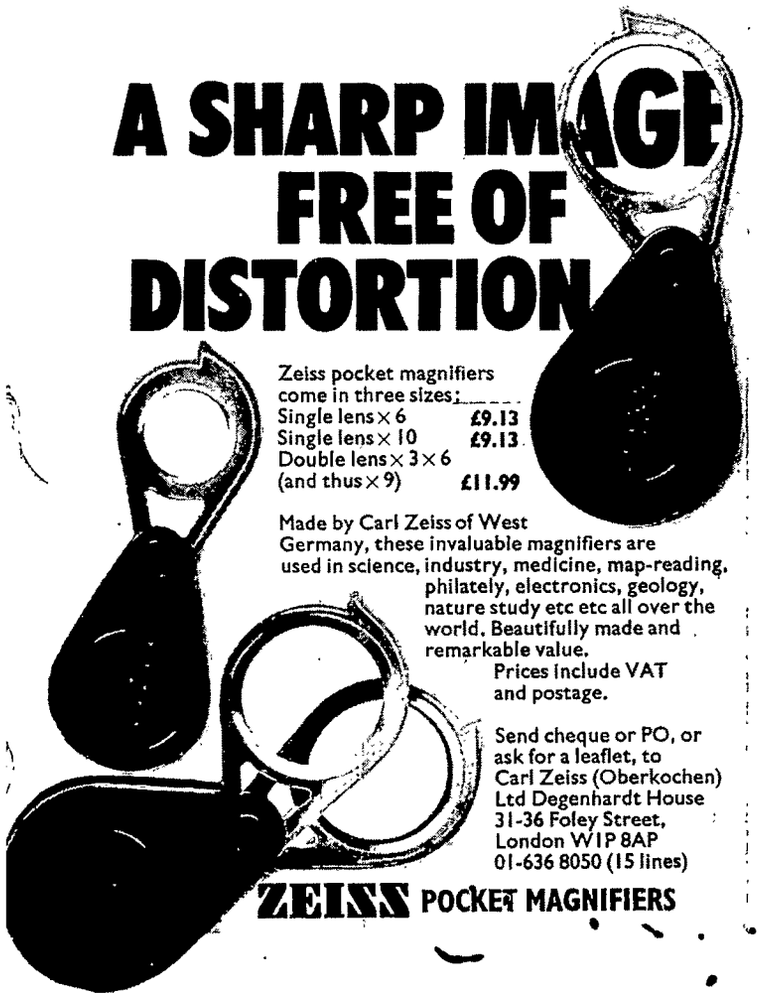
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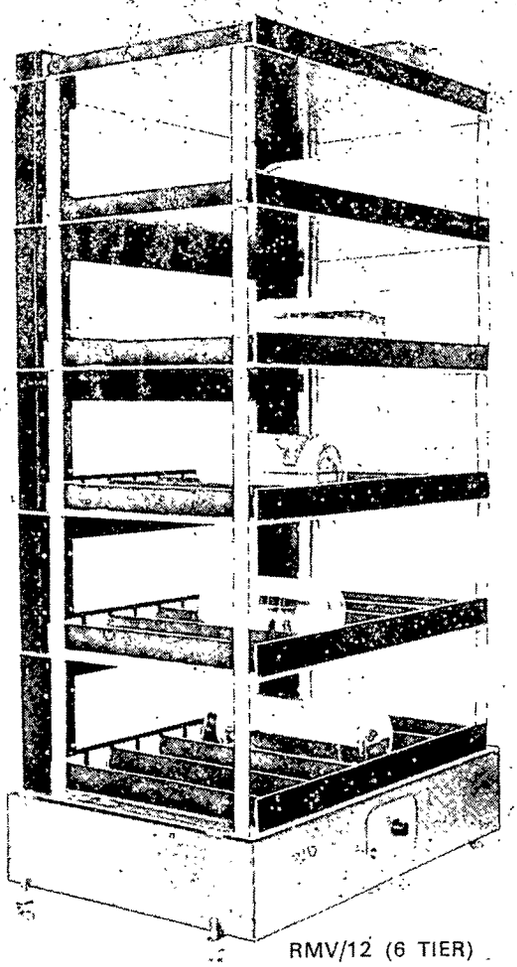
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